PROCEEDINGS OF THE FIFTY-EIGHTH WESTERN POULTRY DISEASE CONFERENCE

March 23-25, 2009 Sacramento, California

58TH WPDC SPECIAL RECOGNITION AWARD

GEORGE L. COOPER

The Western Poultry Disease Conference (WPDC) is honored to present the 58th WPDC Special Recognition award to Dr. George Cooper.

George was born and raised in San Diego, CA. He received his BS degree from San Diego State University in 1971. After a year of surfing, he was accepted into the School of Veterinary Medicine at the University of California, Davis, and received his DVM in 1976.

Dr. Cooper began his veterinary career in a mixed animal practice, but after four years, he decided to work for the California Department of Food and Agriculture as a veterinary medical officer in the Turlock laboratory. He continued at this position when the University of California, Davis, School of Veterinary Medicine, took over administration of the laboratory system in 1988. Having no specific training in poultry, George became a quick learner of poultry diseases and diagnostics. He developed a keen interest in veterinary microbiology, and in 1991, he received his MS degree from California State University, Stanislaus. Furthermore, George became board certified as a diplomate of the American College of Veterinary Microbiologists (Bacteriology/Mycology) in 1990. In addition, in 1992, he obtained his second board certification, but this time as a diplomate in the American College of Poultry Veterinarians. George retired from the laboratory system in 2006 as an associate professor.

His mentors at the Turlock lab included Drs. Dave Bristow and Art Bickford. George was one of the first to research the transmission of *Riemerella anatipester* infection in turkeys and found circumstantial evidence that mosquitoes are possible vectors of this disease in California. In addition, he was the first to report on enteritis in turkeys associated with *Cochlosoma anatis.* He is an author on more than 35 scientific papers and book chapters.

George continues to work part-time at the Turlock lab. He enjoys spending time with his two children, playing softball and travels as much as possible.

SPECIAL ACKNOWLEDGMENTS

The 58th Western Poultry Disease Conference (WPDC) is honored to acknowledge the many contributions and support to the Conference. The financial contributions provide support for outstanding presentations and to help pay for some of the costs of the Conference, thus helping us to maintain a relatively low registration fee for an international conference. More than 45 organizations, companies and individuals have once again given substantial financial support. Many companies and organizations, including some that also contribute financially, send speakers at no expense to the Conference. We thank all these people, and acknowledge their support and contribution.

We are extremely pleased to give a special acknowledgement two supporters at the Benefactor level. They are the **American Association of Avian Pathologists, Inc.** and **Schering-Plough/Intervet.** Once again, our distinguished Patrons, Donors, Sustaining Members, and Friends of the Conference are just as important and are listed on the following pages. We greatly appreciate their generosity and sincerely thank them and their representatives for supporting the WPDC.

Dr. Victoria Bowes would like to thank the members of the WPDC Executive Committee who were always insightful, encouraging, and quick to respond. Dr. Rich Chin deserves special recognition for his strong guidance and commitment to ensuring that the WPDC continues to be an exceptional scientific meeting. A special thank you goes out to the invited speakers; no one hesitated to accept the task and provide this year's program with a variety of timely and interesting presentations. Thanks to all presenters and participants who make the WPDC the conference we all look forward to each spring.

Many have provided special services that contribute to the continued success of this conference. The WPDC would like to thank Ms. Helen Moriyama of the Fresno branch of the California Animal Health and Food Safety Laboratory System (CAHFS), for her secretarial support. For this year's meeting, the WPDC has contracted Campus Events & Visitor Services, of the University of California, Davis, for providing budgetary support for the conference. We would like to thank Ms. Teresa Brown, Ms. Alison Byrum and Ms. Katrina Evans for their work with our conference.

We thank Dr. David Frame for editing and producing another outstanding Proceedings of this meeting. Dr. Frame is indebted to Ms. Sherry Nielson, Staff Assistant III of Utah State University Extension, for her seemingly endless hours of proofreading and formatting the Proceedings for publication. We express our gratitude to all authors who submitted manuscripts. We again acknowledge and thank *Ominpress* (Madison, WI) for the handling and printing of this year's Proceedings. Once again, we acknowledge Bruce Patrick (Graphic Communications, Brigham Young University) for the cover design of the printed proceedings and Dr. Rocio Crespo (CAHFS-Fresno) for original design of the CD label.

58th WPDC CONTRIBUTORS LIST

2009 BENEFACTORS

American Association of Avian Pathologists
Jacksonville, FL

2009 PATRONS

Applied Biosystems Animal Health Group
Austin, TX

Fort Dodge Animal Health
Fort Dodge, IA

Hygieia Biological Laboratories
Woodland, CA

IDEXX Labs, Inc.
Westbrook, ME

Intervet/Schering-Plough Animal Health
Millsboro, DE

Lohmann Animal Health International
Winslow, ME

Merial, Inc.
Gainesville, GA

Nippon Biologicals, Inc.
Tokyo, Japan

Pfizer Poultry Health Division
Durham, NC

Synbiotics Corporation
Kansas City, MO

2009 DONORS

Alpharma, Inc.
Fort Lee, NJ

ANECA
México DF, México

CEVA - Biomune
Lenexa, KS

Charles River
Storrs, CT

Cobb-Vantress, Inc.
Siloam Springs, AR

Cutler Associates International
Moorpark, CA

Eli Lilly and Company / Elanco Animal Health
Indianapolis, IN

G. Yan Ghazikhanian, DVM, PhD, DACPV
Sonoma, CA

Mrs. May Mayeda
(In memory of Dr. Mayeda)
Sacramento, CA

NOVUS International, Inc.
St. Louis, MO

Mrs. Kay Ogasawara
(In memory of Dr. Rosy)
Davis, CA

Phibro Animal Health
Fairfield, NJ

Mrs. Joan Rosenwald
(In memory of Dr. Rosy)
Davis, CA

2009 DONORS (Cont.)

Sunrise Farms, Inc.
Catskill, NY

Vega Farms
Davis, CA

Veterinary Service, Inc.
Salida, CA

2009 SUSTAINING MEMBERS

Aviagen, Inc.
Huntsville, AL

Bayer Animal Health
Shawnee Mission, KS

California Poultry Federation, Inc.
Modesto, CA

Diestel Turkey Ranch
Sonora, CA

Robert Edson, DVM, PhD
Lewisburg, WV

Marion Garcia, DVM
Team Borlaug MNF-W, USARC

Marion A. Hammarlund, DVM
Riverside, CA

Hy-Line International
Dallas Center, IA

ImmunoBiosciences, Inc.
Raleigh, NC

Pacific Egg & Poultry Association
Sacramento, CA

Walco International, Inc.
Ceres, CA

Zacky Farms
Fresno, CA

2009 FRIENDS OF THE CONFERENCE

Arthur A. Bickford, VMD, PhD
Turlock, CA

AgriStats, Inc.
Fort Wayne, IN

Canadian Poultry Consultants Ltd.
Abbotsford, BC, Canada

DSM Nutritional Products
Siloam Springs, AR

Marion Hammarlund, DVM
(In memory of Dr. Mayeda)
Riverside, CA

Mr. & Mrs. Jack Krout
(In memory of Dr. Mayeda)
Petaluma, CA

J.S. West Milling Company
Modesto, CA

Preserve International
Turlock, CA

Richard Yamamoto, PhD
Davis, CA

The American Association of Avian Pathologists

Supports the Educational Mission of the

Western Poultry Disease Conference

The AAAP's newest publication is the

The 3rd Edition of

Avian Histopathology

Just released January 2009

Only $75 plus 10% for shipping in US.

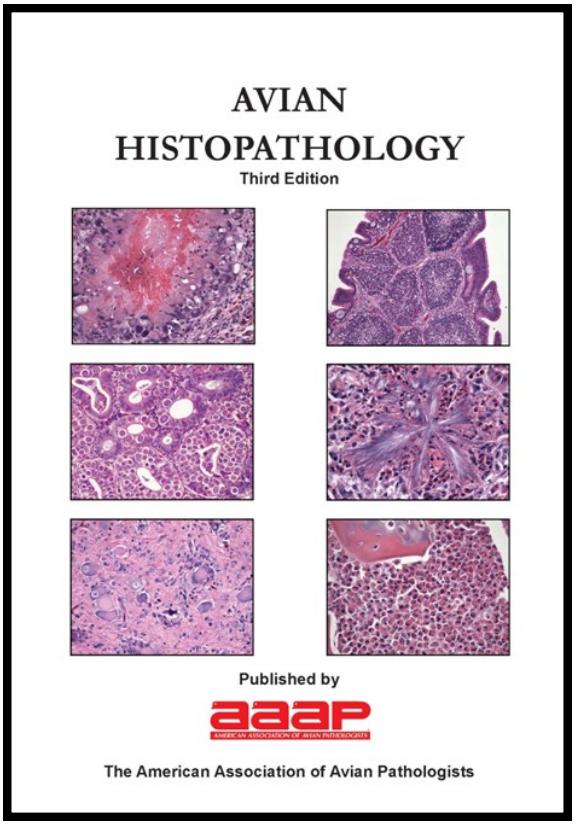
International shipping rates vary.

Order at www.aaap.info

under "publications" or

Call the AAAP business office at

904-425-5735



58th WESTERN POULTRY DISEASE CONFERENCE OFFICERS

PRESIDENT
Dr. Rocio Crespo
California Animal Health & Food Safety
Laboratory System - Fresno
2789 S. Orange Ave.
Fresno, CA 93725-1919

PROGRAM CHAIR
Dr. Victoria Bowes
Animal Health Centre
1767 Angus Campbell Road
Abbotsford, BC V3G 2M3

PROGRAM CHAIR-ELECT
Dr. Nancy Reimers
PO Box 336
Gustine, CA 95322

PROCEEDINGS EDITOR
Dr. David Frame
Utah State University
Cooperative Extension
325 West 100 North
Ephraim, UT 84627

SECRETARY-TREASURER
Dr. Richard P. Chin
California Animal Health & Food Safety
Laboratory System - Fresno
2789 S. Orange Ave.
Fresno, CA 93725-1919
rpchin@ucdavis.edu

CONTRIBUTIONS CHAIR
Dr. Yan Ghazikhanian

58th WPDC PROCEEDINGS

The Proceedings of the 58th Western Poultry Disease Conference are <u>not</u> refereed, but are presented as a service and a source of information to those attending the conference and to others who wish to gain some insight as to the information presented. Copies of the Proceedings are available in either hardcopy or electronic (CD) formats.

Copies of these Proceedings are available from: Dr. R. P. Chin
CAHFS-Fresno
University of California, Davis
2789 S. Orange Ave.
Fresno, CA 93725-1919
rpchin@ucdavis.edu

Price per copy (includes shipping & handling): **<u>Book and CD (sold together)</u>**
US$20.00 for USA shipment.
US$17.00 for AAAP members and orders of 5 or more for USA.
US$20.00 for Canada and Mexico.
US$25.00 for all other countries.

<u>Book or CD (sold separately)</u>
US$15.00 for USA shipment.
US$12.00 for AAAP members and orders of 5 or more for USA.
US$18.00 for Canada and Mexico.
US$22.00 for all other countries.

Make checks payable to: UC Regents
Still available…

50th WPDC Anniversary CD-ROM. This CD contains all printed proceedings of the first fifty Western Poultry Disease Conference meetings. Copies can be purchased from the AAAP: E-mail: aaap@aaap.info. Web: http://www.aaap.info.

Five-year Compilation (2000–2006) Proceedings of the WPDC. This CD contains the printed proceedings of the 51st through the 55th Western Poultry Disease Conferences. Copies can be purchased from the WPDC Secretary-Treasurer.

WESTERN POULTRY DISEASE CONFERENCE (WPDC) HISTORY

YEAR	PRESIDENT	PROGRAM CHAIR	DEDICATION	RECOGNITION
1st WPDC – 1952		A. S. Rosenwald		
2nd WPDC – 1953	P. D. DeLay	A. S. Rosenwald		
3rd WPDC – 1954	C. M. Hamilton	Kermit Schaaf		
4th WPDC – 1955	E. M. Dickinson	W. H. Armstrong		
5th WPDC – 1956	D. E. Stover	E. E. Jones		
6th WPDC – 1957	D. V. Zander	H. E. Adler		
7th WPDC – 1958	H. E. Adler	E. E. Jones		
8th WPDC – 1959	R. D. Conrad	L. G. Raggi		
9th WPDC – 1960	L. G. Raggi	A. S. Rosenwald		
10th WPDC – 1961	A. S. Rosenwald	D. V. Zander		
11th WPDC – 1962	D. V. Zander	R. V. Lewis		
12th WPDC – 1963	R. V. Lewis	Walter H. Hughes		
13th WPDC – 1964	W. H. Hughes	Bryan Mayeda		
14th WPDC – 1965	B. Mayeda	R. Yamamoto		
15th WPDC – 1966	R. Yamamoto	David S. Clark (1st sign of Contributors)		
16th WPDC – 1967	D. S. Clark	Roscoe Balch		
17th WPDC – 1968	R. Balch	Richard McCapes		
18th WPDC – 1969	R. McCapes	Dean C. Young		
19th WPDC – 1970 4th Poultry Health Sym. (PHS)	D. C. Young	W. J. Mathey	1st combined WPDC & PHS	1st listing of distinguished members
20th WPDC – 1971 5th PHS	W. J. Mathey	Ramsay Burdett		
21st WPDC – 1972 6th PHS	R. Burdett	Marion Hammarlund		
22nd WPDC – 1973 7th PHS	M. Hammarlund	G. W. Peterson		
23rd WPDC – 1974 8th PHS	G. W. Peterson	Craig Riddell		
24th WPDC – 1975 9th PHS	C. Riddell	Ralph Cooper		
25th WPDC – 1976 10th PHS	R. Cooper	Gabriel Galvan		
26th WPDC – 1977 11th PHS	G. Galvan	Don H. Helfer	Hector Bravo	
27th WPDC – 1978 12 PHS	D. H. Helfer	Art Bickford		
28th WPDC – 1979 13th PHS	A. Bickford	J. W. Dunsing		
29th WPDC – 1980 14th PHS 5th ANECA	J. W. Dunsing Angel Mosqueda T.	G. Yan Ghazikhanian	P. P. Levine	
30th WPDC – 1981 15th PHS	G. Y. Ghazikhanian	Mahesh Kumar		
31st WPDC – 1982 16th PHS	M. Kumar	Robert Schock		
32nd WPDC – 1983	R. Schock	George B. E. West		
33rd WPDC – 1984	G. B. E. West	Gregg J. Cutler		
34th WPDC – 1985	G. J. Cutler	Don W. Waldrip		Bryan Mayeda

YEAR	PRESIDENT	PROGRAM CHAIR	DEDICATION	RECOGNITION
35th WPDC – 1986 11th ANECA	D. W. Waldrip Jorge Basurto	Duncan A. McMartin Mario Padron	J. A. Allen A. Tellez-G. Rode	
36th WPDC – 1987	D. A. McMartin	Marcus M. Jensen		
37th WPDC – 1988	M. M. Jensen	Barry Kelly	A. S. Rosenwald	
38th WPDC – 1989	B. Kelly	Masakazu Matsumoto		Louise Williams
39th WPDC – 1990	M. Matsumoto	Jeanne M. Smith		Dean Young
40th WPDC – 1991 16th ANECA	J. M. Smith Martha Silva M.	Richard P. Chin David Sarfati M.	A. S. Rosenwald A. S. Rosenwald	
41st WPDC – 1992	R. P. Chin	Rocky J. Terry	Marcus Jensen	Henry E. Adler * *(posthumous) R. A. Bankowski C. E. Whiteman
42nd WPDC – 1993	R. J. Terry	A. S. Dhillon	W. W. Sadler	Royal A. Bagley
43rd WPDC – 1994	A. S. Dhillon	Hugo A. Medina		G. B. E. West
44th WPDC – 1995	H. A. Medina	David D. Frame	W. M. Dungan* *(posthumous)	A. J. DaMassa Gabriel Galvan Walter F. Hughes W. D. Woodward R. Yamamoto
45th WPDC – 1996 21st ANECA	D. D. Frame R. Salado C.	Mark Bland G. Tellez I.	Don Zander M. A. Marquez	Pedro Villegas Ben Lucio M. Mariano Salem Victor Mireles Craig Riddell
46th WPDC – 1997	Mark Bland	James Andreasen, Jr.	Bryan Mayeda	Roscoe Balch Paul DeLay J. W. Dunsing Don Helfer D. E. Stover
47th WPDC – 1998	J. Andreasen, Jr.	H. L. Shivaprasad	W. J. Mathey	Marcus Jensen Duncan Martin
48th WPDC – 1999	H. L. Shivaprasad	R. Keith McMillan		
49th WPDC – 2000	R. K. McMillan	Patricia Wakenell	R. P. Chin	Ralph Cooper Robert Tarbell
50th WPDC – 2001	P. Wakenell	Ken Takeshita		Don Bell Art Bickford
51st WPDC – 2002 27 ANECA	K. Takeshita J. Carillo V.	Barbara Daft Ernesto P. Soto	Hiram Lasher	Bachoco S.A. de C.V. Productos Toledano S.A.
52nd WPDC – 2003	B. Daft	David H. Willoughby		Roland C. Hartman
53rd WPDC – 2004	D. H. Willoughby	Joan Schrader		G. Yan Ghazikhanian
54th WPDC – 2005	J. Schrader	Stewart J. Ritchie	W.D. Woodward	R. Keith McMillan
55th WPDC – 2006	S. J. Ritchie	Peter R. Woolcock		M. Hammarlund
56th WPDC – 2007	P.R. Woolcock	Bruce Charlton	R. Keith McMillan	M. Matsumoto
57th WPDC – 2008 33rd ANECA	B. Charlton M. A. Rebollo F.	Rocio Crespo Maritza Tamayo S.	A. S. Rosenwald* *(posthumous) A. S. Rosenwald*	B. Daft Ernesto Ávila G.
58th WPDC – 2009	R. Crespo	Victoria Bowes		G.L. Cooper
59th WPDC - 2010	V. Bowes	Nancy Reimers		

MINUTES OF THE 57TH WPDC ANNUAL BUSINESS MEETING

President Charlton called the meeting to order on Thursday, 10th April 2008, at 3:20 PM, at the Sheraton Buganvilias, Puerto Vallarta, Jalisco, Mexico. There were 20+ people in attendance.

APPROVAL OF 56th WPDC BUSINESS MEETING MINUTES

The minutes from the 56th WPDC business meeting were reviewed and a motion was carried to approve them as printed in the Proceedings of the 57th WPDC.

ANNOUNCEMENTS

President Charlton acknowledged all the contributors; in particular, those contributing at the Benefactor level, which included the American Association of Avian Pathologists and Shering-Plough/Intervet. He also thanked all the contributors for their generous donations. President Charlton acknowledged the efforts of the current WPDC officers for their work and participation in the organization of this year's meeting. President Charlton asked that we have a moment of silence for Dr. "Rosy" Rosenwald, Violet Hughes (Dr. Walt Hughes's wife) and Dr. Roscoe Balch, all of whom passed away this past year.

REPORT OF THE SECRETARY-TREASURER

Dr. R.P. Chin presented the Secretary-Treasurer report. There were 273 registrants for the 56th WPDC held at the Riviera Hotel and Casino, Las Vegas, NV, March 27-29, 2007. Contributions for the 56th WPDC were $29,800, with a total income of $79,896. There were expenses of $76,983.51 for WPDC for the meeting, resulting in a net gain of $2,912.49. The current balance in the WPDC account was $72,952.52.

Contributions for this year's meeting (57th WPDC) were $25,575. Dr. Chin thanked Dr. Ghazikhanian for a wonderful job as contributions chair. As of the business meeting, we had approximately 500 registrants in Mexico.

REPORT OF THE PROGRAM CHAIR

Dr. Crespo reported that, on behalf of WPDC, she invited three speakers, Drs. Bland, Fletcher and Linares. In addition, WPDC agreed to pay expenses of another speaker if he gave a 30-minute presentation. (ANECA also invited four speakers.) There were a total of 129 papers presented, 88 oral and 41 posters. Of the 88 oral papers, 54 were WPDC and 34 were ANECA. Of the 41 posters, 17 were WPDC and 24 were ANECA. Only four titles were rejected and seven were withdrawn. Drs. Crespo and Soto initially wanted 50% of the titles from WPDC and 50% from ANECA. However, there ended up slightly more from WPDC because of the limited number of titles submitted to ANECA.

REPORT OF THE PROCEEDINGS EDITOR

Dr. D. Frame presented the Proceedings Editor report. Omnipress produced the Proceedings hard copy at an approximate cost of about $ 18.38 per book; up significantly from last year especially due to the size. The master CD was produced by Dr. Frame and they were duplicated in Mexico by Dr. Victor Mireles and his company.

OLD BUSINESS

None discussed.

NEW BUSINESS

President Charlton reported that the WPDC Executive Committee nominated Dr. Nancy Reimers for Program Chair-elect of the 59th WPDC in 2010. There were no other nominations and Dr. Reimers was elected unanimously as Program Chair-elect. President Charlton nominated the following officers for 2008-2009:

Program Chair: Dr. Victoria Bowes
President: Dr. Rocio Crespo
Past-President: Dr. Bruce Charlton
Contributions Chair: Dr. Yan Ghazikhanian
Proceedings Editor: Dr. David Frame
Secretary-Treasurer: Dr. Richard Chin
Program Chair-elect: Dr. Nancy Reimers

Nominations for all offices were closed and all nominees were approved unanimously.

In 2009, the 58th WPDC will be held in Sacramento, CA, March 23-25, 2009. As usual, we will schedule an ACPV workshop for Sunday, March 22, 2009.

There was discussion as to the location of future WPDCs. The WPDC Executive Committee recommended a rotation of various locations, such as Sacramento, Vancouver, BC, Mexico, and Las Vegas, be considered for future meetings. It was decided to hold the meeting in Sacramento every other year, and to rotate Canada, Mexico, and another location within the western USA the other years. Hence, the tentative schedule will be:

2009: Sacramento, CA
2010: Vancouver, BC
2011: Sacramento, CA
2012: Western USA city (e.g., Phoenix/Scottsdale, AZ; Salt Lake City, UT; Seattle, WA; San Diego, CA)
2013: Sacramento, CA
2014: Mexico

It was unanimously approved.

Dr. Cutler agreed to continue as the WPDC CE Coordinator. He and Dr. Chin will look at obtaining CE credits directly from the California Board of Examiners.

President Charlton passed the presidency to Dr. Rocio Crespo who thanked those involved in the organization of the meeting. President Crespo adjourned the meeting at approximately 3:53 PM.

TABLE OF CONTENTS

Note: Both the oral and poster presentations of the 58th WPDC are listed below in alphabetical arrangement by presenter. Authors and titles of all papers scheduled for presentation at the conference are listed.

Abbassi, H.	Significance of Antibody Detection in the Diagnostic of Chicken Cryptosporidiosis	33
Annett, C. B.	Interesting Diagnostic Cases in Western Canada	96
Avellaneda, G.	Challenge Studies to Assess Protection of Infectious Bursal Disease Virus Vaccine Against Field Challenge: The Role of SPF Controls	103
Azzipour, A.	A Study on Production Status of *Mycoplasma Gallisepticum*-Free and MG-Infected Commercial Layer Flocks in Tabriz Region of Iran	68
Bano, L.	Prevalence of NETB Gene in *Clostridium Perfringens* Field Strains Isolated from Chickens	105
Bautista, D. A.	Impact of In-House Litter Composting on Coccidiosis, Necrotic enteritis, and Broiler Performance	51
Blake, J. P.	Evaluation of Litter Treatment Regimens at Three Usage Levels	49
Bland, M. C.	Interactive Problem-Solving of Field Cases Involving Commercial Poultry – An Audience Participation Presentation	34
Broussard, C.	A New Approach to Predict the Impact of Coccidiosis Using the Standard Industry Intestinal Survey	29
Catelli, E.	Epidemiology of Avian Metapneumovirus Infection in Italy	107
Cerdá, R.	Experimental Dual Challenge with *Ornithobacterium Rhinotracheale* and *Mycoplasma Synoviae* in Broilers	63
Cha, R. M.	Phenotype and Functions of T Cells Isolated from Mucosal Linings of Turkeys Exposed to Avian Metapneumovirus Subtype C (AMPV/C)	84
Charlton, B. R.	*Enterococcus Cecorum* Osteomyelitis	98
Chowdhury, E. H.	Development of Slide Micro-Agglutination System for the Rapid Diagnosis of *Salmonella* Infection in the Chicken	7
Chowdhury, E. H.	Investigation on Outbreaks of Low Pathogenic Avian Influenza Virus in Poultry Farms in Bangladesh	21
Christensen, V. L.	How Incubation of Turkey Eggs can Affect Poult Quality	36
Cookson, K.	Crossprotection Study of a Modified Live *E. coli* Vaccine against Three Heterologous APEC Serotypes in Commercial Broiler Chickens	60
Cox, W.	BC Poultry Industry Biosecurity Initiative: The Evolution of a "Necessary Evil"	87
Crespo, R.	Isolation and Distribution of West Nile Virus in Embryonated Chicken Eggs	55

Day, J. M.	Enteric Virus Status of Turkey Flocks Over Time: Molecular Diagnostic Studies Beginning on the Day of Placement	109
Dhillon, A. S.	Examination of *Campylobacter jejuni* Putative Adhesions in Host Cell Binding and Chicken Colonization	10
Dibner, J. J.	Mechanisms of Intestinal Barrier Failure in Subclinical Enteritis	26
Dunn, P.	Interesting Diagnostic Cases from Pennsylvania	93
Fitz-Coy, S. H.	A Look at Several Live Coccidia Vaccines	30
Flamenco, V.	Evaluation of Productive Parameters after Using an O78 *E. coli* Vaccine in Broilers	111
Franca, M.	Fibrinonecrotic Typhlitis in Turkey Poults in California	99
Gallazzi, D.	Unusual Avian Mycobacteriosis in Commercial Brown Layers	113
Gallazzi, D.	Pathogenicity Markers of *Clostridium* spp. in Commercial Turkeys	115
Garcia-López, D.	Comparison between an Anticoccidial Treatment and an Immunoglobulin Y-Based Treatment in Broilers Challenged with *E. tenella*	31
Gauthier, R.	Organic Acids and Essential Oils: A Viable Alternative to Antibiotic Growth Promoters in Poultry Production	74
Gustafson, C.	Efficacy Study of a Live *E. coli* Vaccine in Commercial Turkeys Raised in Floor Pens	117
Hafez, H. M.	Efficacy of Different Doses of Paromomycin in the Feed against *Histomonas meleagridis* In Experimentally Challenged Turkey Poults	78
Hafez, H. M.	Molecular Typing of Infectious Bronchitis Virus	81
Halvorson, D. A.	New Approaches to Poultry Disease Control	1
Karunakaran, D.	Control of Avian Pathogenic *E. coli* in Commercial Poultry	58
Keirs, R. W.	A Novel Hatching Method on Application to an Existing Commercial Hatchery System Results in a Marked Decrease in Late Embryo Loss	45
Leung, F. C.	Time-Scaled Phylogenies of Both Genome Segments of Infectious Bursal Disease Virus Suggest Multiple Re-Assortment Events in its Evolutionary History	86
Leung, F. C.	Identification of *Campylobacter jejuni* in a Local Poultry Farm with Study of Transmission Route	118
Leung, F. C.	Avian H5N1 – An Animal Virus! (2008)	124
Ley, D. H.	*Mycoplasma iowae* Associated with Skeletal Lesions in Commercial Turkeys	100
Lucio-Mattinez, B.	Antibody Response to H5 Pox-Vectored Vaccine in Unconventional Poultry	19
Macklin, K. S.	The Efficacy of Biosealed in Preventing *Salmonella* spp. from Colonizing Concrete Surfaces	119
Marusak, R. A.	Comparison of Turkey Grower Management Practices on Flock Health and Performance	46

Morales Garzon, A.	Serologic Response against H5N2 Oil Emulsion AI Vaccine in Domestic Ducks	119
Munakata, Y.	Application of *Mycoplasma synoviae* Vaccine (MS-H) in Layers	65
Nagano, T.	Field Trials to Test Safety and Efficacy of a Live Attenuated Vaccine of Avian Pathogenic *Escherichia coli* Serovar O78 in Broiler Chickens	121
Nagaraja, K. V.	Role of *Clostridium perfringens* and *Clostridium septicum* in Cellulitis in Turkeys	11
Nagy, E.	Construction of a Recombinant Fowl Adenovirus 9 Expressing the Hemagglutinin Gene of Influenza A Virus	19
Piccirillo, A.	Infectious Laryngotracheitis in Italy: Clinical Cases, Diagnosis, and Characterization by PCR-RFLP	24
Ojkić, D.	Isolation and Identification of an Adenovirus from Ducklings with a Proliferative Tracheitis in Ontario, Canada	103
Olson, T. R.	It's the Feed! Vitamin E Deficiency in Turkeys	77
Pasick, J.	An Introduction to Avian Influenza Virus Molecular Epidemiology	12
Perozo, F.	Protection against Variant Strains Conferred by the Recombinant HVT-IBDV Vaccine Vaxxitek®	85
Powell, C.	A Crisis Communications/Media Relations Primer Are You Media Ready?	3 4
Reimers, N.	*In Vitro* Bio-Mos Agglutination of *Salmonella* Isolates from California Poultry Farms	6
Ritchie, S. J.	Platinum Brooding	42
Shane, S. M.	Assessing the Financial Impact of Diseases of Egg Producing Flocks	56
Singh, S.	Evaluation of the $CD8^+$ T Cell Memory Response to Adenovirus Expressing AIV Hemagglutinin	17
Slacum, G.	The Compatibility of HVT Recombinants with Other Marek's Disease Vaccines	84
Spencer, J. L.	Inactivation and Degradation of Influenza Virus and Newcastle Disease Virus during Composting	122
Stoute, S. T.	Case Report Documenting an Outbreak of Very Virulent Infectious Bursal Disease in Northern California Pullets	87
Stoute, S. T.	Trichomoniasis Primarily Involving the Respiratory Tract of Pigeons in Northern California	101
Tamayo, M.	Marek's Disease Virus Detection in the Field by PCR Method	123
Teeter, R. G.	Effective Caloric Value of Bird Health and Management	71
Thachil, A. J.	A Field Study to Control Cellulitis in Turkeys Using an Inactivated Toxoid	11
Thapa, N. K.	Hematobiochemical Changes of Layer Chickens in Experimental Aflatoxicosis	79

Trites, J. Safety of Feeding Ractopamine to Finishing Turkeys 77

Zsak, L. Prevalence of Parvovirus Infection in Chicken and Turkey Flocks in the United States 54

PROCEEDINGS OF THE FIFTY-EIGHTH WESTERN POULTRY DISEASE CONFERENCE

NEW APPROACHES TO POULTRY DISEASE CONTROL

David A. Halvorson

University of Minnesota, 1971 Commonwealth Avenue, Saint Paul, Minnesota 55108
Halvo002@umn.edu

INTRODUCTION

In 1992 exotic Newcastle disease (END) struck a flock of range turkeys on a farm in North Dakota. Authorities quickly moved in to quarantine the affected flock. They were killed with CO_2, pushed into a pit and burned on the farm. At that point it was discovered that there was a second flock on the farm. This flock had been vaccinated for Newcastle disease and had experienced no clinical signs. It was sent to market. Later the flock service person wryly commented that both approaches, mass animal destruction (MAD) and controlled marketing (CM) had been successful. It was the comment about two successful control methods used in this outbreak that stimulated the thinking behind this presentation.

HISTORY OF SOME POULTRY DISEASE OUTBREAKS

In 1924 the fowl plague epidemic became the first and most widespread serious poultry disease in the U.S. It began in New York City live poultry markets and ultimately involved poultry in 10 states, spreading all the way to Saint Louis, Missouri. The disease was recognized as a serious outbreak in the fall of 1924 and was eradicated by the spring of 1925. The disease was largely controlled and eradicated by enforcement of an embargo of incoming poultry into the live poultry markets and sanitation measures directed at conveyances of poultry. This was the last time in the U.S. that a foreign animal disease caused a large multi-state poultry outbreak.

In 1970 END affected several hundred thousand laying hens on four commercial farms, many back yard flocks and hundreds of wild birds in the El Paso, Texas, and Chamberino, New Mexico, area. By the time the disease was recognized it had apparently killed or immunized all the wild and domestic birds in the area and it quietly disappeared.

In 1971-74 a large END outbreak affected millions of chickens in southern California and was successfully controlled and eradicated by application of movement control, quarantine, sanitation and MAD. Over 12 million chickens were killed.

In 1978 turkey growers in Minnesota experienced a large outbreak of low pathogenicity avian influenza (LPAI) due largely to H6N1, but H4N8, H6N2, H6N8 and H9N2 infections were also detected. One hundred thirty market turkey flocks, 11 breeder flocks and three egg laying chicken flocks were involved. This outbreak was successfully controlled by isolation of affected flocks, traffic control, sanitation and CM.

In 1983-84 Pennsylvania poultry producers in the Lancaster area experienced a LPAI H5N2 outbreak from which a highly pathogenic avian influenza (HPAI) virus emerged. This HPAI outbreak was controlled and eradicated by application of quarantine, movement controls, sanitation and MAD. Seventeen million birds were destroyed.

In 1988 and again in 1991 Minnesota turkey growers experienced large LPAI outbreaks involving 258 turkey flocks and one broiler breeder flock in 1988 and 110 flocks of turkeys in 1991. Subtypes H1, H2, H4, H5, H6, H7, H8, and H9 were detected. Even though LPAI H5 and H7 had been detected, turkey growers, seemingly unaware of the potential danger of HPAI emerging, utilized isolation of affected flocks, traffic control, sanitation and CM.

More recent large poultry disease outbreaks include the following:

- 1995 LPAI in Minnesota successfully controlled - CM
- 1995 LPAI in Utah successfully controlled - vaccination and CM
- 2000-3 LPAI in California successfully controlled - vaccination and CM
- 2002-3 END in California successfully controlled – MAD
- 2002 LPAI in Virginia successfully controlled - MAD
- 2003 LPAI in Connecticut successfully controlled - vaccination and CM
- 2004 HPAI in British Columbia successfully controlled - MAD.

Lessons learned from historical outbreaks. The lesson seems to be that both MAD and CM are associated with successful disease control.

If we look at more recent small outbreaks of H5 or H7 AI, there is a similar trend:

- 2007 LPAI in West Virginia successfully controlled – MAD
- 2007 LPAI in Minnesota successfully controlled – CM
- 2007 LPAI in South Dakota successfully controlled – MAD

- 2007 LPAI in Nebraska successfully controlled – CM
- 2007 LPAI in Virginia successfully controlled - MAD

All these small outbreaks were controlled on the index farm.

The origin of MAD has its roots in the beginning of the 18th century when Pope Clement XI decreed that clubbing and deep burial of affected cattle would be used to eradicate what we now know as Rinderpest. This divine procedure was soon followed by other European countries and later by the United States as well (2). Mass animal destruction of animals in a disease outbreak has been called "stamping out" by O.I.E.

A lot has happened since 1711. Microbiology was discovered; our arsenal of disease control tools has expanded; agriculture has evolved; dramatic sights on television shock consumers; and the ethics of culling healthy animals is being questioned.

According to the National Highly Pathogenic Avian Influenza Response Plan: "When HPAI outbreaks occur in poultry, the preferred eradication and control methods are quarantine, enforcement of movement restrictions, and depopulation (culling) of all infected, *exposed, or potentially infected birds*, with proper disposal of carcasses and rigorous cleaning and disinfection of farms and surveillance around affected flocks." (emphasis mine)

With the advent of LPAI control plans, similar goals and language appear in some state control plans as well. So if both approaches are associated with success, the natural question is: "What do MAD and CM have to do with control?"

It appears that after the disease is under control either approach can be used successfully. There are cases when neither strategy has been particularly successful, and those cases involve actively infected birds being transported to market or being depopulated. So when birds are actively infected either strategy can fail; when the infection is under control either strategy can be successful. It would appear that MAD and CM are disposal strategies rather than control strategies.

Even though the public finds it distasteful, there is support in the veterinary community for stamping out when applied to emergency diseases. This support is strong but not universal, but what happens if healthy flocks are involved? It is often said that stamping out is the most cost effective strategy; however, recent stamping out programs involving poultry disease all eclipsed the $100 million mark in their total costs (Virginia, California, Italy, the Netherlands and British Columbia). It was recently pointed out that stamping out programs for low path AI may cost 10 to 100 times more than controlled marketing (1).

A PROPOSAL

It is questionable whether the modern poultry industry can tolerate the expense and drama of MAD in the control of LPAI, and it is questionable whether some developing countries can tolerate this method in the control of HPAI. The question of whether an alternative strategy would have been more effective has not been asked. In the absence of research trials to document the advantage of this archaic approach, regulatory officials should examine and document instances where emergency diseases were satisfactorily brought under control with a different approach. Low pathogenic avian influenza outbreaks have been effectively controlled by vaccination and CM as well as MAD, but for substantially less money.

Because industry-driven CM programs as well as government-driven MAD programs have been successful, a thoughtful examination of MAD programs leads to the idea that their success is related, not to the destruction of infected, susceptible and convalescent poultry, but to the enforced downtime, designation of infected zones, imposition of quarantines, and intensive monitoring. There is nothing special about killing and burying or burning poultry because disease outbreaks have been stopped by alternative means. Thus we can infer that it is the government's authority to quarantine, order cleaning and disinfecting, monitor and permit repopulation that accounts for its success in controlling disease. These strengths in government programs match up well with the major weakness of industry programs.

The modern poultry industry is driven by the companies' needs for meat and eggs. The weakness of industry-driven disease control is that this need for a continuous supply of meat and eggs may cause companies to act in ways that do not contribute to disease control and may actually contribute to disease spread.

A new hybrid disease control program is proposed that encompasses the best that industry and government programs have to offer. Industry, State and APHIS veterinarians, in a cooperative arrangement, could initiate well-thought out measures when a disease outbreak occurs. These measures would include biosecurity, flock scheduling, processing, vaccination, and area repopulation.

CONCLUSION

CM and MAD are disposal strategies. Successful disease control strategies have more to do with the universally accepted isolation or quarantine, movement control and sanitation rather than to one disposal technique or another. There is widespread agreement on the importance of isolation, traffic control and sanitation even if agreement is lacking on what to do with infected, exposed, potentially infected, and convalescent birds.

REFERENCES

1. Halvorson, D.A., *et al*. The economics of avian influenza control. Proceedings of the 52nd Western Poultry Disease Conference. Pp 5-7, March 8-11, 2003. Sacramento, California. 2003.
2. Kaleta, Erhard. A critical time for poultry diseases. Proceedings of the XIIIth Congress of the World Veterinary Poultry Association. July 19-23, 2003. Denver, Colorado. 2003.

A CRISIS COMMUNICATIONS/MEDIA RELATIONS PRIMER

Charlie Powell

Senior Public Information Officer, College of Veterinary Medicine,
Washington State University, Pullman, WA 99164-7010
Phone (509) 335-7073 (24 hrs.), Email: cpowell@vetmed.wsu.edu

Sound media relationships are a three-way business relationship between you, your stakeholders/ audience, and the media that will deliver your message. Your goal is to provide media with *saleable information* designed to go *through* them and *to* your audience. Anything less is destined to be ineffective at best (unheard message) and a failure (negative reaction) at worst.

What is a crisis? Any unscheduled event that can or will result in intense media scrutiny on an accelerated timeline and that could most likely negatively impact the primary concerns of a business or institution. Recall too, the lay public and media have a much more liberal definition of what they *feel* is a crisis and that evolves into a reality by broadcast. An emerging crisis can involve many conceivable things, especially one or more of the following:

- Crime;
- Negligence;
- Unethical conduct;
- Accidents;
- Public or personal health;
- Emergencies;
- Natural disasters;
- Catastrophes.

Successful crisis management requires:

- Prior planning;
- Pre-determined, trained, spokespeople;
- Open lines of communication established beforehand, including "new" media, i.e. blogs, webcasts, MySpace, YouTube, Facebook, etc.;
- Effective, purely honest media relations developed over a long period of time;
- Dedication;
- Attention to detail;
- An unwavering ethic to be reasonable, forthcoming, and truthful at all costs.

Important truths about media relations and crisis communications:

1. Even in the direst cases, each media contact is an extraordinary opportunity to reach your audience with your message. Rarely is it sound to pass up a media contact. If legal tells you otherwise, you need to build a better working relationship with them before a crisis. Welcome and work with media in a sound business manner.
2. Always, always, always confirm for yourself the details of a crisis as quickly as possible before speaking.
3. "Smoldering crises"—multiple studies have revealed that 80 percent of all "crises" involve issues or events known well in advance to those who must deal with them or who will be most negatively impacted by them.
4. "Perception is reality"—Well sort of. It's true for the most part, but perceptions are best fueled to realities by some element of fact also being present. That fact *may or may not be* related to the real issue. Nonetheless, you will have to address it and it will emotionally move your stakeholders.

5. "Truth and trust go hand in hand"—The only thing we all have in reality is credibility with others. If you lie, people don't trust you. Once people don't trust you, the truth no longer has any value to you. Once your character and credibility is called into question or worse yet exposed as fraudulent, you're done in the business.

6. "No comment" is not an appropriate answer nor will it help to resolve a crisis in your favor. Such a response is typically received by the public as, "they have something to hide." Empower those you prefer not talking to media by teaching them to say: "I'm not the person that has the information on that. Here is the contact information for the person who does. If you problems reaching them, call me back and I will help track them down."

7. Be willing to question any information you are given from any source before you convey it to the public as fact or advice. Have appropriate expertise speak to their areas of expertise.

8. The media is a necessary part of modern business—deal with them at each and every opportunity and develop sound business relationships early.

9. Develop a sound working relationship with your legal counsel in advance as this is growing in importance.

10. The best handling of crisis and risk communication occurs when all the players know each other BEFORE the crisis. There is no substitute for reaching out and getting to know all who might be involved as well as conducting training exercises.

11. Prepare for the long haul. Media interest will continue in a crisis as long as the story remains saleable to the public.

ARE YOU MEDIA READY?

Charlie Powell

Senior Public Information Officer, College of Veterinary Medicine,
Washington State University, Pullman, WA 99164-7010
phone 509-335-7073 (all hours), fax 509-335-2132
email: cpowell@vetmed.wsu.edu, website: www.vetmed.wsu.edu

Media relations are the most visible, successful, and frequently used public relations tool. This guide will help you develop skills to become media ready.

Tips on Speaking with Media Representatives:

- When a reporter calls, buy time. Ask when their deadline is and tell them you will call back in 20 or 30 minutes because you are speaking with someone right now.
- Use the time to develop your message. Three to five simple, declarative sentences, 20 words or less.
- Always provide a business card. If the subject is complicated, add a bulleted fact sheet.
- Be yourself.
- Be conversational, rather than formal in your approach. Speak in short, concise sentences, 20 words or less.
- Express passion for your topic.
- Be honest about your knowledge and skill levels.
- Never say anything that you don't want to see in print or hear on the air. There is no such thing as **off the record especially in today's world where people produce their own news**.
- Be prepared for the interview by having a comment ready for each of the topics and issues that touch your area of expertise.
- Each interview needs well thought out ideas. Take a second to think before speaking. If you don't understand the question, say so and it will be rephrased.
- Choose one or two messages that are important for the public to know. Emphasize those points through repetition and comments such as "This is very important."
- Offer to help the interviewer with as much information as you may have available. By feeding them information, you will have a good idea of what questions will be asked of you.
- Remember that people in the listening or viewing audience don't usually know industry jargon. Substitute common terms. Pretend you are talking to an aging friend.

- If a statement is made that is not true, refute it immediately and politely.
- This is not an oral examination or a court deposition. When you have delivered your message simply and clearly, stop talking. If asked again, make your statement again and then smile and stop talking.
- Get additional media relations training at each opportunity.

Television Tips:

- Choose an appropriate background.
- Direct your attention to the person conducting the interview—not the camera.
- Wear clean, conservative colors and clothing. Avoid harsh contrasts in color and patterns. Present a professional image.
- Make sure your hands and fingernails are clean.
- Try to keep movements to a minimum and underplay your gestures. Sit or stand still in a natural and relaxed manner.
- Visual aids are always helpful.
- Be yourself! Remember, TV is an intimate medium that seeks emotion above all else.

Radio Tips:

- Listen to the interview or show ahead of time to get to know your host and audience.
- Have facts and examples written on note cards for easy reference.
- Be relaxed; you have time to make your point.
- Be careful what you say during commercial breaks, as your microphone might still be on.

Newspaper Tips:

- Always ask for the reporter's deadline.
- Be flexible and prepared to change your schedule to accommodate the reporter.
- Make friends with the reporter—not enemies.
- You can ask if the reporter knows when the story will run; however, usually the reporter will not be able to give you an exact date.
- Reporters are simply a conduit for information; you are really talking to an audience.

Telephone Tips:

- Ask for the topic and the angle of the story.
- If you need time to prepare, ask if you can call the reporter back.
- Ask the reporter if responses are going to be taped directly off the phone.
- Answer the questions briefly and to the point. Remember nothing is **off the record**.

New media:

- This includes blogs, podcasts, RSS feeds, mobile feeds, and online video content **not just** your website
- Journalists today expect these other sources to be available
- People opposed to your enterprise will use these communication channels
- It can have profound influence
- In crisis and risk communication, these media are essential.

For Every Interview:

- Be personable
- Be accurate
- Be accessible
- Be careful
- Be exciting
- Be direct
- Be available

Most importantly…

Always remember you are talking THROUGH media to YOUR AUDIENCE. You are NOT talking TO media exclusively. All messages must be saleable for media's audience AND address your needs to your stakeholders.

IN VITRO BIO-MOS AGGLUTINATION OF *SALMONELLA* ISOLATES FROM CALIFORNIA POULTRY FARMS

Nancy Reimers, Kyle Newman, Francisco Uzal, and Gregg Cutler

SUMMARY

Twenty individual isolates of *Salmonella* spp. were selected from cases submitted to the California Animal Health and Food Safety Laboratory System. The cases represented 11 unique accessions from four companies in California. All companies routinely utilize a *Salmonella* vaccination program and occasionally include mannin-oligo-sacharride products in their rations.

All isolates were non-group D *Salmonellas*. Eighty-five percent of the submitted isolates were from routine chick paper screening and 15% were from mortality – one direct swab and two intestinal pools. BIO-MOS agglutinated 70% of the isolates. BIO-MOS agglutinated 100% of the mortality isolates and 65% of the chick paper isolates.

(The full-length article will be published in *Avian Diseases*.)

Sample Number	Agglutination by BIO-MOS	Source	Company
1A	Yes	Chick Papers	A
1B	Yes	Chick Papers	A
1C	Yes	Chick Papers	A
1D	Yes	Chick Papers	A
2A	No	Chick Papers	A
2B	No	Chick Papers	A
3A	Yes	Mortality - Direct Swab	B
4A	Yes	Chick Papers	C
5A	Yes	Chick Papers	C
6A	Yes	Chick Papers	C
6B	No	Chick Papers	C
7A	Yes	Chick Papers	C
7B	Yes	Chick Papers	C
8A	No	Chick Papers	C
9A	Yes	Chick Papers	C
9B	Yes	Chick Papers	C
10A	No	Chick Papers	D
10B	No	Chick Papers	D
11A	Yes	Mortality - Intestinal Pool	D
11B	Yes	Mortality - Intestinal Pool	D

DEVELOPMENT OF SLIDE MICRO-AGGLUTINATION SYSTEM FOR THE RAPID DIAGNOSIS OF *SALMONELLA* INFECTION IN THE CHICKEN

R. Parvin, E. H. Chowdhury, M. Asaduzzaman, N. Jannat, M. G. Haider, and M. A. H. N. A. Khan

Department of Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh
emdad001@yahoo.com

ABSTRACT

The cultural method to identify avian *Salmonella* infections is laborious and expensive, thus a rapid, sensitive and cost-effective method for the diagnosis of salmonellosis is anticipated. *S. pullorum* was isolated and a stained *Salmonella* somatic antigen was prepared. The protein concentration of stained antigen was measured and adjusted to 3000 ng/µL. Preservative was used to increase its shelf life. Slide agglutination tests were carried out with un-diluted and diluted anti-sera having known ELISA titre and end point agglutination titre was determined. The standard curves were generated using 2- and 10-fold dilutions of sera to determine the titre of the unknown field sera. It was observed that the newly developed stained somatic antigen produced distinct tiny clumps with positive anti-sera of *Salmonella* spp. The present slide agglutination system was found to be easy, sensitive, reliable, cost and time effective and needs very small amount of antigen, sera and as well as accessories. This method may be used to screen the *Salmonella* infections in the poultry farms and to calculate the titre of the anti-salmonella antibody in the infected and vaccinated chickens under farm conditions. A test kit containing stained antigen along with positive and negative control sera may be prepared for commercial uses.

INTRODUCTION

Salmonellae including pullorum disease, fowl typhoid and other infections may cause varieties of clinical signs from acute systemic disease and gastrointestinal symptoms in poultry flocks to embryonic problem in hatchery (5). In recent years, diagnostic laboratories have been concerned with reducing the time required for diagnosis of *Salmonella* infections. The current standard laboratory procedure to culture and identify *Salmonella* takes approximately four to seven days. Even these methods are tedious, time consuming and confer little guarantee of sensitivity and species specificity. Tube agglutination test for pullorum disease was described by Jones's in 1913 (8) and subsequent introduction of a rapid plate serum agglutination (RSA) test (9) and a stained antigen for whole blood test (11) provided a practical basis. But none of these tests were found sensitive and was solved by the development of the enzyme-linked-immunosorbent assay (ELISA) (4) and its application to the measurement of antibody response to specific infections (13). The application of ELISA assays for *Salmonella enteritidis* have been described (3,7,12). However, ELISA test is expensive, time consuming, needs skilled manpower and not easy to perform in the field condition. The microagglutination (MA) system (microtest) had been adapted for a wide variety of serological procedures due to its better sensitivity (1,15). It has been cited for detecting agglutinations of several *Salmonella* serotypes (15) in the field condition as well as a routine diagnostic test for the detection of chronic carriers of *S. pullorum* and *S. gallinarum* (14). Antigen produce from local isolate is always more sensitive and will be economically cheaper than the imported one. Therefore, the present study was undertaken with the following objectives: Preparation of stained colored *Salmonella* antigen with a local isolate and development of slide microagglutination (MA) system for the rapid diagnosis of *Salmonella* infection in chickens in the field condition.

MATERIALS AND METHODS

Preparation of antigen. The locally isolated *S. pullorum* was used for production of the *Salmonella* colored antigen. Test tubes containing samples on nutrient broth were incubated for 24 h at 37°C. From the nutrient broth, subcultures were also made on Brilliant Green agar, Salmonella Shigella agar, MacConkey agar, EMB agar, TSI agar, LB agar, and nutrient agar, and incubated at 37°C for over night. On the basis of colony and staining characters, and biochemical tests the organisms were isolated and identified. The organism was further confirmed by PCR as described in Saha *et al*. (10). Single colony of *Salmonella pullorum* was inoculated into 50 mL of LB broth. Then the flasks were placed in incubation for 48 h. 50 mL broth culture was divided into two conical flasks containing each 25 mL. Then tetrazolium salt was added aseptically in the amount of 0.5% in each

broth culture and incubated one flask for 2 h and another flask for 24 h. After incubation, 0.5% phenol was added in each flask and was incubated for 1 h and 2 h, respectively. The stained broth suspension was filtered through sterile gauge and poured in to eppendorf tube. Afterwards these were centrifuged at 16000 rpm for 15 min, supernatant was decanted, and cells were suspended in 0.5% phenolized saline, 0.5% fomalized saline and 0.09% sodium azide. The suspension was vortex vigorously with a few sterile glass beats and transferred it into another eppendorf tube. Then the solution kept in 4°C as neotetrazolium stained antigen for future use. The total protein concentration of the stained antigen was measured by the Folin Phenol method of Lowry *et al.* (6). 20 µL of stained antigen and 20 µL chicken sera were placed on a sterile glass slide by a micropipette and mixed thoroughly by stirring with tips. The agglutinations titer of unknown sera were determined and compared with known ELISA titer. Correlation curve were prepared to determine the antibody titer of unknown field sera. Finally shelf life was determined for newly developed *Salmonella* antigen.

RESULTS AND DISCUSSION

Preparation of *Salmonella* colored antigen. In the present study a sensitive neotetrazolium stained *Salmonella pullorum* antigen was prepared from a local isolates and a micro-agglutination procedure for the detection of *S. pullorum* antibody was developed. The protein concentration of the colored antigen was adjusted to 3000 ng/µL with the help of BSA standard curve. A suitable preservatives 0.5% phenolized saline was chosen.

Determination of antibody titer. The collected known positive sera were diluted as 10-fold and 2-fold pattern and agglutination test was performed with the newly developed *Salmonella* colored antigen. In 10-fold dilution, the positive agglutination was recorded maximum up to 10^{-8} dilution with antibody having titer 11870, while it was up to 10^{-1} dilution when antibody titer was 1570. To check it further, 2-fold dilutions were made from a panel of antibodies having ELISA titer 335-2311. In case of 2-fold diluted sera the positive agglutination recorded maximum up to 2^{-7} dilution with antibody having titer 2311, while it was up to 2^{-2} dilution with antibody having titer 676 and no agglutination with antibodies having titer 335.

Standard curve. From the 10-fold and 2-fold dilution it was observed that the end point agglutination titer of serum sample having ELISA titer 1570 was found in 10^{-1}th dilution, while in 2-fold dilution end point agglutination titer was found in 2^{-5}th dilution with serum sample having ELISA titer 1630. This indicates a positive correlation between 2- and 10-fold dilution (Figure 1).

Validation with field sample. Among the seven flocks, the flock number 49 showed the higher prevalence as 35% confirmed case, 35% suspect and 30% negative. The flock number 50 and 51 also showed positive agglutination in 20% and 10% cases, respectively. The flock number 52, 53, 54, and 55 did not show any positive result but 30%, 10%, 15%, and 10% cases respectively, were found suspect. Detail of the results may be seen in Table 1. The agglutination percentage of total number of sera from seven flocks was also calculated. Among 180 sera it was shown that 13 sera were confirmed as positive, 31 suspect, and 76 negative. The ELISA titer of unknown sera were calculated from the standard curve that was almost similar to the instruction of the ELISA kit (GUILDHAY, UK).

Sensitivity and Specificity. The tetrazolium stained *Salmonella* antigen from a local isolate was successfully developed which could be used to screen the *Salmonella* infection in the poultry flocks at the farm premises. Although this antigen was developed from the local isolate of *Salmonella pullorum* it also reacts with antibodies of *Salmonella gallinarum* and other *Salmonella enteritidis*. The neotetrazolium stained *Salmonella* antigen showed enhanced sensitivity than the conventional agglutination test and the specificity of the stained antigen was high as it did not reacted with the negative serum and water control. The results are in agreement with a previous study (14).

This method also may be used to calculate the titer of the anti-*Salmonella* antibody in the infected and vaccinated chickens under farm conditions. Finally a kit was organized named "BAU-Path S Antigen Kit" contain 1mL of developed stained antigen that was sufficient for 50 test, positive serum, negative serum, and information sheet However, cautions have to be taken with the reading of the results. With increase time, the stained antigen may react with the non-specific antibody present in the serum. Therefore, results within one minute were suggested as suspected case of salmonellosis. The antigen must be shaken before use. Always keep away from the light.

REFERENCES

1. Anonymous. A selected bibliography of micro methods in microbiology with special emphasis on microtiter techniques. Cooke Engineering Co., Alexandria, Va. 1970.

2. Cheesbrough, M. District Laboratory Practice in Tropical Countries, part- 2. Cambridge low price edn. Cambridge University Press, UK. pp. 64-65. 2000.

3. Cooper, G.L., R.A. Nicholas, and C.D. Bracewel. Serological and bacteriological investigations of chickens from flocks naturally infected with *Salmonella enteritidis*. *Vet. Res.* 125: 567-572. 1989.

4. Engvall, E. and P. Perlman. Enzyme-linked immunosorbent assay (ELISA). Quantitative Assay of Immunoglobulin G. Immunochemistry, 8: 871-874. 1971.

5. Gast, R.K. Paratyphoid infections. In: Calnek, B.W., H.J. Barnes, C.W. Beard, L.R. McDoughand, and Y.M. Saif, (eds). *Diseases of Poultry*, 10th edn. Lowa State University Press. Ames, IA. Pp. 97-121. 1997.

6. Lowry O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. Protein measurement with the folin phenol reagent. *J Biol Chem; 193:265-75.* 1951.

7. Nicholas, R.A. and G.A. Cullen. Development and application of an ELISA for detecting antibodies to *Salmonella enteritidis* in chicken flocks. *Vet.Res.*128: 74-76. 1991.

8. Rettger, L.F. Septicaemia among young chickens. N.Y. *Med. J.* 71: 803-805. 1990.

9. Runnels, R.A., C.J. Coon, H. Farley, and F. Thorp. An application of the rapid-method agglutination test to the diagnosis of bacillary white diarrhoea infection. *J. An. Vet. Med. Ass.* 70: 660-662. 1927.

10. Saha S.S., E.H. Chowdhury, S.M. Rhaman, S. Sultana, M.G. Haider, and M.R. Islam. Detection of *Salmonella gallinarum* using polymerase chain reaction. Paper submitted to Bangladesh Veterinary Journal. 2007.

11. Schaffer, J.M., A.D. McDonald, W.J. Hall, and H. Bunyea. A stained antigen for the rapid whole blood test for pullorum disease. *J. Am. Vet. Med. Ass.*79: 236-240. 1931.

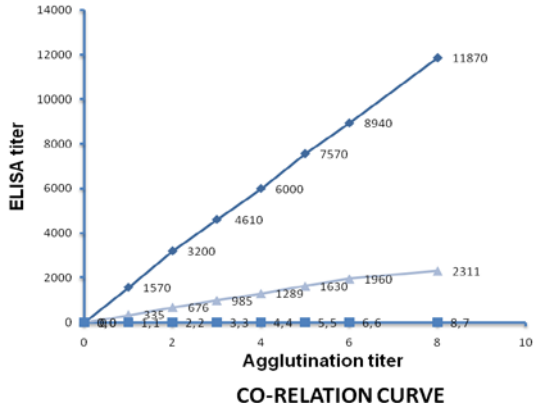
12. Timoney, J.F., N. Sikora, H.L. Shivaprasad, and M. Opitz. Detection of antibody to *Salmonella enteritidis* by a gm flagellin-based ELISA. *Vet. Res.* 127: 168-169. 1990.

13. Voller, A., D. Bidwell, and A. Bartlett. Microplate enzyme immunoassays for the immuno-diagnosis of virus infections. Manual of Clin. Immunol. ASM, Washington, D.C.: 506-512. 1976.

14. Williams, J.E. and A.D. Whitemore. Serological diagnosis of pullorum disease with the microagglutination system. *Applied Microbiol.* 21: 394-399. 1970.

15. Witlin, B. Detection of antibodies by microtitration techniques. *Mycopathol. Applied Microbiol.* 33: 41-257. 1967.

Figure 1. Standard curve between ELISA and agglutination titer (10-fold and 2-fold).



Legend:
Series 1: 10-fold agglutination titer and ELISA titer
Series 2: Dilution scale
Series 3: 2-fold agglutination titer and ELISA titer

Table 1. Field sample showing the agglutination result with newly developed stained antigen.

Flock no.	No. Of serum	Age in week	Agglutination result					
			Positive(+)		Suspect (±)		Negative(-)	
49	20	54.6	07	35%	07	35%	06	30%
50	20	54.6	04	20%	06	30%	10	50%
51	20	52.1	02	10%	05	25%	13	65%
52	30	42.2	0	0%	06	30%	24	80%
53	30	36.2	0	0%	02	10%	28	93.3%
54	30	36.2	0	0%	03	15%	27	90%
55	30	34.2	0	0%	02	10%	28	93.3%

EXAMINATION OF *CAMPYLOBACTER JEJUNI* PUTATIVE ADHESINS IN HOST CELL BINDING AND CHICKEN COLONIZATION

A. Singh Dhillon and Michael E. Konkel

Washington State University, Avian Health and Food Safety Laboratory, 2607 W. Pioneer, Puyallup, WA 98371

Campylobacter jejuni colonization of chickens is dependent upon surface exposed proteins termed adhesins. Putative *C. jejuni* adhesins include CadF, CapA, JlpA, MOMP, PEB1, Cj1279c, and Cj1349c. We examined the genetic relatedness of ninety-seven *C. jejuni* isolates recovered from human, poultry, bovine, swine, ovine, and canine sources by multilocus sequence typing (MLST) and examined their profile of putative adhesin-encoding genes by dot blot hybridization. To assess the individual contribution of each protein in bacteria-host cell adherence, the *C. jejuni* genes encoding the putative adhesins were disrupted by insertional mutagenesis. The phenotype of each mutant was judged by performing *in vitro* cell adherence assays with chicken LMH hepatocellular carcinoma epithelial cells and *in vivo* colonization assays with broiler chicks. MLST analysis indicated that the *C. jejuni* isolates utilized in this study were genetically diverse. Dot blot hybridization revealed that the *C. jejuni* genes encoding the putative adhesins, with the exception of *capA*, were conserved among isolates. The *C. jejuni* CadF, CapA, Cj1279c, and Cj1349c proteins were found to play a significant role in the bacterium's *in vitro* adherence to chicken epithelial cells, while CadF, PEB1, and Cj1279c were determined to play a significant role in the bacterium's *in vivo* colonization of broiler chicks. Because Cj1279c promotes the binding of *C. jejuni* to host cells, plays a significant role in *C. jejuni* colonization of chickens, and harbors fibronectin Type III domains, we have termed the product encoded by the *Cj1279c* gene FlpA for Fibronectin-like protein A.

ROLE OF *CLOSTRIDIUM PERFRINGENS* AND *CLOSTRIDIUM SEPTICUM* IN CELLULITIS IN TURKEYS

K. V. Nagaraja, A. J. Thachil, and D. A. Halvorson

Department of Veterinary and Biomedical Sciences, University of Minnesota,
1971 Commonwealth Ave, St. Paul, MN 55108

Cellulitis in turkeys has been causing a significant economic loss for turkey producers in USA. Its impact has caused significant concern for it to be currently considered as the high priority disease of turkeys in Minnesota and elsewhere. Cellulitis is associated with acute mortality and presence of inflammatory subcutaneous fluid and crepitus, most commonly in commercial male turkeys nearing market age. The mortality is reported to be as high as 2 to 3% per week in the affected flocks.

Cellulitis usually appears at the age of 13 to16 weeks and persists until the birds are marketed. But more recently, even 8-week old birds were found to be affected with cellulitis. The lesions have been seen in various areas of the body, including: the breast, abdomen, legs, thighs, groin, and the back of the bird. Interestingly, in most cases of cellulitis, there appears to be no trauma to the skin. Palpitation of the affected areas often reveals crepitation due to gas bubbles in the subcutis and musculature. At necropsy, there is accumulation of bubbly, serosanguinous fluid in the subcutis. For this reason, cellulitis condition in turkeys appears different from the gangrenous dermatitis (GD) reported in broilers. The underlying musculature may have a cooked appearance in severe cases. The liver and spleen are often enlarged and may contain large necrotic infarcts. The kidneys are usually swollen.

Diagnostic laboratories have consistently isolated clostridial organisms from turkey cellulitis lesions. Organisms isolated from cellulitis lesions in turkeys predominantly include *Clostridium septicum* and *C. perfringens*. Experimental induction of cellulitis lesions and mortality in turkeys is possible with *C. perfringens* and *C. septicum* indicating the significance of clostridia in causing cellulitis. The affected tissues contained large amounts of gelatinous exudates and gas bubbles, most of which are in the subcutaneous and intermuscular connective tissue.

In our study we found *C. perfringens* and *C. septicum* spore culture caused severe cellulitis and mortality in five-week-old turkeys under experimental infection

C. septicum was found to be more lethal in mice as well as in turkeys and are more capable of inducing cellulitis lesions and mortality than *C. perfringens* in poults. The results of this study will be presented.

A FIELD STUDY TO CONTROL CELLULITIS IN TURKEYS USING AN INACTIVATED TOXOID

Anil J. Thachil, David A. Halvorson, and Kakambi V. Nagaraja

Department of Veterinary and Biomedical Sciences,
University of Minnesota, 1971 Commonwealth Ave, St. Paul, MN 55108

Clostridium perfringens and *C. septicum* are currently considered as the organisms responsible for cellulitis in turkeys, a major cause of economic loss to turkey producers over the last few years. The involvement of other species of clostridia including *C. septicum* in the development of cellulitis in turkeys is increasingly reported recently. The objective of our study was to look at the effects of an experimental toxoid vaccine in controlling cellulitis in turkeys using *C. septicum* alone. *Clostridium septicum* was grown and allowed to sporulate producing toxins in a suitable media. The culture supernatants were used to make an experimental *C. septicum* toxoid. The experimental inactivated vaccine was found to be useful in reducing the antibiotic usage and mortality in cellulitis affected birds in the field.

AN INTRODUCTION TO AVIAN INFLUENZA VIRUS MOLECULAR EPIDEMIOLOGY

John Pasick

Canadian Food Inspection Agency, National Centre for Foreign Animal Disease, Winnipeg, Manitoba, Canada R3E 3M4

INTRODUCTION

Avian influenza (AI) outbreaks in gallinaceous poultry are not random; rather, they can usually be related to environmental and host factors that vary with time, place and population subgroup. Molecular epidemiology, which employs molecular techniques to type and subtype avian influenza viruses, is one useful way of providing insight into the potential source and distribution of the viruses responsible for these outbreaks.

The ultimate source of all avian influenza viruses is wild aquatic birds belonging to the orders Anseriformes and Charadriformes. These viruses are characterized by combinations of two surface antigens: the hemagglutinin (HA), of which there are 16 genetically and antigenically diverse subtypes and the neuraminidase (NA), of which there are nine genetically and antigenically diverse subtypes. In addition to these surface antigens, the segmented genome of AI viruses code for an additional eight proteins. Under some situations viruses from this natural reservoir compartment are transmitted to gallinaceous poultry where they may undergo adaptation. It is thought that the evolutionary dynamics of AI viruses within wild aquatic birds and gallinaceous poultry differ significantly. Viruses in wild aquatic birds are thought to be in a state of evolutionary stasis characterized by low rates of evolutionary change (1). By contrast, viruses that are introduced to abnormal hosts like gallinaceous poultry are thought to undergo higher rates of evolutionary change. Influenza viruses undergo genetic change by a number of different mechanisms. These include point mutations, insertions, deletions and, due to the segmented nature of the viral genome, segment reassortment.

BASIC CONCEPTS OF MOLECULAR EVOLUTION

AI viruses have RNA genomes and given the error prone nature of RNA polymerases, mistakes in nucleotide incorporation is a feature associated with the replication of their genes. Because of the degeneracy of the genetic code these nucleotide incorporation errors or substitutions can be either synonymous (i.e. result in no amino acid change) or non-synonymous (i.e. result in an amino acid change). Prior studies have shown that AI viruses from wild aquatic birds have relatively low numbers of nonsynonymous to synonymous substitutions per site (d_N/d_S) which may indicate that strong selective constraints are at work within this population (2). Nucleotide substitutions fall into two major groups. Those which involve a purine (adenosine and guanine) being replaced by another purine or a pyrimidine (cytosine and thymidine/uracil) being replaced by another pyrimidine are referred to as transitions. Those which involve a purine being replaced by a pyrimidine or visa-versa are referred to as transversions. For steric reasons, transitions are more easily made than transversions, a fact that is taken into consideration in many nucleotide substitution models. Other errors in viral gene duplication can result in the deletion or insertion of one or more nucleotides, referred to as *indels*. If the indel involves multiples of three nucleotides, the reading frame of the gene is preserved. However, when the indel involves one or two nucleotides, the reading frame of the gene is disturbed resulting in a truncated and often defective viral protein.

The above changes are random in nature. Their fixation within a population is termed the evolutionary rate. The evolutionary rate for AI viruses has been estimated to be quite high, ranging from 1.8 to 8.4 x 10^{-3} substitutions/site/year (3). In the presence of appropriate selective pressures, changes resulting in an adaptive advantage will increase in frequency and become fixed in a population after fewer generations than if the change were to have neutral effects. This is viewed as an adaptive evolutionary process on which natural selection is based. Alternatively, the neutral theory of evolution views that the majority of genetic changes are the result of the random fixation of neutral mutations. Positive selection still occurs but only a minority of mutations become fixed by this process. Hence, based on the neutral theory of evolution, the majority of mutations that become fixed within a population are a result of random genetic drift. Molecular phylogenetic analysis makes use of nucleotide and amino acid sequence data to infer phylogenetic relationships of AI viruses based on the concepts of natural selection and neutral evolution.

PHYLOGENETIC TREES AND METHODS FOR INFERRING THEM

Evolutionary relationships among AI viruses can be illustrated by a phylogenetic tree. A typical phylogenetic tree and its parts are illustrated in Figure 1.

Phylogenetic analysis is a multi-step process which can be summarized as follows:

1. Alignment of the nucleotide or amino acid sequences of the viral genes of interest.
2. Determine the presence of a phylogenetic signal i.e. the presence of conserved and random positions in an alignment that can be used in phylogenetic inference.
3. Determine the best tree building method for the data set.
4. Choose the strategy to find the best tree under the selected optimality criterion.
5. Scrutinize the tree obtained to determine the level of confidence of the results.

Multiple Sequence Alignment. The alignment of nucleotide or amino acid sequences is an essential first step for most phylogenetic analyses. Alignments can be done manually but are most often accomplished using computer programs like Clustal X. Sequences are aligned one on top of the other so that homologous (descending from a common ancestral residue) nucleotides or amino acids from different sequences line up in the same column. Sequences that are evolutionarily related begin as identical to one another and then diverge over time with the accumulation of substitutions and indels. Gaps are introduced to the alignment with the expectation that they will correspond to the indels, thus leaving the columns maximally aligned (Figure 2).

A commonly used measurement of sequence similarity is **sequence identity** which is defined as the number of identical residues in an alignment divided by the number of aligned positions. One can also count the number of nucleotide differences per homologous site to obtain a measure of sequence divergence. This measure known as the observed distance or **p-distance** in many cases underestimates the true genetic distance or **evolutionary distance** between sequences. This is because multiple hits or more than one mutation may have occurred at a particular site in a sequence. To compensate for this, various nucleotide substitution models are used to infer the evolutionary distance from the data.

Some Methods used for Constructing Phylogenetic Trees. A number of different methods exist for inferring phylogenetic trees. The resulting trees may or may not differ from the true phylogenetic tree. Methods can be grouped based on:

1. Whether they use discrete character states or a distance matrix of pairwise dissimilarities.
2. Whether the method employs stepwise clustering to construct a single tree or carries out an exhaustive search of all theoretically possible trees.
3. Whether or not an explicit model of evolution is employed.

Maximum parsimony (MP) and Maximum likelihood (ML) are both character-based methods that examine the theoretically possible tree topologies for a given number of virus isolates. ML typically utilizes an explicit model of evolution whereas MP does not. The Neighbor-joining (NJ) method is a non-character, distance matrix based method which also utilizes an explicit model of evolution.

In the **neighbor-joining** method (4) the aligned DNA sequences of interest are used to calculate the genetic distances according to the nucleotide substitution or evolutionary model being utilized. This results in a distance matrix in which the character states (A, T, C or G) of the original data matrix are lost. The NJ algorithm analyses this distance data by first grouping the two OTUs (virus isolates) with the smallest distance between them and then progressively adding more distant OTUs to the group or to new groups. The method assumes that the data are additive so that the observed distance between two OTUs in the resulting tree, is equal to the sum of the branch lengths connecting them.

The **maximum parsimony** method (5,6) analyses the character states (A, G, C, T) of the original data matrix using an optimality criterion. This method aims to find the tree topology for a set of aligned sequences that can be explained by the minimal number of character changes or fewest evolutionary steps. Minimization of the evolutionary change necessary to infer a phylogenetic tree reflects the philosophical arguments of "Ockham's Razor" which takes the position of simplicity over unnecessary complexity. The methods used for finding the optimal tree include an exhaustive search but for analyses that involve > 20 OTUs, heuristic methods are usually employed. MP is the method used most frequently by cladists, whose major interests are the ancestor-descendent relationships between organisms.

The **maximum likelihood** method (7) like MP, analyses the original data matrix using an optimality criterion. Phylogeny inference using this method sets out to determine the tree topology, branch lengths and the various parameters of the evolutionary model, such as the transition/transversion ratio, base frequencies and rate of variation among sites, which maximizes the probability of observing the sequences being analyzed. This method produces a large number of different trees, and estimates for each tree, the conditional probability

that it represents the true phylogeny given the data and evolutionary model being used. The "tree space" or number of bifurcated rooted trees for *x* taxa (viral gene sequences) is given by the equation:

$$\frac{(2x-3)!}{[2^{x-2}\,(x-2)]!}$$

A data set of just 10 influenza hemagglutin genes for instance, will generate 34,459,425 rooted trees that have to be examined if an exhaustive search is employed. This becomes computationally prohibitive for data sets > 10 necessitating the use of alternative strategies to search the "tree space" which may not guarantee that the best possible tree is examined.

The goal of any tree reconstruction method is to select the one tree that actually represents the historical branching order of the sequences that are being analyzed. One must understand that the trees obtained, regardless of the method that was used in their construction, are only estimates of the true tree and therefore the reliability of those estimates must somehow be measured. The most widely used method for doing this is the **bootstrap** method although other methods also exist. Bootstrapping involves generating artificial data sets by randomly choosing columns from the original alignment to create a new alignment. Each column in the original alignment can be selected more than once or not at all until the new set of sequences or bootstrap replicate is the same length as the original. A new tree is then constructed and the process repeated. The proportion of OTUs that cluster together among all bootstrap replicates is computed and this proportion gives an indication of the statistical significance of a cluster's monophyletic origin.

APPLICATIONS OF PHYLOGENETIC ANALYSIS OF AVIAN INFLUENZA VIRUSES

There are numerous examples of how phylogenetic analysis has enhanced our understanding of avian influenza outbreaks in poultry. It can provide clues as to the origin of an isolate – whether its ancestor was responsible for previous outbreaks in poultry or whether it was the result of a separate introduction from the wild bird reservoir. For instance, phylogenetic analysis carried out on H7 avian influenza viruses isolated between 1994-98 from live bird markets in the northeast USA (8), inferred that that the majority of the outbreaks were the result of a single virus introduction. These viruses continued to circulate within those markets where they underwent a progressive adaptive evolution to poultry. The LPAI H7N3 which was later responsible for the outbreak that occurred in the spring and summer of 2002 in Virginia, West Virginia and North Carolina was shown to be genetically related to the H7N2 viruses circulating in those live bird markets (9). A similar analysis of H5 subtype viruses isolated between 1998 and 2002 from US poultry (10), showed that they clearly differed from HPAI A/chicken/Pennsylvania/1370/83 (H5N2) forming a different clade that contained two distinct subgroups or clusters A and B. This clustering was based on analysis of the HA and NS genes of these isolates. Although these H5 viruses shared high sequence similarity in their HA genes, the genetic evidence suggested that they were likely the result of separate introductions from the wild bird reservoir rather than extended circulation within poultry. Epidemiologic, serologic and molecular phylogenetic methods (Figure 3) were used to implicate the wild bird reservoir as the most likely source of virus responsible for the HPAI H7N3 outbreak on a Saskatchewan broiler breeder farm in September 2007.

The LPAI H3N2 viruses identified as responsible for moderate to severe drops in egg production in turkey breeder layer flocks in the USA and Canada since 2004, were shown to be most closely related to triple reassortant H3N2 viruses that had circulated in the U.S. swine population since 1998 (11,12,13). In many cases the affected turkey flocks were in close proximity to swine farms supporting the conclusions of the inferred viral phylogeny. Phylogenetic analysis showed that genome of these viruses is comprised of human-like HA, NA and PB1 genes, swine-like M, NS and NP genes and avian-like PA and PB2 genes.

Finally, phylogenetic analyses have been essential to our understanding of the continued evolution and spread of Eurasian H5N1 HPAI viruses. These viruses, which have spread to three continents, have undergone numerous reassortments with other avian influenza viruses generating many different genotypes in the process. The HA protein, however, has not been replaced and forms the basis for comparing the different viral isolates (14). Based on phylogenetic analysis performed on all of the publicly available H5 HA sequences that have evolved from the 1996 goose/Guangdong H5N1 isolate, the currently existing Eurasian H5N1 isolates have been grouped into 19 clades. Clade designation is based of a hierarchical numbering system which is being advocated for universal acceptance by researches when referring to the currently circulating HPAI H5N1 viruses.

REFERENCES

1. Evolution and ecology of influenza A viruses. Microbiol. Rev. 56: 152-179. 1992.

2. Widjaja, L., S.L. Krauss, R.J. Webby, T. Xie, and R.G. Webster. Matrix gene of influenza A viruses isolated from wild aquatic birds: ecology and

emergence of influenza A viruses. J. Virol. 78: 8771-8779. 2004

3. Chen, R. and E.C. Holmes. Avian influenza virus exhibits rapid evolutionary dynamics. Mol. Biol. Evol. 23: 2336-2341. 2006.

4. Saitou, N. and M. Nei. The neighbour-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 4: 406-25. 1987.

5. Farris, J.S. Estimating phylogenetic trees from distance matrixes. Am. Nat. 106: 645-668. 1970.

6. Fitch, W.M. Toward defining the course of evolution: Minimum change for a specific tree topology. Syst. Zool. 20: 406-416. 1971.

7. Felsenstein, J. Evolutionary trees from DNA sequences: A maximum-likelihood approach. J. Mol. Evol. 17: 368-376. 1981.

8. Suarez, D.L., M. Garcia, J. Latimer, D. Senne, and M. Perdue. Phylogenetic analysis of H7 avian influenza viruses isolated from the live bird markets of the northeast United States. J. Virol. 73: 3567-3573. 1999.

9. Senne, D.A. Avian influenza in North and South America, 2002-2003. Avian Dis. 50:167-173. 2007.

10. Lee, C.-W., D.A. Senne, J.A. Linares, P.R. Woolcock, D.E. Stallknecht, E. Spackman, D.E. Swayne, and D.L. Suarez. Characterization of recent H5 subtype avian influenza viruses in US poultry. Avian Path. 33:288-297. 2004.

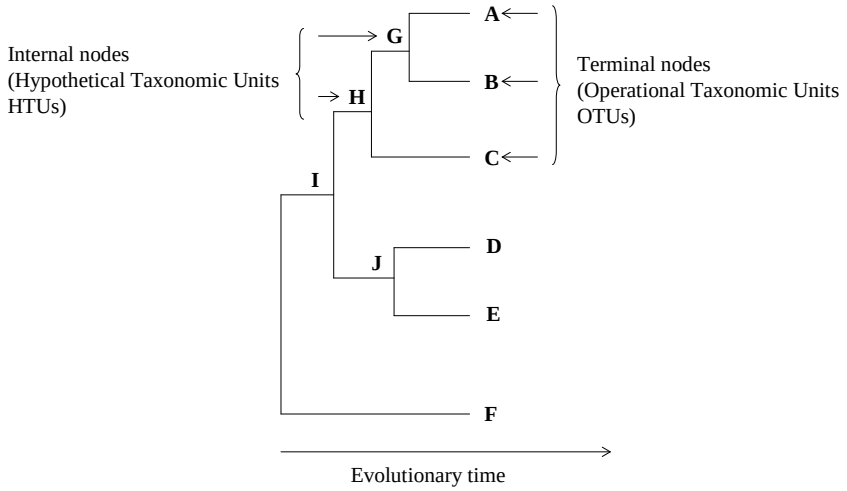
11. Tang, Y., C.W. Lee, Y. Zhang, D.A. Senne, R. Dearth, B. Byrum, D.R. Perez, D.L. Suarez, and Y. M. Saif. Isolation and characterization of H3N2 influenza A virus from turkeys. Avian Dis. 49:207-213. 2005.

12. Choi, Y.K., J.H. Lee, G. Erickson, S.M. Goyal, H.S. Joo, R.G. Webster, and R.J. Webby. H3N2 influenza virus transmission from swine to turkeys, United States. Emerg. Infect. Dis. 10:2156-2160. 2004.

13. Olsen C.W., A.I. Karasin, S. Carman, Y. Li, N. Bastien, D. Ojkic, D. Alves, G. Charbonneau, B.M. Henning, D.E. Low, L. Burton, and G. Broukhanski. Triple reassortant H3N2 influenza A viruses, Canada 2005. Emerg. Infect. Dis. 12:1132-1135.

14. http://www.who.int/csr/disease/avian_influenza/guidelines/nomenclature/en/

Figure 1. Evolutionary relationships among AI viruses.



The OTUs A, B, C, D, E and F represent individual AI virus isolates. The HTU H represents a hypothetical AI virus from which A, B and C evolved. Since A, B and C cluster together they are said to have a monophyletic origin.

Figure 2. Three sequences which are derived from a common ancestral sequence mutate and diverge. Mutations include transitions, transversions and a three-nucleotide deletion.

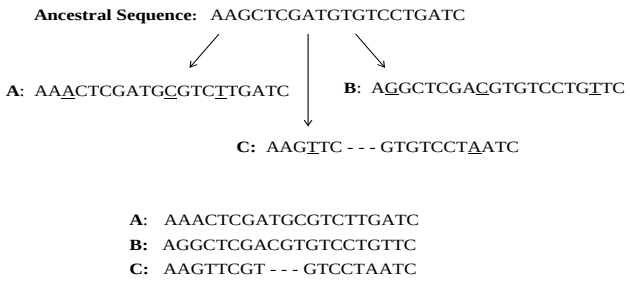
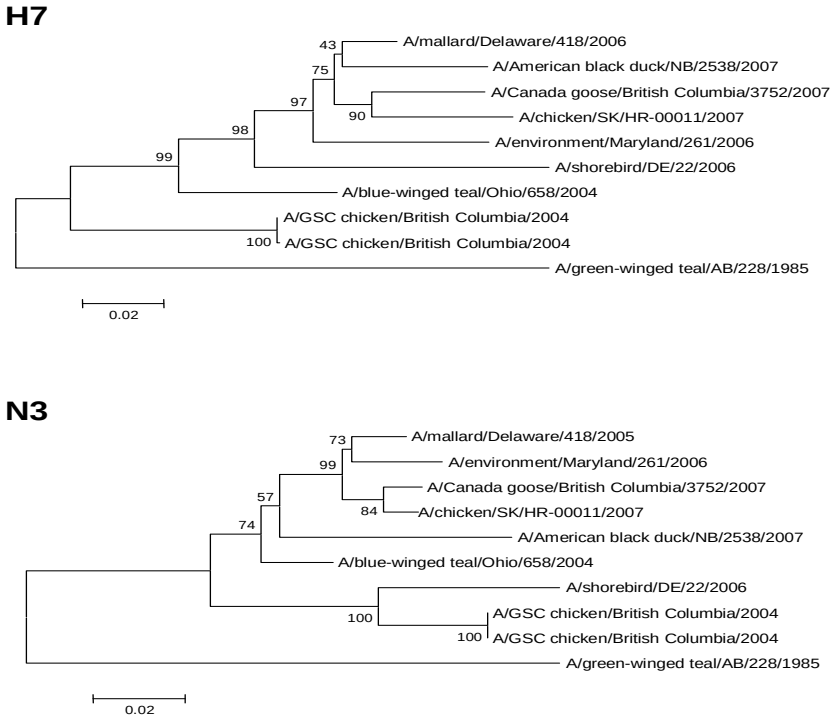


Figure 3. Phylogenetic analysis of H7 and N3 genes of A/chicken/Saskatchewan/HR-00011/2007 and H7N3 viruses recently isolated from wild aquatic birds. The trees were generated using the neighbor-joining method. Evolutionary distances were computed using the method of Nei-Gojobori. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.



EVALUATION OF THE CD8$^+$ T CELL MEMORY RESPONSE TO ADENOVIRUS EXPRESSING AIV HEMAGGLUTININ

Shailbala Singh[A], Worthie E. Briles[B], Haroldo Toro[C], De-chu C. Tang[D], Zongkai Shi[D], and Ellen W. Collisson[A,E]

[A]Texas A&M University, College Station, TX 77843
[B]Northern Illinois University, DeKalb, IL 60115
[C]Auburn University, Auburn, AL 36846
[D]Vaxin Inc., Birmingham, AL 35203,
[E]Western University of Health Sciences, Pomona, CA 91766

ABSTRACT

Non-replicating human adeno-virus vectored vaccine encoding the hemagglutinin from H5N9 avian influenza virus (AIV) (AdTW68.H5) has been shown to protect chickens from highly pathogenic viruses, with production of protective humoral immunity. The goal of the current study was to characterize the effective memory CD8$^+$ T lymphocyte response against the virus. Chickens were inoculated with AdTW68.H5 and the memory T cell responses were evaluated after ex-vivo re-stimulation with virus infected antigen presenting cells. MHC-I restricted effector T cell responses against the virus could be detected 10 days p.i. and memory T cell responses were detected at three weeks p.i. The peak response mostly mediated by CD8$^+$ T cells, was observed at five weeks p.i. and gradually decreased with time. The response was similar to that observed following inoculation of chickens with DNA plasmid expressing HA.

INTRODUCTION

Avian influenza virus (AIV) with its potential to mutate and evolve into highly pathogenic virus is a grave concern for the poultry industry. Additionally, its potential to evolve into a zoonotic pathogen also makes it a public health concern (1). Strategies to control the virus include vaccination in cases of low pathogenic viruses and culling of infected flocks in cases of a highly pathogenic virus infection. Currently poultry in affected areas is vaccinated either with inactivated whole virus vaccine (8,11) or with fowlpox vectored vaccines (10). Limitations to the efficacy of whole virus vaccine is the inability to distinguish infected from vaccinated birds (DIVA) and pre-existing immunity to the fowlpox virus (9). Viral vectored vaccines have an advantage over the use of whole virus vaccine since the risk of generation of new or virulent viruses is absent and DIVA is possible. Efficacy of vaccines is further enhanced if they are able to elicit cellular immune responses mediated by CD8$^+$ T lymphocytes which cause destruction of infected cells and thus additional viral clearance (5). Furthermore, cell mediated immune response has been shown to provide cross protection against heterologous viruses (7). A non-replicating human adenovirus vector encoding HA from H5N9 AIV (AdTW68.H5) has been developed and found to elicit an effective humoral immune response and protect chickens against highly pathogenic virus challenges (12,13). In mice recombinant adenovirus vaccine vectors have been shown to generate protective CD8$^+$ T cell immune response (2,3). In the current study CD8$^+$ T cell response to the AdTW68.H5 vaccine, shown to induce protective immunity, was demonstrated in chickens.

EXPERIMENTAL DESIGN

The AdTW68.H5 vectored vaccine was administered i.m. to B19/B19 MHC-I haplotype chickens at a dose of 1 x 10^8 ifu (12,13). PBMCs were collected at varying times post inoculation and T lymphocytes were purified as described by Seo and Collisson (6). Chicken kidney cells infected with the virus were used as antigen presenting cells. Purified T cells were restimulated *ex-vivo* by culturing with infected antigen presenting cells. The activation of T cells was determined by nitric oxide production as described by Karaca *et al.* (4).

RESULTS

The CD8$^+$ effector T cell response was detected at 10 days post-inoculation. This response was MHC-I restricted. Memory CD8$^+$ T cell response emerged at three weeks post-inoculation and were highest at five weeks post-inoculation. This T lymphoycte response was MHC-I restricted and specifically directed against AIV infected cells. The memory response did gradually decrease with time.

CONCLUSION

AdTW68.H5 vectored AIV vaccine induced CD8$^+$ T cell mediated immune response in chickens

when administered i.m. The non-replicating adenovirus expressing the HA provides a rational effective vaccine candidate since it induces both humoral as well as cell mediated immunity. Its practical application will require further studies.

REFERENCES

1. Fauci, A.S. Emerging and re-emerging infectious diseases: influenza as a prototype of the host-pathogen balancing act. Cell 124:665-670. 2006.

2. Gao W., A.C. Soloff, X. Lu, A. Montecalvo, D.C. Nguyen, Y. Matsuoka, P.D. Robbins, D.E. Swayne, R.O. Donis, J.M. Katz, S.M. Barratt-Boyes, and A. Gambotto. Protection of mice and poultry from lethal H5N1 avian influenza virus through adenovirus-based immunization. J Virol.80:1959-1964. 2006.

3. Hoelscher, M.A., Jayashankar, S. Garg, V. Veguilla, X. Lu, N. Singh, J.M. Katz, S.K. Mittal, and S. Sambhara. New pre-pandemic influenza vaccines: an egg- and adjuvant-independent human adenoviral vector strategy induces long-lasting protective immune Clin Pharmacol Ther. 82:665-671. 2007.

4. Karaca, K., I.J. Kim, S.K. Reddy, and J.M. Sharma. Nitric oxide inducing factor as a measure of antigen and mitogen-specific T cell responses in chickens. J Immunol Methods 192:97-103. 1996.

5. , G.F., R.A. , and A.D. . Influenza virus-specific cytotoxic T lymphocytes: a correlate of protection and a basis for vaccine development. Curr Opin Biotechnol. 18:529-36. 2007.

6. Seo, S.H. and E.W. Collisson. Specific cytotoxic T lymphocytes are involved in *in vivo* clearance of infectious bronchitis virus. J. Virol. 71:5173-5177. 1997.

7. Seo, S.H. and R.G. Webster. Cross-reactive, cell-mediated immunity and protection of chickens from lethal H5N1 influenza virus infection in Hong Kong poultry markets. J Virol.75:2516-2525. 2001.

8. Stone, H., B. Mitchell, and M. Brugh. *In ovo* vaccination of chicken embryos with experimental Newcastle disease and avian influenza oil-emulsion vaccines. Avian Dis. 41:856-863. 1997.

9. Swayne, D.E., J. R. Beck, and N. Kinney. Failure of a recombinant fowl poxvirus vaccine containing an avian influenza hemagglutinin gene to provide consistent protection against influenza in chickens pre-immunized with a fowl pox vaccine. Avian Dis. 44:132-137. 2000.

10. Swayne, D.E., M. Garcia, J.R. Beck, N. Kinney, and D.L. Suarez. Protection against diverse highly pathogenic H5 avian influenza viruses in chickens immunized with a recombinant fowlpox vaccine containing an H5 avian influenza hemagglutinin gene insert. Vaccine 18:1088-1095. 2000.

11. Tollis, M. and L. Di Trani. Recent developments in avian influenza research: epidemiology and immunoprophylaxis. Vet J. 164:202-215. 2002.

12. Toro, H., D.C. Tang, D.L. Suarez, J. Zhang, and Z. Shi. Protection of chickens against avian influenza with non-replicating adenovirus-vectored vaccine. Vaccine 26:2640-2646. 2008.

13. Toro, H., D.C. Tang, D.L. Suarez, M.J. Sylte, J. Pfeiffer, and K.R. Van Kampen. Protective avian influenza *in ovo* vaccination with non-replicating human adenovirus vector. Vaccine 25:2886-2891. 2007.

CONSTRUCTION OF A RECOMBINANT FOWL ADENOVIRUS 9 EXPRESSING THE HEMAGGLUTININ GENE OF INFLUENZA A VIRUS

Dan Hui Yang[A], Juan C. Corredor[A], Peter J. Krell[B], and Éva Nagy[A]

[A]Departments of Pathobiology and [B]Molecular and Cellular Biology,
University of Guelph, Guelph, Ontario, N1G 2W1, Canada

SUMMARY

Fowl adenoviruses (FAdVs) are about 70-90 nm in diameter, non-enveloped viruses with icosahedral symmetry and they are in the family *Adenoviridae*, genus *Aviadenovirus*. FAdVs are classified into five species, each including one or more serotypes: *Fowl adenovirus A* (FAdV-1), *Fowl adenovirus B* (FAdV-5), *Fowl adenovirus C* (FAdV-4 and -10), *Fowl adenovirus D* (FAdV-2, -3, -9 and -11), and *Fowl adenovirus E* (FAdV-6, -7, -8a and 8b). FAdV genomes are about 10 kb longer than the 30-36 kb of the mastadenoviruses. Fowl adenoviruses have a worldwide distribution and appear to be ubiquitous in poultry farms. Certain FAdVs are more pathogenic than others, and diseases, such inclusion body hepatitis, are more often associated with isolates belonging to serotypes 2, 4, 6, 8 and 11. Low pathogenic FAdVs are good candidate as vector viruses for use in poultry.

We developed a system for construction of recombinant viruses based on fowl adenovirus 9 (FAdV-9). In our earlier work we showed that the tandem repeat region 2 (TR-2) which is on the right end of the genome can be deleted and replaced with a foreign gene. We also demonstrated that chickens can be immunized with the recombinant virus and the virus could be administered through the drinking water and feed. From these results we felt that FAdV-9 could be used as a vaccine against poultry diseases. To identify additional sites for insertion we conducted a deletion analysis and identified a 2.4 kb non-essential region. Here we report on the generation of a recFAdV-9 expressing the full-length hemagglutinin (HA) gene of an avian influenza A virus. Two recombinant viruses, FAdV-9ΔL-HA-R and FAdV-9ΔL-HA-L, carrying the HA coding sequence replacing this 2.4 kb region in two orientations were generated by homologous recombination and subsequent transfection of chicken hepatoma (CH-SAH) cells. Expression of the HA protein was detected by Western immunoblotting using anti-avian influenza A (H5N1) HA polyclonal antibody. The activity of the recombinant HA protein was also examined by hemadsorption and hemagglutination assays. Further characterization of the recombinant viruses, such as stability is currently under way.

(The full-length article will be published in *Vaccine*.)

ANTIBODY RESPONSE TO H5 POX-VECTORED VACCINE IN UNCONVENTIONAL POULTRY

Benjamín Lucio-Martínez

Avian Diagnostic Laboratory, Animal Health Diagnostic Center
College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

SUMMARY

Inactivated water-in-oil (W/O) vaccines have been widely used to prevent economic losses in chickens and turkeys exposed to low-pathogenicity avian influenza (LPAI) in Mexico, Pakistan, Guatemala, El Salvador, Italy, USA, and Hong Kong (1). These vaccines are well tolerated by chickens and turkeys, but may not be acceptable for use in other poultry species, frequently raised outdoors and at an even a higher risk of exposure to avian influenza.

Objections to the use of O/W vaccines in non-conventional poultry may include behavior modification due to pain, and formation of granulomatous lesions in the muscle as is the case in cattle (5). In addition, vaccines in game birds are more

likely to be applied by untrained personnel, increasing the risk of accidental auto-injection of O/W vaccines, resulting in severe lesions in humans (2,4). Fowl pox-vectored avian influenza H5 vaccines have also been used extensively in chickens (1), and reported to induce immunity in cats (3). This study was done to determine the antibody response of ring-neck pheasant, chuckar partridge, turkey, white Peking duck, peafowl, and Guinea fowl after one or two subcutaneous injections of a fowl pox-vectored avian influenza H5 vaccine (Trovac-AIV H5, Merial Select, Inc., Athens GA).

MATERIALS AND METHODS

Birds. Ring-neck pheasants, chuckar partridge, turkey, peafowl, and Guinea fowl were obtained from commercial sources. White Peking ducks were obtained from Cornell's specific-pathogen-free flocks. The birds were raised in isolation from one day of age at Cornell University.

Experimental procedure. At experimental day 0 (21 days of age) the birds were bled and randomly assigned to one of four groups. Each group was made of five birds. The first group was used as negative control and was given no treatment. The remaining 15 birds were vaccinated subcutaneously with a calculated dose of 1 million infectious doses in 0.5 mL. At experimental day 21, negative control and vaccinated birds were bled. The vaccinated groups were injected again subcutaneously with a calculated dose of 1 million infectious doses in 0.5 mL. The birds were bled on experimental day 42, and humanely euthanized using CO2.

RESULTS

Hemagglutination-inhibition (HI). HI tests were performed using 4 HA units of inactivated A/tky/Ire/83 H5N8. Negative control groups had titers of 1.2 log2 or less in all cases. The vaccine induced antibodies in all vaccinated birds as shown in Table 1.

DISCUSSION

These results indicate that fowl pox-vectored H5 avian influenza vaccines induced a good antibody response in turkey, chuckar partridge, white Peking duck, ring-neck pheasant, and peafowl. In Guinea fowl the response was considerably lower. Whether these levels of antibodies will protect against disease is an open question, and it will likely depend on the pathogenicity of the challenging virus.

ACKNOWLEDGMENTS

Our deep appreciation to Dr. Kemal Karaca who provided the vaccine used in these studies, and to Dr. David Swayne for the inactivated H5 antigen.

REFERENCES

1. Capua, Ilaria and D.J. Alexander. Avian influenza: recent developments. Avian Pathology 33:393-404. 2004.

2. Fukumi, H. Effectiveness and untoward reactions of oil adjuvant influenza vaccines. Symp. Series Immunobiol. Stand. 6:237. 1967.

3. Karaca, K., D.E. Swayne, D. Grosenbaugh, M. Bublot, A. Robles, E. Spackman, and R. Nordgren. Immunogenicity of Fowlpox Virus Expressing the Avian Influenza Virus H5 Gene (TROVAC AIV-H5) in Cats. Clin. Diagn. Lab. Immunol. 12(11): 1340–1342, 2005.

4. Ogonuki,H., S. Hashima, and H. Abe. Histopathological tests of tissues in the sites of local reactions caused by injection of oil adjuvant cholera vaccine. Symp. Series Immunobiol. Stand. 6:125. 1967.

5. O'Toole, D., L. Steadman, M. Raisbeck and R. Torpy. Myositis, lameness, and recumbency after use of water-in-oil adjuvanted vaccines in near-term beef cattle. J. Vet. Diag. Inv. 17:23-31. 2005.

Table 1. Hemagglutination inhibition response in several unconventional poultry species vaccinated with fowl pox-vectored H5 avian influenza.

	Experimental Day		
Species	**0**	**21**	**42**
Turkey	0	4.0[A]	3.3
Chuckar	0	4.8	2.9
Duck	0	3.1	5.0
Pheasant	0	4.0	4.9
Peafowl	0	4.3	5.2
Guinea fowl	0	2.2	2.9

[A] Geometric mean of the reciprocal $\log_2$ of the highest dilution to inhibit 4 hemagglutination units.

INVESTIGATION ON OUTBREAKS OF LOW PATHOGENIC AVIAN INFLUENZA VIRUS IN POULTRY FARMS IN BANGLADESH

N. Jannat, E. H. Chowdhury, R. Parvin, M. A. H. N. A. Khan, and M. R. Islam

Department of Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh
emdad001@yahoo.com

ABSTRACT

Since March 2007, an epidemic of high and low pathogenic avian influenza occurred in backyard and commercial poultry in Bangladesh. The agents were pathotyped as highly pathogenic H5N1 and low pathogenic H9N2. This investigation reports outbreaks of low pathogenic H9 in commercial parent stock chickens. The morbidity and mortality were 50% and 3%, respectively during two to three months. The egg production decreased to 37% and hatchability reduced to 10%. The clinical signs include less feed and water intake, nasal and ocular discharges and severe respiratory distress. On postmortem, samples including larynx, trachea, and lungs were collected in 10% neutral buffered formalin and tracheal swabs, larynx, and tracheal tissues were collected in falcon tube containing 50% buffered glycerin with antibiotic. Histopathology was conducted using routine procedure. RNA extraction and RT-PCR was done using Qiagen RNA extraction and one step RT-PCR kits. Grossly, hemorrhagic nasal septum, sinuses, eyelids, larynx, and trachea were found. Lungs were congested. Histologically, in larynx and trachea loss of mucosal epithelium, hemorrhages and mononuclear cells infiltration in lamina propria, blood tinged exudates in laryngeal and tracheal lumen. In few cases, goblet cells were hypertrophied and mucosal glands were prominent that formed cystic spaces. RT-PCR confirmed the presence of 244 bp product of matrix protein gene and 488 bp fragment of H9 subtype specific hemagglutinin gene in the suspected samples. Cloning and sequencing of the virus is in progress.

INTRODUCTION

Avian influenza is an infectious disease of birds caused by influenza virus type A strains (2). It belongs to the *Orthomyxoviridae* family (9). Orthomyxoviruses are spherical or pleomorphic, enveloped and 80-120 nm in diameter (17). This is an RNA virus having the negative-sense segmented ssRNA and having eight segments. Wild aquatic birds, notably members of the orders *Anseriformes* and *Charadriiformes*, are carriers of the full variety of influenza virus A subtypes, and thus, most probably constitute the natural reservoir of all influenza A viruses (4,20). Serious problems have been reported in recent years associated with widespread outbreaks of viruses of H9N2 subtype, not only in Pakistan and Iran, but also in the Middle East and Asian countries through China. Influenza A viruses of subtype H9N2 are now considered to be widespread in poultry (16). H9N2 influenza viruses are endemic in many Asian countries (6). LPAI H9N2 appears to have spread across the whole of Asia in that time and has become endemic in poultry in many of the affected countries (1). Infections of domestic avian species with low pathogenic avian influenza (LPAI) viruses can be asymptomatic or cause a wide range of clinical signs varying from mild respiratory disease to more severe diseases affecting the respiratory and enteric systems. Details of clinicopathologic study of a naturally infected low pathogenic avian influenza virus (LPAI) is very limited. This study has reported the natural H9 infections in commercial chickens using RT-PCR and described the clinical, gross and histopathological findings.

MATERIALS AND METHODS

A parent stock farm was selected for this study. The history of the outbreak and clinical signs were recorded from the veterinarian of the farm. Routine necropsy was done and lesions were recorded. Tissue samples from larynx, trachea, and lungs were collected and preserved in 10% neutral buffered formalin immediately after postmortem for histopathology. Specimens of larynx, trachea, and lung tissues were processed and stained with hematoxylin and eosin (12). Tracheal swabs or tissues were collected in falcon tube containing sterile transport medium. Total RNA was isolated from the field samples using Qiagen RNeasy kit. One-step RT-PCR was done using Qiagen RT-PCR kit. RT-PCR products were analyzed by 1.5% agarose gel, stained with ethidium bromide and examined against UV light using an image documentation system. Appropriate primer sequences for RT-PCR were selected to amplify matrixprotein (MP) gene (4) and H9 hemagglutinin gene (11) of AI viruses. The primers targeting the NP genes are common for all

Type A influenza viruses, but the primers for H genes were designed for specific subtype (H9).

RESULTS AND DISCUSSION

The morbidity and mortality were 50% and 3%, respectively. The egg production decreased to 36% and hatchability decreased to 10%. Mild rales and gasping were observed in infected chickens. Less feed and water intake was observed. Hemorrhagic nasal septum, sinuses, eyelids were found. Hemorrhagic exudates in larynx and trachea with congested Lungs were found. Peritoneum, air sac, and pericardium were thickened and cloudy. In larynx and trachea, loss of mucosal epithelium, exudates containing desquamated, degenerated and necrotic epithelial cells and red blood cells in the lumen of the trachea and larynx, inflammatory cells were accumulated around the blood vessels in the sub-mucosa. No lesions were found in the lungs and air sacs. This type of histopathological lesions were supported by different authors for avian influenza (8,13,14,15). The hemagglutination (HA) test was performed to determine the presence of virus in allantoic fluid as AI virus has hemagglutinin. The suspected virus samples were serially diluted and agglutinated the chicken RBC up to 2^8 dilution. Results indicated that the suspected virus posses hemagglutinin antigens.

RT-PCR. The samples belong to H9 subtypes of AI viruses were first tested with RT-PCR for MP gene. Samples were tested positive and yielded a product of about 244 base pair (bp) as expected (Figure 1). The RT-PCR for H9 hemagglutinin gene worked perfectly as it amplified a product of about 488 bp from the field samples (Figure 2).

The one-step, reverse-transcriptase polymerase chain reaction (RT-PCR) was applied for the detection of avian influenza virus in field specimens by different authors (5,10,18,19). The paper confirms the LPAI infection in the parent stock farms in Bangladesh much before (September/06) the official declaration of HPAI outbreak in Bangladesh. However, N subtyping was not yet done, but presence of H9N2 virus has been already confirmed in the poultry farm in Bangladesh either as single or concurrent infection with H5N1. Continued surveillance of poultry for avian influenza infection is critical to minimize the magnitude of outbreak and thus limit the risk of human infection. H9 subtype AIV can infect human and thus this virus needs special attention. Detail molecular study like cloning and sequencing is necessary to understand the molecular epidemiology of the virus.

REFERENCES

1. Alexander, D.J. An overview of the epidemiology of avian influenza. *Vaccine.* 25:5637-44. 2007.

2. Ergin, S. Avian influenza as an emerging infection. Veteriner-Fakultesi-Dergisi-Istanbul. 32: 1-11. 2006.

3. Fouchier, R.A.M., T.M. Bestebroer, S. Herfst, L. van der Kemp, G.F. Rimmelzwaan, and A.D.M.E. Osterhaus. Detection of influenza A viruses from different species by PCR amplification of conserved sequences in the Matrix gene. *J. Clin. Microbiol.* 38: 4096-4101. 2000.

4. Fouchier, R.A.; B. Olsen, and T.M. Bestebroer. Influenza A virus surveillance in wild birds in Northern Europe in 1999 and 2000. *Avian Dis.* 47: 857-60. 2003.

5. Horimoto, T. and Y. Kawaoka. Molecular changes in virulent mutants arising from avirulent avian influenza viruses during replication in 14-day-old embryonated eggs. *Virology*. 206: 755-9. 1995.

6. Kim, J.A., S.H. Cho, H.S. Kim, and S.H. Seo. H9N2 influenza viruses isolated from poultry in Korean live bird markets continuously evolve and cause the severe clinical signs in layers. *Vet. Microbiol.* 118:169-76. 2006.

7. Krauss, S., D. Walker, S.P. Pryor, L. Nile, L. Chenghong, V.S. Hinshaw, and R.G. Webster. Influenza A viruses of migrating wild aquatic birds in North America. *Vector Borne Zoonotic Dis.* 4: 177-89. 2004.

8. Kwon, Y.K., Y.J. Lee, J.G. Choi, E.K. Lee, W.J. Jeon, O.M. Jeong, M.C. Kim, S.J. Joh, J.H. Kwon, and J.H. Kim. An outbreak of avian influenza subtype H9N8 among chickens in South Korea. *Avian Pathol*. 35: 443-447. 2006.

9. Lamb, R.A. and R.M. Krug, Orthomyxoviridae: the viruses and their replication. In: Fieldds, B.N., Knipe, D.M., Howley, P.M., Chanock, J.L., Melnick, R.M., Momath, T.P. and Roizman, B. (Eds.), *Fields. Virol*, 3rd ed., Lippincott-Raven, Philadelphia, PA. 1996.

10. Lee, C.W. and D.L. Suarez. Application of real-time RT-PCR for the quantitation and competitive replication study of H5 and H7 subtype avian influenza virus. *J Virol Methods.* 119: 151-8. 2004.

11. Lee, M.S., P.C. Chang, J.H Shien, M.C. Cheng, and H.K. Shieh. Identification and subtyping of avian influenza viruses by reverse transcription-PCR. *J. Virol. Methods*. 97: 13-22. 2001.

12. Luna, L.G. Manual of Histologic Staining Methods of the Armed Forced Institute of Pathology. 3rd edition. McGraw Hill Book Company, New York. 1968.

13. Nili, H. and K. Asas. Natural cases and an experimental study of H9N2 avian influenza in commercial broiler chickens of Iran. *Avian Pathol.* 31: 247-52. 2002.

14. Nili, H. and K. Asasi. Avian influenza (H9N2) outbreak in Iran. *Avian Dis.* 47: 828-31. 2003.

15. Nili, H., K. Asasi, H. Dadras, and M. Ebrahimi. Pathobiology of H9N2 avian influenza virus in Japanese quail (*Coturnix coturnix japonica*). *Avian Dis.* 51: 390-2. 2007.

16. Peiris, M., W.C. Yam, K.H. Chan, P. Ghose, and K.F. Shortridge. Influenza A H9N2: Aspects of Laboratory Diagnosis. *J. Clin. Microbiol.* 37:3426-3427. 1999.

17. Quinn, P.G., B.K. Markey, M.E. Carter, W.J. Donnelly, and F. C. Leonard. *Orthomyxoviridae.* In: Veterinary Microbiology and Microbial Disease by. Blackwell Science Ltd. Iowa State University Press. pp. 375-380. 2002.

18. Starick, E. and O. Werner. Detection of H7 avian influenza virus directly from poultry specimens. *Avian Dis.* 47: 1187-1189. 2003.

19. Starick, E., O.A. Roemer, and O. Werner. Type- and subtype-specific RT-PCR assays for avian influenza A viruses (AIV). *J. Vet. Med. Series B.* 47: 295-301. 2000.

20. Widjaja, L., S.L. Krauss, R.J. Webby, T. Xie, and R.G. Webster. Matrix gene of influenza a viruses isolated from wild aquatic birds: ecology and emergence of influenza a viruses. *J. Virol.* 78: 8771-9. 2004.

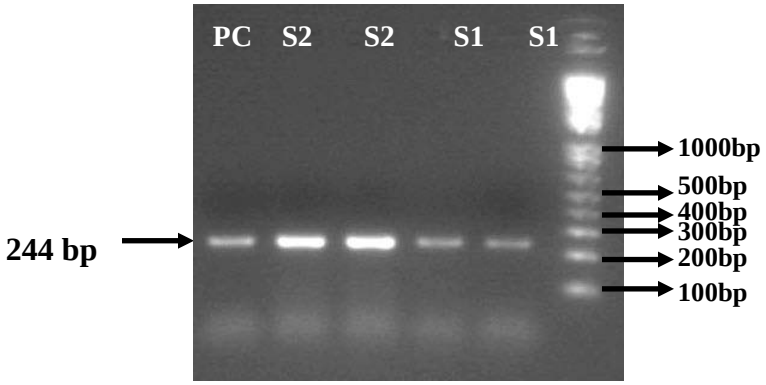


Figure 1. Amplification of the fragment of matrix protein gene of Type A influenza virus by RT-PCR using primers designed by Fouchier *et al.* (4). M = Marker, S1 = Field sample-1, S2 = Field sample-2, PC = Positive control (RNA extracted from inactivated H9 virus received from VLA, England).

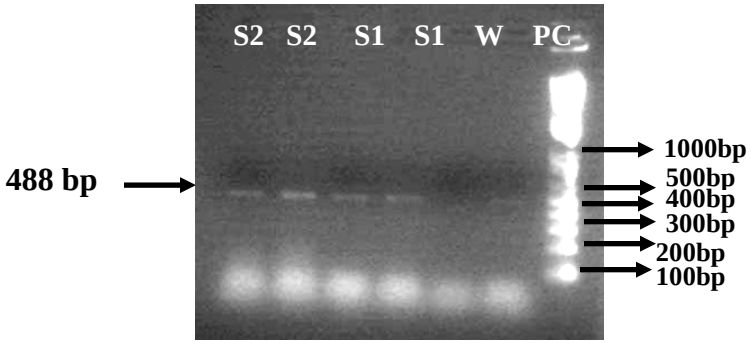


Figure 2. Amplification of the fragment of H9 gene by RT-PCR using primers of Lee *et al.* (11). M = Marker, PC = Positive control (RNA extracted from inactivated H9 virus received from VLA, England), W = Water control, S1 = Field sample-1, S2 = Field sample-2.

INFECTIOUS LARYNGOTRACHEITIS IN ITALY: CLINICAL CASES, DIAGNOSIS, AND CHARACTERIZATION BY PCR-RFLP

A. Piccirillo[A], E. Morandini[B], L. Gavazzi[C], R. Ceruti, P. Pesente[B], D. Giovanardi[B], A. Mondin[A], and A. Moreno[D]

[A]Department of Public Health, Comparative Pathology and Veterinary Hygiene, Veterinary Medicine Faculty, University of Padua - Viale dell'Università, 16, 35020 Legnaro (PD), Italy
[B]Veronesi Verona Spa., Via Valpantena, 18, 37034 Verona, Italy
[C]Gesco Consorcio Operativo, Via del Rio, 400, 47020 San Vittore, Cesena (FC), Italy
[D]Virology Department - Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Via A. Bianchi, 9, 25124 Brescia, Italy

INTRODUCTION

Infectious laryngotracheitis (ILT) is an acute respiratory disease of chickens caused by *Gallid herpesvirus 1*, a member of the subfamily *Alphaherpesvirinae*, family *Herpesviridae*. Although infectious laryngotracheitis virus (ILTV) has been the first poultry pathogen controlled by vaccination, ILT is still one of the most important diseases in the poultry industry, especially in areas where high density of poultry populations are reared (3). Mild and severe forms of the disease were commonly described in layers worldwide, but in the last years numerous cases of mild disease have been described in broilers in many countries (7,8).

During the period 2007 to 2008, several cases of mild ILT were observed in broilers in North Italy. The causative agent was ILTV in all outbreaks. The present paper reports the diagnostic findings from 36 cases and the molecular characterization of 17 field ILTV strains and four ILTV CEO vaccines by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis. In addition, a preliminary genomic characterization based on the sequencing of gE, gG, TK, ICP18.5 and ORFB-TK genes was carried out on three field strains and one CEO vaccine in order to better understand the eventual correlation between ILTV field and vaccine strains.

MATERIALS AND METHODS

Clinical cases. From May 2007 to October 2008, 36 cases exhibiting upper respiratory disease were observed in broiler flocks located in North Italy, divided in the following provinces: 18 in Brescia; five in Cremona; three in Teramo; two in each of these sites: Bergamo, Forlì, Verona, and Padova; and one in each of these sites: Mantova and Treviso. Out of these, 32 occurred in birds between 40 to 50 days of age and only four in younger animals. Thirty outbreaks occurred in industrial farms whereas six from dealer farms. No ILTV vaccination was applied in any industrial farm. In dealer farms, where layers and broilers, although in separate units, were reared in close proximity, only layers were vaccinated against ILTV.

Viruses. Seventeen ILTV field isolates and four live attenuated ILT vaccines were analyzed in this study. Only four vaccines are authorized in Italy and they are all of chicken embryo origin (CEO): Nobilis® Laringovac (Intervet), Poulvac ILT (Forte-Dodge Animal Health), Bio Laringo PV (Merial), and LAR-VAC (Fatro).

Virus isolation and identification. Tracheal tissue homogenates from 17 outbreaks were inoculated into nine to eleven day-old SPF chicken embryonated eggs (CEE) by chorionallantoic membrane (CAM) route. The CAMs were observed for the presence of pocks lesions after several days and passages and the presence of ILTV was evaluated by polymerase chain reaction (PCR). The presence of other respiratory pathogens, like infectious bronchitis virus (IBV), Newcastle disease virus (NDV) and avian influenza virus (AIV) were evaluated on all homogenates by previously described procedures (1,4,9).

DNA extraction. Viral DNA was extracted using the High Pure Viral Nucleic Acid Kit (Roche Diagnostics Corporation) from 200 µL of a suspension of the infected CAMs and reconstituted vaccines according to the manufacturer's instructions.

Real-time PCR. Tracheal tissue homogenates from all the outbreaks were analyzed by real-time Taqman® PCR assay for the detection of ILTV as previously described (2).

PCR-RFLP. PCR-RFLPs were performed as described by Kirkpatrick *et al.* (5). Except for ICP4 gene, our study analyzed gE, gG, TK, ICP18,5 and ORFBTK genes. The ORFB-TK region consists of several unique genes, including ORFB, ORFC, ORFD and ORFE. PCR products were digested with the

specific restriction endonuclease enzymes (New England Biolabs) i.e. EaeI for gE, MspI for gG and TK, HaeIII for ICP18.5 and FokI for ORFB-TK. After SybrSafeTM DNA Gel Stain (Invitrogen) staining, RE DNA fragments were visualized by Gel DocTM XR (Bio-Rad). Molecular weights of both PCR products and RE fragments were calculated by using Quantity One® 1-D Analysis Software (Bio-Rad).

Sequence analysis. Amplicons from three field isolates and one vaccine strain were purified using High Pure PCR Purification kit (Roche Diagnostics Corporation) and submitted to BMR Genomics (Padua, Italy) for sequencing. Analysis of the chromatograms, assembly of nucleotide sequences and the prediction of amino acidic sequences were performed using the Chromas Pro Software v. 1.42 (Technelysium Pty Ltd., Australia). Alignment of both nucleotide and amino acidic sequences was performed with CLUSTALW Software version 2.0 (6).

RESULTS

Clinical signs and pathology. Clinical sings included reduction of feed and water consumption, gasping with expectoration of blood-stained mucus, evident swelling of the infra-orbital sinuses, conjunctivitis, closed and watery eyes, and persistent nasal discharge. Low mortality (>10%) was reported. Gross lesions observed in tracheal and laryngeal tissues were variable from mild tracheitis with excess of mucus to severe with hemorrhagic or diphtheritic changes. Other lesions such as edema and congestion of conjunctiva and infraorbital sinuses were observed.

Real-time PCR and virus isolation. Tracheal homogenates from all 36 outbreaks resulted positive by ILTV real-time PCR. Of these, 17 field samples and four vaccine strains were propagated on CAMs of CEE and the presence of ILTV was demonstrated in all samples by real-time PCR. All cases were negative for NDV, AIV, and IBV.

PCR-RFLP. For all ILTV field isolates and vaccine strains, specific PCR products were generated for each individual gene (gE, gG, TK, ICP18.5 and ORFB-TK). All the field isolates and the vaccine strains under study produced PCR products of the same molecular weights and of the expected sizes as indicated by Kirkpatrick et al. (5), except for ICP18.5 which was about 6,400 bp instead of 5,890 bp. Restriction endonuclease digestion of all the genes produced identical patterns in all the field isolates and the vaccine strains. These patterns consisted of three bands of 1.10, 0.49, and 0.26 kb for gE PCR products, eleven bands of 0.28, 0.26, 0.23, 0.20, 0.18, 0.08, 0.07, 0.05, 0.04, 0.03, and 0.02 kb for gG PCR products, six bands of 0.89, 0.56, 0.24, 0.18, 0.13, and 0.12 kb for TK PCR products, ten bands of 2.00, 1.00, 0.80, 0.70, 0.45, 0.42, 0.35, 0.32, 0.24, and 0.22 kb for ICP18.5 PCR products and seven bands of 2.40, 0.90, 0.54, 0.35, 0.23, 0.13, and 0.06 kb for ORFB-TK PCR products. In some cases, the analysis of RFLP patterns was difficult for the presence of additional bands which sizes suggested that they could be due to partially digested products.

Sequencing and sequence analysis. The comparison of the nucleotide sequences and successive analysis of restriction sites of the field isolates and vaccine strain confirmed the patterns obtained by PCR-RFLPs of each individual gene. ClustalW2 analysis demonstrated 100% homology between the three field isolates for all the genes sequenced. Homologies among field and vaccine ILTV strains were 100% for gE and TK, 99.9% for gG, 99.9% for ICP18.5 and 99.8% for ORFB-TK genes. Deduced amino acid (aa) sequences revealed one aa substitution in ORFE (A135 to G) and ICP18.5 (H715 to R), and one additional aspartic acid residue at position 67 in ORFC in the field strains.

DISCUSSION

In 2007-2008 thirty six cases of ILTV were reported in broilers in North Italy in an area characterized by a high density of industrial poultry (broilers and layers) farms and dealer farms. These are the first cases observed in broiler population in this area of the country. ILTV was demonstrated by real-time PCR in all 36 tracheal homogenates submitted to the laboratory whereas other respiratory viruses AIV, NDV and IBV were excluded as the cause of the disease. The clinical signs were classified into mild forms of ILT in all cases. Most of the outbreaks (89%) occurred in broilers of 40 to 50 days of age. These flocks were “mixed sex” and in many cases it has been observed that clinical ILTV appeared one to two wks after the partial depopulation of female broilers and could be related to contact with contaminated fomites, trucks or personnel. Also the close proximity between farms and the spreading of contaminated litter neighboring the susceptible farms could likely have spread ILTV.

The characterization by PCR-RFLP of gE, gG, TK, ICP18.5 and ORFB-TK revealed the same RFLP patterns for all the ILTV strains under investigation. These results suggest a close relationship between the field isolates and the vaccine strains. However, the comparison of full sequences of these five genes for three field and one vaccine strains revealed some interesting results. The sequences of the five genes of the three field isolates showed 100% of homology at nucleotide level. The nucleotide sequences of gE and TK genes were identical among field isolates and vaccine strain whereas some differences either at

nucleotide and amino acidic level of the other examined genes/genomic regions were evidenced between them. On the basis of these preliminary results it could be hypothesized that field isolates are closely related to CEO vaccine strains but not identical.

A further genomic characterization of the other field isolates described in this study should be undertaken in order to verify if the same differences are presented in all field strains. In addition, further sequence analysis will be carried out to provide a better comprehension if these amino acid mutations could be involved in the virulence of ILTV strains and in the rapid spread of the disease during this epidemic. Since the PCR-RFLP analysis used in this study could not discriminate between field isolates and vaccine strains, Authors emphasize the necessity to characterize the ILTV strains by comparing RFLP patterns with gene sequencing.

REFERENCES

1. Adzhar A., R.E. Gough, D. Haydon, K. Shaw, P. Britton, and D. Cavanagh. Av Pathol, 26:625-640. 1997.
2. Callison S.A., S.M. Riblet, I. Oldoni, S. Sun, G. Zavala, S. Williams, R.S. Resurreccion, E. Spackman, and M. Garcia. J Virol Meth, 139:31-38. 2007.
3. Guy, J.S. and M. Garcia. Diseases of Poultry, Iowa State Press, Ames, pp.137-152. 2008.
4. Kho, C.L., M.L. Mohd-Azmi, S.S. Arshad , and K. Yusolff. J Virol Meth, 86: 71-83. 2000.
5. Kirkpatrick N.C., A. Mahmoudian, D. O'Rouke, and A.H. Noormohammadia. Avian Dis., 50:28-34. 2006.
6. Larkin, M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson, and D.G. Higgins. Bioinformatics, 23: 2947-8. 2007.
7. Ojkic, D., J. Swinton, M. Vallieres, E. Martin, J. Shapiro, B. Sanei, and B. Binnington. Av Pathol, 35: 286-292. 2006.
8. Sellers, H.S., M. Garcia, J.R. Glisson, T.P. Brown, J.S. Sander, J.S. Guy J.S. Av Dis, 48: 430-436. 2004.
9. Spackman, E., D.A. Senne, T.J. Myers, L.L. Bulaga, L.P. Garber, M.L. Perdue, K. Lohman, L.T. Daum, and D.L. Suarez. J Clin Microbiol, 40:3256-60. 2002.

MECHANISMS OF INTESTINAL BARRIER FAILURE IN SUBCLINICAL ENTERITIS

J.J. Dibner, M.A. Quiroz, and C.D. Knight

Novus International, Inc., 20 Research Park Drive, Missouri Research Park, St. Charles, Missouri 63304

SUMMARY

One common consequence of enteritis, regardless of its cause, is a breakdown in the barrier function that normally protects the animal against invasion by commensal and pathogenic gut microbiota. The objective of this review is to describe the structure and function of the intestinal barrier that prevents invasion of the host, to summarize the evidence that loss of barrier function accompanies oxidative stress associated with enteritis, and to discuss possible consequences of the resulting bacterial translocation.

INTRODUCTION

Subclinical enteritis is an increasing problem in the poultry industry. It can be caused by a number of factors, including any of a variety of relatively mild parasitic, bacterial and viral infections as well as transient toxic challenges (1). As the name implies, it rarely causes death but can be associated with poor feed digestibility and absorption, resulting in poor performance. It is also becoming clear that activation of the innate immune system and the associated generation of reactive oxygen metabolites (ROM) plays a role in the normal host response to its microbiota (2) and is essential in the development of homeostatic balance between the host and its microbiota early in life. It has been suggested that the ROM response can exceed the host's ability to control it, resulting in oxidative stress that itself can become a pathogenic factor in the further development of chronic intestinal inflammation (3).

The gastrointestinal tract is the primary site of entry for any orally administered compound, including dietary ingredients. The functions of this organ system include digestion, absorption, and protection, and the structure of the gut is well adapted to perform these functions. The mucosa of the gut is the first tissue to encounter dietary ingredients and contaminants, and studies of its macroscopic and microscopic structure

have been used to clarify the initial response of the animal to these materials. Research has demonstrated the enormous changes in the structure and function of this organ system during the first week after hatching and in its adaptation to environmental and dietary changes later in life (4). Results suggest that the more mature gut is still responsive to microbial changes, environmental conditions, toxins and dietary factors, and that the initial response to these various events is general subclinical enteritis, i.e. intestinal inflammation. Thus, studying the consequences of intestinal inflammation is to some extent independent of the causative factors. This review will focus on barrier failure as a general effect of enteritis itself rather than the factors that can initiate intestinal inflammation.

OXIDATIVE STRESS

Animal nutritionists typically include antioxidants in their finished feed formulations to prevent oxidative damage to the fat portion of the feed, including fat soluble vitamins and pigments. Controlling oxidation in the feed is important, but it should be recognized that there are endogenous sources of ROM. First, the active metabolism of gut epithelium is itself a source of ROM, associated with activity of the electron transport chain (5). The reactive species produced include the superoxide anion (O_2^-), and hydrogen peroxide (H_2O_2). These are considered to be an inevitable result of oxidative phosphorylation by mitochondria (6). Another endogenous source of oxidative stress includes the nitric oxide (NO) generated by the gut innate and acquired immune systems as they react to the numerous commensal and pathological microbial species that are inevitably introduced during ingestion of feed and water.

Cells are protected from damage by these ROM through the action of endogenous antioxidant defenses such as mitochondrial-Mn-dependent superoxide dismutase, Cu-Zn SOD and glutathione (3). These systems can be overwhelmed, however, if the oxidant stress and antioxidant capacity become unbalanced, and under such conditions tissue damage can be extensive (7). If O_2^-, H_2O_2, and NO are not rapidly detoxified they can generate more damaging free radicals such as the hydroxyl radical (·OH). This species is more dangerous because there is no enzymatic path to detoxify it. Oxidative stressors are additive, therefore ingestion of oxidized feed ingredients can tip the oxidative balance in the intestine. It is clear then that supplemental antioxidants preserve the feed but can also reduce the damage associated with constitutive and metabolic sources of ROM.

INTESTINAL BARRIER STRUCTURE AND FUNCTION

The gut provides a barrier to the invasion of the commensal and opportunistic microflora. The physical barrier consists of mucin overlying the gut epithelium, the epithelial cells themselves with their impermeable cell to cell tight junctions and toll-like receptors (TLR), intraepithelial leukocytes, and the basal lamina. For the dissemination of bacteria to other organs, these barriers, as well as those represented by the innate and adaptive immune cells of the lamina propria must be overcome.

The first structural layer of the barrier is extracellular mucin. Mucin is a mixture of high molecular weight proteoglycans that forms a continuous layer overlying and connected to the gut epithelial cells. The role of the mucin in barrier preservation is to restrict interaction between the microbiota and the gut epithelium itself, reducing the likelihood of attachment and subsequent activation of the innate immune response mediated by the TLR of the gut epithelium (8).

Figure 1 shows another component of the barrier, the gut epithelial cells. This epithelium consists of a single layer of columnar epithelial cells. These cells are tightly adherent to one another by virtue of cell to cell junctions at the apical and basolateral regions. The junctions consist of several parts: the apical tight junction (zonula occludens) is the most important in regulating paracellular permeability. Tight junctions consist of a continuous band of branching cytoskeletal proteins that form a perijunctional actin ring of integral membrane proteins (9). The function of these continuous interepithelial tight junctions is to control the movement of water, solutes, and electrolytes, in addition to members of the microbiota.

Oxidative stress in the GI system, regardless of cause, is associated with loss of barrier function. This can be demonstrated using inhibitors of inducible nitric oxide synthase (10). The mechanisms responsible for this loss of barrier function may include denaturation of the protein component of the enterocyte junctional complexes (11) resulting in failure of both cell-cell adhesion and apical membrane integrity (11). The importance of the association of barrier failure and oxidative stress in the gut is that it could be a cause of dissemination of CP or other opportunistic pathogens into other organs that does not necessarily require pathogen invasion or toxic damage to occur. Other contributors to oxidative stress, including oxidized dietary ingredients could also be involved. Thus, the maintenance of balance between total oxidative stress in the gut and total antioxidant capacity can be augmented with the use of dietary antioxidants. Because enteritis and barrier failure result in bad performance, lack of skin pigmentation and has the potential of killing the animal, the relationship of them to oxidative stress is an

important additional reason to include dietary antioxidants in all diets, even those supplemented with fresh fat.

CONCLUSIONS

Barrier function failure has numerous causes, but oxidative stress should not be one of them. There are two very important ways to reduce the likelihood of systemic disease associated with bacterial translocation. First is the control of gut oxidative stress. The association between it and barrier failure means that antioxidant supplementation should always be a part of the formulation, even in diets supplemented with stabilized or fresh fat sources. The second way is to reduce the likelihood that a barrier failure will result in translocation of such potentially deadly anaerobes as CP. This means that homeostasis of the gut microbial populations needs to be protected. Addition of organic acids or enzymes that improve nutrient availability can have benefits beyond performance; it can reduce the acid-sensitive CP population, reducing the likelihood that it will be disseminated by gut barrier failure from any cause, including subclinical enteritis.

REFERENCES

1. Hoerr, F. Pathogenesis of enteric diseases. Poult. Sci. 77: 1150-1155. 1998.

2. Rumbo, M., and Schiffrin, E. Ontogeny of intestinal epithelium immune functions: Developmental and environmental regulation. Cell. Mol. Life Sci. 62: 1288-1296. 2005.

3. Aw, T. Molecular and cellular responses to oxidative stress and changes in oxidation-reduction imbalance in the intestine. Am. J. Clin. Nut. 70: 557-565. 1999.

4. Dibner, J., and Knight, C. Early feeding and nutritional programming in hatchling poultry. In: Proceedings Arkansas Nutrition Conference, pp. 1-9. Fayetteville, AK. 2001.

5. Ojano-Dirain, C., Tinsley, N., Wing, T., Cooper, M., and Bottje, W. Membrane potential and H_2O_2 production in duodenal mitochondria from broiler chickens with low and high feed efficiency. Comp. Biochem. Physiol. Part A. 147: 934-941. 2007.

6. Chance, B., Sies, H., and Boveris, A. Hydroxide metabolism in mammalian organs. Physiol. Rev. 59: 527-609. 1979.

7. Weiss, S. Tissue destruction by neutrophils. N. Engl. J. Med. 3220: 365-376. 1989.

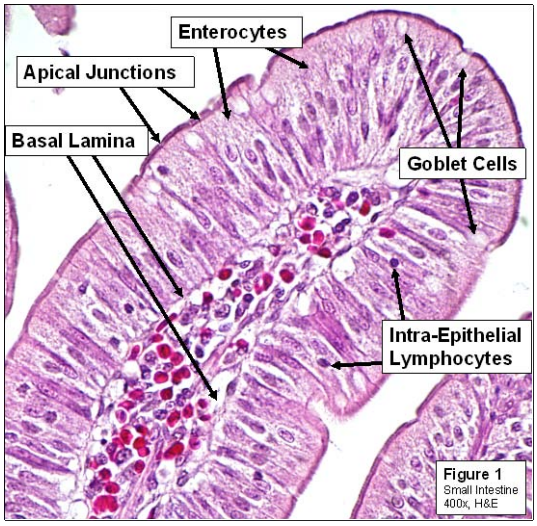
8. Swank, G., and Deitch, E. Role of the gut in multiple organ failure: Bacterial translocation and permeability changes. World J. Surg. 20: 411-417. 1996.

9. Gonzalez-Mariscal, L., Betanzos, A., and Avila-Flores, A. MAGUK proteins: Structure and role in the tight junction. Seminars Cell Devel. Biol. 11: 315-324. 2000.

10. Wingler, K., Muller, C., Schmehl, K., Florian, S., and Brigelius-Flohe, R. Gastrointestinal glutathione peroxidase prevents transport of lipid hydroperoxides in CaCo-2 cells. Gastroenterology. 119: 420-430. 2000.

11. Blikslager, A., Moeser, A., Gookin, J., Jones, S., and Odle, J. Restoration of barrier function in injured intestinal mucosa. Physiol. Rev. 87: 545-564. 2007.

Figure 1.



A NEW APPROACH TO PREDICT THE IMPACT OF COCCIDIOSIS USING THE STANDARD INDUSTRY INTESTINAL SURVEY

Charles Broussard[A], RG Teeter[B], and Linnea Newman[A]

[A]Intervet/Schering-Plough Animal Health, Summit, NJ
[B]Department of Animal Science, Oklahoma State University, Stillwater, OK
E-mail: chares.broussard@sp.intervet.com

SUMMARY

Since the introduction of in-feed anticoccidials to the poultry industry, the standard method of determining the efficacy of these products has been to perform an intestinal health survey. Typically these surveys involve the necropsy of several chickens (usually five birds) to give a representation of coccidial challenge for a house or multi-house farm. Bird samples of various ages are submitted to represent the range of potential coccidiosis challenge in the field. The focus of the intestinal health survey is to determine the efficacy of the current anticoccidial program to the coccidial challenge. The scoring method developed by Reid and Johnson (1) has been adopted as the standard method to assess severity of gross cocci lesions. In addition a standardized method for the ranking of microscopic scoring for coccidial development has been developed by Fitz-Coy (2). This has been shown to be extremely helpful with evaluating low level challenges of some coccidial species especially *E. maxima*.

A limitation of the current intestinal survey has been the interpretation of the data collected. The data is usually reported as the severity of gross and microscopic lesions for each pathogenic species of *Eimeria* affecting broiler performance. This data is summarized and expressed as a percentage of affected birds. Then this information is extrapolated to the greater population of chickens in the field operation to represent the current coccidial challenge for the anticoccidial program being evaluated.

The current methodology of evaluating only the severity of coccidial lesions ignores the impact of the timing of the coccidial challenge during the broiler growth cycle. Recent studies by Teeter *et al.* (3) demonstrated the importance of timing of coccidial challenges on broiler performance. This work showed coccidial challenges of equivalent severity had more negative impact on chickens if occurring later in the growth cycle. This correlation also held true for birds exhibiting low level coccidial challenge (+1 and +2 scores). This work substantiates the need in the field to more accurately assess coccidial challenge by evaluating both the severity and timing of coccidial challenges.

Does the current intestinal survey data collection method allow a more sensitive method to evaluate efficacy of anticoccidial programs and to predict the impact of these programs on broiler performance? The use of statistical modeling has allowed predictability in laboratory situations of coccidiosis challenge on broiler performance (3). Can this approach be taken one step further to accurately predict the impact of coccidiosis at the field level and allow the poultry industry to begin a proactive approach to coccidial disease management versus the current reactive approach?

REFERENCES

1. Reid, W.M. and J. Johnson. Pathogenicity of Eimeria acervulina in light and heavy coccidial infections. Avian Diseases 14:156-177. 1970.

2. Fitz-Coy, S. Diseases of Poultry 12th Edition. Coccidiosis. 1068-1085. 2008.

3. Teeter, R.G., A. Beker, C. Brown, C. Broussard, S. Fitz-Coy, J. Radu, and L. Newman. Calorimetry applications quantify the variable cost of subclinical at various points in the broiler growth curve. 57th Western Poultry Disease Conference Proceedings 99-101. 2008.

A LOOK AT SEVERAL LIVE COCCIDIA VACCINES

Steve H. Fitz-Coy

Poultry Pathologist-Parasitologist, Intervet/Schering-Plough Animal Health
steve.fitz-coy@sp.intervet.com

SUMMARY

The *Eimeria tenella* was apparent in all vaccines; this was confirmed by the bloody dropping pans. The *E. maxima* in vaccine D appeared to be relatively pathogenic or the number of *E. maxima* oocysts per dose was high; this was based on the pathological signs exhibited by affected birds. The coccidia organisms in vaccine A were more sensitive to the drugs tested than the coccidia isolates in the other vaccines. Of the live coccidia vaccines tested, the organisms in product A was less pathogenic to the host.

INTRODUCTION

Coccidia are very prolific, hardy, ubiquitous, and highly antigenic, but susceptible to drying. For the coccidia susceptible host, these agents can be pathogenic leading to weight loss, impaired feed utilization, poor pigmentation, and even mortality. The coccidia life cycle follows an intricate but yet direct pathway. It is during these developmental pathways of the parasites that they may destroy vast numbers of intestinal cells in the affected birds. The level of damage in the host may be related to several factors: (a) the number of coccidia ingested that have completed their life cycle in the host; (b) the species of coccidia ingested, for example, *E. maxima* is more pathogenic than *E. acervulina*; and (c) pathogenicity of the isolate, some isolates with in a species may be more pathogenic than others.

Maintaining good bird health and productivity is dependent on good coccidia control. A low parasite burden in the host is necessary, this is particularly important during the critical periods of the bird's growing curve. Judicious use of anticoccidial drugs and managing anticoccidial drug responsiveness is essential in maintaining effective long-term coccidiosis control. During the 1960s and 1970s, there was one approved live coccidia vaccine. By the 1980s there were two live coccidia vaccines and in 2009 there are five approved products available for broiler chickens in the US.

MATERIALS AND METHODS

Commercial broiler chickens obtained from a local hatchery were used in these studies; birds were six to 10 days before being placed in experimental pens. The coccidiosis vaccines were coded A, B, C, D, and E. The dose levels were 0, 20x and 33x doses per bird via oral inoculation. Parameters measured were growth, feed utilization (FCR), consistency of feces, lesion scores, parasite burden, and oocysts output. The drugs tested were those commonly used by the broiler industry and at the levels recommended.

RESULTS AND DISCUSSION

Between 117 and 121 h post-inoculation (pi), *E. acervulina* type oocysts were seen in samples from birds that had received the vaccines. Feces were normal for the control birds and those given vaccine A. But the feces for those birds that got D were bloody, watery and mucoid. Feces produced by the birds given B were bloody and watery. By 129 h pi, *E. acervulina* type seen in the samples from birds given vaccines A, C and D; *E. tenella* seen in fecal samples from birds given vaccine D. By 141 h pi, *E. acervulina* type seen in sample from birds given vaccine A. *E. acervulina* type and *E. tenella* seen in feces from birds given vaccine C. *E. acervulina* type, but *E. maxima* and *E. tenella* seen with vaccine D. Feces were normal for the control birds, those birds given vaccine A had watery and bloody feces. The birds given vaccine B had watery feces and those given D had watery and mucoid feces. By 164 h pi, *E. acervulina* type, *E. tenella* and *E. maxima* were seen in all the fecal samples.

Birds given vaccine A or C grew at rates comparable to that of the controls when measured between 96-120 h pi, 35 g, 33 g, or 30 g per bird, respectively. However, birds given vaccine D grew at a rate of only 6 g per bird during the same period. When growth was measured between 120-144 h pi, the controls grew at a rate of 34 g per bird, whereas the birds given vaccine A or C grew at rates of 27 g and 29 g, respectively. But the birds that were given vaccine D grew at a rate of 14 g during the same period. Growth rate for the controls between 144-164 h pi was 62 g and the growth rate for those birds given vaccine A or C grained 47 g. The growth rate of the birds given D had lowest gain during that period, 28 g per bird. The control group had the lowest FCR, followed by those given vaccines A, C and D, respectively (1.59, 1.64, 1.69, and 1.96).

The coccidia in vaccine A were sensitive to the anticoccidial drugs tested. Those organisms from

vaccine B were relatively sensitive and those from vaccines C and D showed signs of drug tolerance. Vaccine D appeared to be the most pathogenic among the vaccines tested; this vaccine caused severe growth suppression, impaired FCR, and relatively high parasite burden. The organisms in vaccine C appeared to be the most prolific as measured by oocysts produced per dose; followed by vaccines D and A, respectively.

COMPARISON BETWEEN AN ANTICOCCIDIAL TREATMENT AND AN IMMUNOGLOBULIN Y-BASED TREATMENT IN BROILERS CHALLENGED WITH *E. TENELLA*

Donají García López, Ruth I. Larios Castillejos, J. Andrés Morales Garzón, and Eduardo Lucio Decanini

Investigación Aplicada, SA de CV., 7 Norte No 416, Colonia Centro, Tehuacán, Puebla, CP 75700, México
E-mail: dgacia@grupoidisa.com

SUMMARY

The goal was compare the effect of an immunoglobulin Y product (Supracox®), liquid and powder, obtained from hyperimmunization of hens with multiple species of *Eimeria* oocysts vs. liquid anticoccidial with toltrazuril 2.5% like active ingredient (TZ) face to challenged of *Eimeria tenella* (Et). All immunoglobulin Y-based groups reduced fecal oocyst shedding in comparison with positive control group. TZ group fecal oocyst shedding was not constant and some values were higher than positive control. Better group in score lesions was powder treatment B. We conclude that immunoglobulin Y-based treatment is an effective option against coccidia challenge infection.

RESUMEN

Comparación entre un tratamiento a base de inmunoglobulinas-Y y un anticoccidiano, al ser administrados en un desafío con *Eimeria tenella.* Se comparó el efecto de las inmunoglobulinas Y (Supracox®), tanto líquidas como en polvo, obtenidas de la hiperinmunización de aves con oocistos de diferentes cepas de *Eimeria*, contra un anticoccidiano líquido que contenía como principio activo el toltrazuril al 2.5% (TZ) ante un brote de *Eimeria tenella* (Et), Las dosis para Supracox fueron de de 0.02 g/ave en el tratamiento en polvo y de 2 mL/ave en el tratamiento líquido, mientras que para el toltrazuril se siguieron las recomendaciones del fabricante. El 100% de los grupos tratados con Supracox presentaron una disminución en la excreción de oocistos, por debajo del control positivo. El grupo tratado con TZ tuvo una excreción de oocistos variable y en ocasiones mayor a la del control positivo. El 66.7% de los grupos tratados con Supracox tuvieron mayor ganancia de peso que el grupo tratado con TZ. De los tres tratamientos de Supracox el denominado 2 (producto en polvo) fue el que mejor resultados brindo comparándolo con todos los grupos, ya que fue el que obtuvo la mayor ganancia de peso y su calificación de lesiones fue de 1.

INTRODUCTION

Poultry coccidiosis is a widespread, economically important disease characterized by poor weight gain or feed conversion, depigmentation, and a slight increase in mortality. The higher economic effects are on the applied control methods, such as drug treatment or vaccination strategies. The occurrence of resistance in different *Eimeria* species (3) and the authorities restrictions give us the opportunity of researching new options (1). The use of egg yolk derived immunoglobulin like as an alternative to antibiotic treatment for control in several animal species has been proved (2).

MATERIAL AND METHODS

Chickens and experimental design. Seventy male one-day-old commercial broilers were randomly assigned to six groups (11 birds/group) and were fed throughout the trial with standard diet without anticoccidials. Each bird was vaccinated with NDV at first day of age. Group 1 and 2 were A and B immunoglobulin-Y powder, group 3 was liquid immunoglobulins, group 4 was toltrazuril; groups 5 and 6 were positive and negative controls. The animals were necropsied at eight days post challenge; the rest of each group until 13 days post challenge to continue the fecal oocyst counts.

***Eimeria* and challenged.** Animals were orally inoculated with 4.5×10^4 sporulated wild-type strain of *E. tenella* oocysts, 13 days post-challenge; chickens

were necropsied and checked for characteristic *E. tenella* cecal lesions.

Inmunoglobulin Y-based treatment: was supplemented for three days, from five to seven day-post infection. Powder A and B refers two different schedules of hyperimmunization.

Anticoccidial product. Liquid with toltrazuril 2.5% like active ingredient observing the dose rates and specifications recommended for maker lab.

Parameters. Fecal oocyst counts from three d post infection to 13 d. Table 1 shows the counts of oocyst per g, and scores lesions using the Johnson and Reid method.

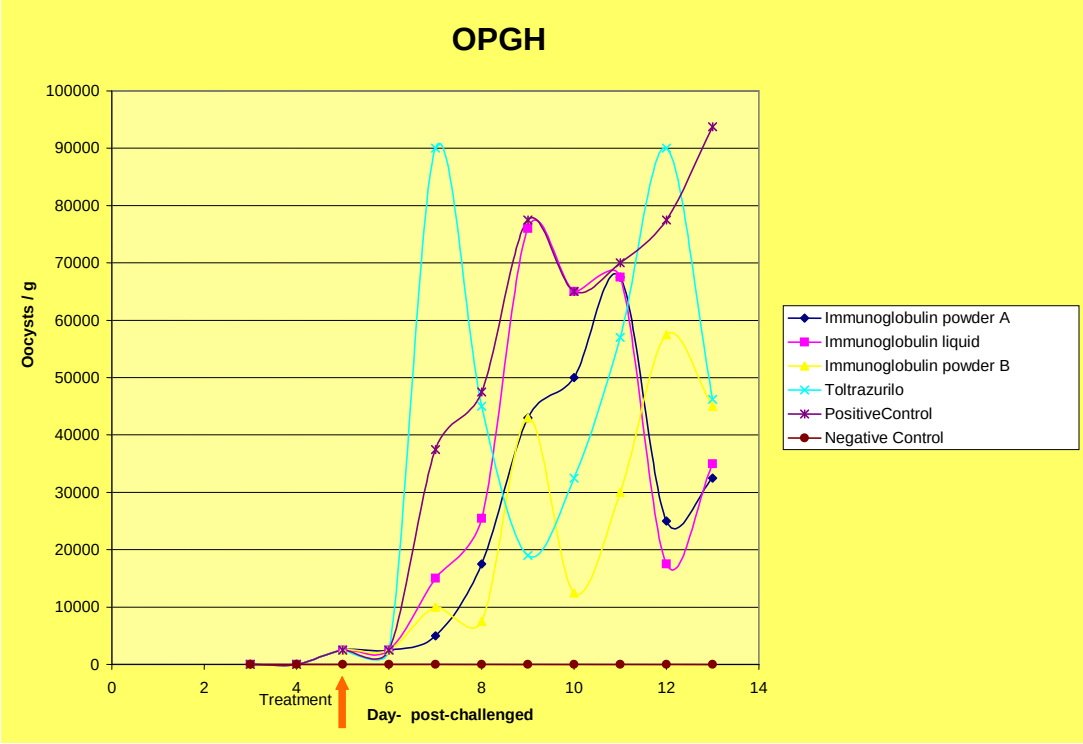
RESULTS AND DISCUSSION

All groups began to shed oocysts from five d post infection; immunoglobulin Y-based groups reduced fecal oocyst shedding in compare with positive control group. TZ group fecal oocyst shedding was not constant, and some values were higher than positive control (Figure 1). Better group in lesion scores was powder treatment B. In the present trial, best protection was afforded by the powder immunoglobulin B; this one reduced fecal oocyst shedding compare with all groups. Powdered product had no statistical difference in lesion score or fecal oocyst counts with the control group (Tukey test), although a numerical difference existed. The probable reason was that the animals were in battery units and did not get reinfected. Passive immunization of chickens with specific Ig-Y is a good alternative against coccidiosis control.

REFERENCES

1. Dallaoul, R.A. and H.S. Lillehoj. Recent advances in immunomodulation and vaccination strategies against coccidiosis. Avian Dis. 49: 1-8. 2005.
2. Ikemori,Y., M. Kuroki, R.C. Peralta, H. Yokohama, and Y. Kodama. Protection of neonatal calves against fatal enteric colibacillosis by administration of egg yolk powder from hens immunized with K-99- piliated enterotoxigenic Escherichia coli. Am. J. Vet. Res. 53:2005-2008. 1992.
3. Vertomen, M.H., H.W. Peek, and A. van der Laan. Efficacy of toltrazuril in broilers and development a laboratory model for sensitivity testing of Eimeria field isolated. Vet Q. 12: 183-192 .1990.

Figure 1. Fecal oocyst counts.



SIGNIFICANCE OF ANTIBODY DETECTION IN THE DIAGNOSTIC OF CHICKEN CRYPTOSPORIDIOSIS

Hayet Abbassi[A] and Muriel Naciri[B]

[A]College of Food, Agricultural, and Natural Resource Sciences, Animal Science Department, University of Minnesota, 1364 Eckles Avenue, Saint Paul MN 55108.
[B]INRA, Station de Pathologie Aviaire et de Parasitologie, 37380, Nouzilly, France

ABSTRACT

The kinetics of anti-*Cryptosporidium baileyi* antibodies were compared with the oocyst shedding in four groups of SPF chickens. Group I received orally *C. baileyi* oocysts at 11 days of age (Day11) and a challenge with the parasite at Day 60. The second group II, did receive in addition a very virulent strain of infectious bursal disease virus (vvIBDV) at Day 7. Two control groups, one (group III), had the vvIBDV at Day 7 and the parasite at day 60, and the other (group IV), had only the parasite at Day 60. A fifth group of chicken had only placebos and served as uninfected control group. The first group shed oocysts during 24 days, showed a seroconversion for IgM and IgA 10 dpi that last 24 days and over 52 days respectively. The IgG seroconversion was evident 17 dpi reached a peak 38dpi and stayed relatively high until the end of the experiment, Day 83. In the group IV, seroconversions were evident for all immunoglobulin isotypes (IgG, IgM and IgA) 10 dpi, and reach a peak 17dpi. Both IgG and IgA were positive until the end of the experiment 23dpi (Day 83), while IgM was not anymore detectable at that time. The oocyst shedding, last only five days in this group. Compared to group I the birds in group II shed about the double amount of oocysts during a longer period of time (45 *versus* 24 days); however, they did not show any detectable antibodies for all isotypes at all time points. More surprisingly, the birds in group III, having the virus at one week of age and a primo-infection with the parasite 53 days later shed lower quantity of *C. baileyi* oocysts during five days when compared to the group IV, but no antibodies were detected in these birds. These results were not expected, knowing that the follicular regeneration in the bursa of Fabricius takes place few weeks after an infection with vvIBDV.

The results in this study allow as having new considerations for our interpretations of the serology. Taken together with our previous findings (1,2), we start to build a clearer idea about the significance of each antibody isotype in the diagnostic of a cryptosporidial infection. The current question is: What will be the effect of an IBDV strain that is less virulent? Also, what will be the effect of variant IBDV strains that are very different from the vvIBDV strains in many aspects?

(The full-length article will be published in *Avian Diseases*.)

REFERENCES

1. Abbassi, H., F. Coudert, Y. Chérel, G. Dambrine, J. Brugère-Picoux, and M. Naciri. Renal Cryptosporidiosis (Cryptosporidium baileyi) in specific-pathogen free chickens experimentally coinfected with Marek's disease virus. Avian Dis. 43:738-744.1999.

2. Abbassi, H., F. Coudert, G. Dambrine, Y. Chérel, and M. Naciri. Effect of Cryptosporidium baileyi in specific pathogen free chickens vaccinated (CIV988/Rispens) and challenged with HPRS-16 strain of Marek's disease virus. Avian Pathol. 29:623-634. 2000.

INTERACTIVE PROBLEM-SOLVING OF FIELD CASES INVOLVING COMMERCIAL POULTRY – AN AUDIENCE PARTICIPATION PRESENTATION

Mark C. Bland

Cutler Associates, Napa California

My plan is to share with you, the WPDC audience, several field cases involving small commercial poultry operations where background information will be provided regarding each case and with WPDC audience participation, we will try and formulate a plan to find the cause and resolve the issue at hand be it through proper diagnosis and treatment or through necessary management changes to mitigate the problem. This is to be an interactive presentation by both the speaker and the audience. Space is provided for you to write down your answers and comments for the presentation. I suggest for those in the audience who wish to participate (residents, interns, poultry science students, etc.) that you may find sitting in the first few rows helpful for both speaker and participants.

Case 1: Commercial brown meat pullets/ cockerels experiencing late mortality.

History: A multi-age commercial facility growing brown-feathered pullets and cockerels for the specialty markets was experiencing increased late mortality in flocks from 12 to 17 weeks of age. Daily mortality ranged from 0.3 to 0.75 %. At any one time the farm had day-old chicks to market-aged birds on site. Clinical signs included high mortality and morbidity, coughing, snicking, and with approximately 1 to 2% of the flock experiencing swollen hocks and lameness. The owner states that this and his second farm (located nearby) have had continual problems for the past year or so – more so in the late fall to early spring. The owner has been spending several thousand dollars each month on medication in the older flocks.

While you are accumulating historical information you are informed that there may be a second problem involving higher than normal mortality during the brooding period. Mortality has ranged from 5 to 15% for the first two weeks with very few if any flocks have first two-week mortality of less than 5%. In addition, the owner points out that several months ago birds were submitted to the lab and *Mycoplasma synoviae* was diagnosed. The owner is not sure what this all means to the overall problem(s) he is facing.

There are 10 production buildings on site. All are of conventional design, with curtains on the sidewalls, dirt floors, nipple drinkers and automated pan feeder system. Chick placements are approximately 20,000 and placed every other week. Current weather conditions are cold and rainy.

List or identify your possible problems/ differential diagnoses to the high mortality a) during brooding and b) prior to market?

What additional questions would you ask the producer in an attempt to get a clearer picture of the situation?

The following are your observations during the farm visit:

Brooding: First week house temperatures are in the mid-80s. Partial room brooding is in place. An adequate number of radiant stoves are present. There appears to be one feeder flat for every 200+ chicks. Feeder pans are empty. Nipple water system is in place and functioning properly. Numerous chicks are observed with pasted vents.

Grow-out: Litter conditions are very wet, curtains are open, outside weather conditions are cold and rainy, inside house temperature is in the low 50s. Nipple water system is in place and functioning properly. A good number of the older birds are coughing and depressed. You observe a few birds with swollen hocks.

What samples, procedures, tests would you submit?

Based on your farm visit, answers provided to your questions during the initial presentation and lab results what is your tentative diagnosis?

What recommendations would you suggest to the owner, which would help reduce the mortality issues currently on the farm?

What long-term recommendations would you recommend to the owner to help prevent future issues from developing?

Case # 2: Broiler farm with poor production, higher than normal condemnations and late mortality issues.

History: You meet with the live production supervisor. He wants your opinion regarding two of their worst broiler production farms (A and B). The farms are about 1/8 of a mile apart and are located about two miles from a large layer facility and two very large broiler complexes operated by another broiler company. Farm A is placed first with approximately 190,000 broilers over a seven-day period. Then farm B receives about 180,000 broilers about a week later with the same age spread of one week. For the past two years both farms A & B have been "bottom dwellers" in regards to production performance (feed conversion, body weights, mortality, and condemnations at the plant). Recent lab reports from birds grown at both farms indicate bacterial septicemia (*E. coli*) and high serological titers to infectious bronchitis virus (IBV). A Cal 99, IBV virus was isolated. The live production supervisor wants to change the IBV vaccination program to include a stronger IBV vaccine like Ark 99 or Holland strain in hopes of solving the poor performance and wants your "blessing."

So, what do you think?

What other questions or information would you like to know or discuss?

Farm A: Consists of eight buildings, six are approximately 40 x 500 feet, have a high peak roof, are partially insulated, and are curtain sided; two are 50 x 400 feet, with a high peak roof, are partially insulated, and are environmental enclosed. Partial room brooding is in place. Outside temperature is in the mid 50s. It is cold and windy.

Farm visit observations: Farm A:

Flocks range from 24-31 days of age. Brooder stoves are off. Overall flock health in all buildings is OK. Flock uniformity varies in each house. Litter conditions range from poor to barely acceptable. Daily mortality is approximately 0.1%. No other observations of note.

Farm B: Consists of 11 buildings, eight are approximately 50 x 400 feet, have a high peak roof, are partially insulated, and are curtain sided; three are approximately 20-25 x 100 feet and environmental enclosed. Partial room brooding is in place. Outside temperature is in the mid 50s. It is cold and windy.

Farm visit observations: Farm B

Curtain sided buildings, flock ages are 20-24 days of age, and stoves are off.

In two houses (24 days of age) the brooding curtain is up. House temperature is 58°F, chicks are in large groups huddled together throughout the house, and flock is uneven in size. Litter conditions are poor.

In two houses (20 to 22 days of age) the brooder curtain is partially down. House temperature is 70° to 72°F on the brood side and 58° to 60°F on the cool side. Litter conditions are poor on the brood side and good on the cool side. Eighty percent of the birds are located on the brood side and look comfortable. The 20% on the cool side are huddled against the sidewall where the sun is shining.

One house (22 days of age), the brooder curtain is partially down. House temperature is 74°F on the brood side and 60°F on the cool side. Litter conditions are good throughout both sides of the house. Birds are evenly distributed throughout both sides of the house. No huddling is seen. Birds look fairly even.

Environmental houses: Flocks range in age from 19-24 days. Brooder stoves are off.

In three houses the brooder curtain is partially down. House temperature is 74° to 76°F on the brood side and 58° to 60°F on the cool side. One fan is operating on a timer (1 min/12 min) and is located on the brood side farthest away from the brood curtain (minimum ventilation). Litter conditions are poor on the brood side and good on the cool side. Ninety-five percent of the birds are located on the brood side and look comfortable except for the fact that the birds are very crowded around the tube-hanging feeders. There is approximately one round tube-hanging feeder per 200 + birds in the house. The 5% of the birds on the cool side are huddled against the sidewalls.

Do you see any issues with the current management?

What additional questions would you ask the producer in an attempt to get a clearer picture of the situation?

What suggestions or recommendations would you provide to the ranch manager to address the current situation?

Any additional comments that you would like to make to the production supervisor?

Any take home message for the veterinarian and/or the producer?

Case # 3: Central nervous signs in two-week-old Muscovy ducklings.

History: A flock of two-week-old Muscovy ducklings placed on a multi-aged commercial production facility were experiencing signs of central nervous involvement and respiratory distress. Approximately five to seven ducklings appeared to be involved. Current mortality was within normal standards. Two additional flocks, three and four weeks of age, were in the same house showing no clinical signs of distress.

What are your possible diagnoses?

What additional questions would you ask the producer in an attempt to get a clearer picture of the situation?

What samples, procedures, tests would you submit?

What is your tentative diagnosis based on the answers provided to your questions during the presentation?

What is your recommendation to treat the flock and/or affected birds? What steps would you suggest to prevent the problem from occurring in future flocks?

Future outcome.

HOW INCUBATION OF TURKEY EGGS CAN AFFECT POULT QUALITY

Vern L. Christensen

North Carolina State University

The final destiny of the properly managed fertilized turkey egg within incubators in our modern management systems is a viable hatchling poult. Understanding how incubation can affect the viability and quality of modern turkey eggs is essential to the poultry industry because of limited profit margins and economies of scale. Modern-day turkey hatcheries generally have egg setting capacities for thousands of eggs. Fertilized turkey eggs require a 28 day developmental period so the scheduling of eggs settings and removal of hatched poults from the incubator requires careful planning and monitoring of incubation conditions. Sanitation is imperative as well because conditions for bacterial, mold and viral growth are ideal under incubation environments. In fact, human vaccine manufacture is still often accomplished using fertilized poultry eggs.

Our understanding of processes creating a hatchling that is characteristic of the species has improved over that last 30 years because of our understanding of basic biological principles that govern the growth and development of cleidoic eggs. Embryos within cleidoic eggs by definition are packaged with all the nutrients required to develop a new organism of that specie if the egg is treated properly to foster development. The outside elements that are required for development are oxygen and heat. We need to better understand how to use these two elements in the proper proportions.

Fick's First Law of Diffusion. The bird egg is a self-contained life-support system for the developing bird embryo. All the nutrients, minerals, energy sources and water utilized by the embryo during its incubation are already in the freshly laid egg so that the egg requires only warming by the parents and period turning to prevent the adhesion of the embryo to the shell membranes. Still the egg lacks one crucial requirement: oxygen, which drives the metabolic machinery of the embryonic cells so that they can execute the complex maneuvers of development. How does the egg breathe, taking up oxygen from the surrounding atmosphere and discharging carbon dioxide, the waste product of respiration? In the late 1970s the laboratory of Hermann Rahn in the dental school at the State University of New York in Buffalo described in detail how the egg accomplishes respiration. This knowledge suggested principles that can be used in our modern-day incubation systems to ensure poult quality.

Over the typical 28 days of incubation a typical turkey egg weighing 90 g will take about 7 L of oxygen and give off 20 L of carbon dioxide and 15 L of water vapor prior to the 25th day of development (8). The total communication channel or pore occupies only

0.024 of 1% of the total shell surface. The total functional pore area of a normal turkey eggs is only 2.2 mm^2. The embryo itself has no control over its respiration rates or its oxygen-driven metabolic rate. It is totally dependent upon the type of shell that is placed around it during egg formation. Little is known of the mechanisms in the turkey breeder hen that control this process (3). We do know that high altitude reduces the functional pore area of a turkey egg (10).

The movement of gas by diffusion through a permeable barrier depends on the random motion of the gas molecules and the concentration of the diffusing species on one side of the barrier and the concentrations on the other side. Since collisions among gas molecules are more frequent in a concentrated gas than in a dilute one, the molecules will tend to move from the side of higher concentration to the side of lower concentration.

A simplified version of Fick's law of diffusion states that the quantity of a given gas diffusing in a unit of time through the pores of an eggshell will be directly proportional to the area of the pores available for diffusion and to the difference between the concentration of the diffusing gas at one end of the pore and the concentration at the other end. On the other hand, the rate of diffusion will be inversely proportional to the length of the diffusion path (in this case the length of the pores through the eggshell). In other words, the gas conductance of the eggshell depends on the ratio of pore area to pore length. Doubling the area available for diffusion of a gas or doubling the concentration difference of that gas across the shell will double the rate of passage, whereas double the pore length will halve the rate of passage, all other factors remaining equal. Thus, if one could measure the flux of a gas and divide it by the concentration difference of that gas across the pores, one would be able to calculate the conductance of the shell for that gas (8). This calculation is used to estimate the functional properties of eggshells defined as eggshell conductance.

Eggshell conductance theory. Eggshell conductance is then the property of the eggshell that governs how an egg breathes. In an extension of that same idea, it is also the property that drives the metabolism and thereby the rate of growth and differentiation of a turkey embryo into a hatched poult (8). Eggshell conductance creates common characteristics at the time of hatching that are described as follows: 1) The total amount of oxygen that will have been consumed is about 100 mL per g of initial egg mass and equivalent to a caloric expenditure of .5 kcal per g of initial egg mass. 2) The oxygen concentration in the air cell shortly before pipping will have fallen from 21% initially to 14% while the carbon dioxide concentration will have reached a value of about 6%. 3) Eggs will have lost about 15% of their initial mass due to the loss of water vapor, yet their relative water content will still be the same as when they were laid. These common end results require a precise pore geometry of the shell or shell conductance, which in each species is matched to the egg mass, its metabolic rate and incubation time. Much of this information is readily available in a multi-authored review (9).

Egg shell conductance theory predicts a period of time in the development of precocial species when the metabolic rate or oxygen uptake of the embryo will exceed the functional ability of the shell to provide oxygen. This time has been defined as the plateau stage in oxygen consumption and occurs in the turkey embryo at 24 or 25 days of incubation (4). The plateau stage in oxygen consumption plays a major role in maturation of tissues required outside the shell as well as sets up mechanisms leading to successful thermoregulation. One lesser known function is to establish blood acid-base balance prior to emergence from the shell (7). The three conditions mentioned above in the prior paragraph must be met at the plateau stage in oxygen consumption in order to create a hatchling that has the characteristic maturity of the species. The plateau stage is not thought to occur in altricial species although some data suggest that it may (6).

Conductance constants. The third concept that is necessary to clarify how the incubation of eggs can affect poult quality is the conductance constant. Ar and Rahn (1) noted among 96 species of birds that three measurements were interrelated. The three factors were the egg weight, the eggshell conductance and the length of the incubation period. Ar defined the conductance constant as the ratio of the product of eggshell conductance and the length of the incubation period in days divided by the weight of the egg. For all species tested this was calculated to equal 5.13. Therefore, the constant varies directly with conductance and the length of the incubation period but inversely with egg weight. We can use the conductance constant to predict each of the components of the equation. For example, if we know the weight of an egg as well as its eggshell conductance prior to placement into an incubator, we can predict how long to incubate that egg to maximize the maturation of poults prior to hatching.

Practical considerations – hatching times. Conductance constants imply that for each egg weight and eggshell conductance value, there exists an appropriate incubation period. We control the length of the incubation period by the temperature of our incubation cabinets as well as the availability of oxygen (1). The reader is reminded that an egg must consume 100 mL of oxygen per g of initial egg mass to create a hatchling that has the maturity characteristic of

the species (8). Thus, oxygen controls the growth of the embryo, but the temperature controls the rate of that growth (2). This is illustrated in Table 1.

These data suggest that when the incubation period is matched to the egg weight and the eggshell conductance that the "hatching window" is shorter. Most poults will hatch within a 36 hour time period. If the three egg variables are not matched, then the hatch window is much wider. This observation gives us an observable trait to create a good quality poult. Table 2 illustrates why this may be.

Overall the data suggest that short developmental periods require the embryo to remain at internal pipping in a hypoxic environment for a longer time. Extended time in hypoxia may demand more carbohydrate energy for activity than the hepatic and renal enzyme systems can recycle and shuttle back to critical tissues such as the heart and intestine. This may result in immature organ systems or in extreme cases embryo death.

Measure of poult quality affected by conductance constants. Table 3 and Table 4 illustrate how incubation can be used to improve poult quality. The initial table shows the effects of incubating eggs such that they hatch following a short and long incubation period. The first table illustrates eggs weighing nearly 100 g at setting in the incubator.

Table 4 illustrates the effect of eggshell conductance on poult quality from large eggs weighing nearly 100 g.

Eggshell conductance has a greater effect on poult quality of poults hatching from large eggs than does the length of the incubation period. Low eggshell conductance eggs of heavier weights obviously yield poults of reduced quality compared to Average and High conductance eggs.

Physiological and anatomical reasons why the conductance constant may affect poult quality are given in Table 5 and Table 6.

Although the length of the incubation period did not affect the growth of the poults (Table 3), it is clear that longer incubation periods with heavy eggs resulted in differences in both intestinal and thyroid maturation.

CONCLUSIONS

Hatchery-related problems. Carver *et al*., (2002) concluded that the age of the breeder hen and the size of the egg she produced were factors in seven and 14 day poult mortality. Larger eggs produce better poults until late in the laying period when poult quality deteoriated. The data illustrated above suggest some ideas for troubleshooting these problems. The Hatching Window could be measured by doing 36 hour counts as well as the time of hatching. Hatchery-related factors considered and found no significant risks for mortality included age of egg, number of eggs set, percentage of fertile eggs, and percentage of hatched eggs, poult injection, toe clipping and beak trimming. Donaldson et al. (1995) indicated that elevated carbon dioxide (0.4%) caused metabolic effects in newly hatched poults that suggest hypercapnia is a stressor and an additional risk factor for early poult mortality. Poults may be routinely sexed, beak-trimmed, toe-trimmed, and desnooded before shipping. There is abundant evidence these hatchery poult-processing practices contribute to poult mortality (see Carver et al., 2002).

Watch for these ten hatchery-related problems. Don't blame your hatchery man for excessive early mortality - at least not always. The mortality could be the fault of poor brooder house management.

Having said that, however, there are 10 points for growers to watch for in hatchlings - hatchery related factors that could give producers problems with their poults. They are:

1. Dehydrated poults: They generally result from low humidity during incubation, and early hatch or excessively long holding periods in boxes before delivery. Dehydrated poults can be identified upon delivery at the farm by **examining the shanks and feeling the poults**. If a fairly large number of poults have shriveled legs or shanks and bodies feel hard and look angular, give the birds extra good care the first week. The poults should have "easy" access to water and feed and they should be kept comfortable. Do not chill or overheat the poults in the brooder house. **Always check a sample of poults (50 to 100 at random) upon delivery to determine the state of dehydration and then brood accordingly.**

2. Weak poults: Weak poults do not have to be dehydrated poults, and often are not. Weak poults usually result from higher than recommended temperatures during hatching, inadequate ventilation in the hatchers, over-fumigation at hatching time, infection, rough sexing or setting old eggs. Weak poults can be identified easily by pressing down on the poults in the boxes with the palm of the hand. If the poults are strong, they will offer considerable resistance to the pressure of the hand; if they are weak, they can be pushed down easily. **With a little practice (the touch of the master) you can detect weak poults upon delivery. Weak poults need better than average brooder house care.**

3. Large, soft-bodied poults: Large, sluggish poults usually are the result of high humidity during incubation and hatching. They often have a heavy abdomen and feel soft and full of moisture to the touch. They generally ship better when transported long distances. **These poults usually present no serious brooding problems except that they appear sluggish.**

4. Rough navels: The navels of poults always should be checked upon delivery to the farm. A rough or open navel makes the chick more susceptible to infections. Rough or unhealed navels result when the hatch is late (more than 28 days), incubation temperature has been variable and high, or when excessively high humidity was used during hatching. **Poults with rough navels upon delivery probably should receive a broad-spectrum antibiotic in the feed or water for the first week to minimize the possibility of infection and morbidity.**

5. Omphalitis (navel infection): Omphalitis is the result of filth in the hatchers and/or contaminated poult boxes and poult box pads. *E. coli*, *Pseudomonas*, *Proteus*, or occasionally a *Staphylococcus* usually causes it. Sometimes the yolk sac is involved in addition to the navel. Yolk sac contents change from a yellow-green material to a caseous material or to a yellow-brown watery material when contaminated with *E. coli*. The navel opening often has an offensive odor. **Mortality and morbidity will be high with a high percentage of runts among the surviving poults.**

An omphalitis infection means that the hatchery must change its clean-up and egg and hatchery sanitation programs immediately. **A broad-spectrum antibiotic or a nitrofuran may help reduce morbidity and the percentage of runts.** The type of organism involved and drug resistance will affect the type of response one gets to treatment.

6. Poult delivery. Errors in the chick delivery system can injure potentially strong, healthy poults. Damage can occur in several ways; namely, overheating in the delivery van, chilling in the van, poor van ventilation resulting in overheating, chilling, or CO_2 poisoning. Assuming good judgement in programming the load, the driver becomes the "key" to a successful delivery of undamaged poults. Some truck drivers have no feel for the product being delivered. **The salvage process at the brooder house consists of ample feed and water, and a comfortable brooding temperature, along with a tremendous amount of care and attention.** The amount of loss depends on the damage done to the chicks in transit, and the amount of care given them during brooding.

7. Improper toe trimming, wing clipping, snood removal, poultry injection and rough handling during sexing: Quality control and sanitation are the greatest problems a hatchery has with these operations - getting the hatchery personnel to do their jobs properly and uniformly. Improper wing clipping or toe trimming can leave a chronic sore. Many years ago poultrymen recommended wing clipping or toe trimming through a joint taking into account the proper angle, and using a modified beak trimming unit to do the job.

The injection site and injection process should be kept as sterile as possible. Poults should be injected according to directions - meaning proper equipment and needles, recommended dosage of antibiotic mixture, injection in the upper portion of the neck and subcutaneously. If the antibiotic mixture is of the type that puts the chicks to sleep, delivery should be delayed until **all** poults have recovered from the injection.

There is little that farm management can do with poults that have been improperly processed except to talk to hatchery management.

8. Poult grading. All malformed, straddle-legged, and weak chicks should be culled at the hatchery before delivery. A high percentage of abnormal poults will die or be morbid. Most hatcheries do a good job of removing the abnormal poults.

9. Nutritional deficiencies. Breeder rations that are marginal in certain vitamins and/or trace minerals can result in hatched chicks that are weak and marginal in vitamin and/or mineral reserves. Those chicks should be fed a prestarter well fortified with vitamins and minerals. Often when young breeder hens have been on a poorly vitamin and mineral-fortified holding ration, the first two or three hatches of chicks will not start and live as well the first week of brooding as later hatches.

Some of the vitamins and minerals which could be deficient in the breeder ration and which could reflect themselves in the young poult are E, K, riboflavin, biotin, folic acid, pantothenic acid and B_{12}. Some of the minerals would be iodine, potassium, manganese and cobalt. Check to see whether the breeder hens are receiving an adequate diet, if not, feed a prestarter. **A prestarter will have more protein, higher vitamin and mineral fortification and higher levels of growth promotant than the regular starter.**

10. Irregular-sized poults: Irregular-sized chicks result from different age of breeder flocks and age size, variations in incubator temperature and humidity. **If the poults are from a healthy flock, there is little worry since hatching weight is poorly correlated with market weight.**

REFERENCES

1. Ar, A., and H. Rahn. Interdependence of gas conductance, incubation length, and weight of the avian egg. In: Respiratory Function in Birds, Adult and Embryonic, J. Piiper, ed., Springer Verlag, Berlin. Pages 227-236. 1978.

2. Christensen, V.L., L.G. Bagley, J. Prestwich, T. Olson, M. Wineland, and D.T. Ort. Length of the developmental period of turkey eggs affects cardiac physiology and subsequent embryo survival. Int. J. Poult. Sci. 6:95-101. 2007.

3. Christensen, V.L., and G.S. Davis. Maternal

dietary iodide influences turkey embryonic thyroid function. Poultry Sci. 80:1286-1292. 2001.

4. Christensen, V.L., W.E. Donaldson, and K.E. Nestor. Embryonic viability and metabolism in turkey lines selected for egg production or growth. Poultry Sci. 72:829-838. 1993.

5. Christensen, V.L., D.T. Ort, K.E. Nestor, G.B. Havenstein, and S.G. Velleman. Genetic control of embryonic cardiac growth and functional maturation in turkeys. Poult. Sci. 87:858-877. 2008.

6. Dietz, M.W., M. van Kampen, M.J.M. van Griensven, and S. van Mourik. Daily energy budgets of avian embryos: The paradox of the plateau phase in egg metabolic rate. Physiol. Zool. 71:147-156. 1998.

7. Erasmus, B. deW., and H. Rahn. Ontogeny of acid-base balance in the bullfrog and chicken. Respir. Physiol. 11:46-53. 1970/71.

8. Rahn, H. Gas exchange of avian eggs with special reference to turkey eggs. Poult. Sci. 60:1971-1980. 1981.

9. Rahn, H., A. Ar, and C.V. Paganelli. How bird eggs breathe. Sci. Amer. 240:46-55. 1979.

10. Rahn, H., C. Carey, K. Balmas, B. Bhatia, and C.V. Paganelli. Reduction of pore area of avian eggshell as an adaptation to altitude. Proc. Natl. Acad. Sci. 74:3095-3098. 1977.

Table 1. Time of hatching (h of incubation) of turkey embryos developing in eggs at three different temperatures.

	Hours of Incubation				
Temperature	648	654	660	666	672
37.1□C	2.7b	9.0c	39.7c	59.9c	100.0
37.3□C	3.4b	25.0b	44.5b	78.7b	100.0
37.5□C	11.3a	46.2a	65.5a	83.1a	100.0
Mean ± SEM	5.8 ± 0.4	26.7 ± 0.9	49.9 ± 1.0	73.9 ± 0.9	NA
Probability	0.0001	0.0001	0.0001	0.0001	

[a,b,c] Columnar means followed by a different superscript differ significantly ($P < 0.01$). [1]Overall mean ±SEM of pooled data from three trials.

Table 2. Times (h) to attain a stage of development and the time remaining at that stage of poult embryos incubated at three different temperatures.

	Internal pip		**External pip**		**Hatched**
Temperature	Attain	At	Attain	At	Attain
37.1□C	628	8.1c	636	20.1a	657a
37.3□C	626	11.4b	637	18.1a	655ab
37.5□C	624	14.7a	639	14.1b	653b
Mean ± SEM	626 ± 6	11.4 ± 0.5	638 ± 8	17.4 ± 0.1	655 ± 3
Probability	0.1887	0.0001	0.4917	0.0315	0.0568

[a,b,c] Columnar means followed by a different superscript differ significantly ($P < 0.01$). [1]Overall mean ±SEM of pooled data from three trials.

Table 3. Effects of different incubation temperature profiles on growth of poults (large eggs).

	HP	**LP**	**Overall SE**	**P value**
Time to feed (min)	104	144	97.56	NS
Weight d 1(g)	60.6	60.0	8.2	NS
Weight d 3 (g)	94.2	91.1	23.4	0.0200
Weight d 7 (g)	123	125	12.6	NS
Feed per gain	1.28	1.19	0.01	NS

HP = poults from eggs incubated at high temperature profile; LP = poults from eggs incubated at low temperature profile.

Table 4. Effect of different eggshell conductance values on growth of poults hatching from large eggs.

	Low	Average	High	Overall SEM	P value
Time to feed (min)	163[a]	129[b]	81[c]	9.8	0.0387
Weight d 1 (g)	59	60	61	8.2	NS
Weight d 3 (g)	90[b]	93[a]	95[a]	2.3	0.0120
Weight d 7 (g)	118[b]	123[a]	130[a]	4.3	0.0071
Feed per gain	1.33[a]	1.26[ab]	1.12[b]	0.06	0.0387

Low = low eggshell conductance; Average = average eggshell conductance; High = high eggshell conductance.

Table 5. Effect of different incubation profiles on anatomic and physiologic factors determining maturity of poults hatching from large eggs.

	HP	LP	Overall SEM	P value
Body weight at hatching (g)	52.4	53.3	1.81	NS
BW without yolk (g)	48.2	49.3	1.49	NS
Yolk sac weight (g)	4.2	4.2	0.15	NS
Jejunal length (cm)	12.96	13.44	0.80	NS
Jejunal weight (g)	0.455	0.482	0.0009	NS
Relative jejunum weight	0.9	0.10	0.00003	NS
Total maltase activity	327.8[b]	367.5[a]	2.0	0.0393
Total ALP activity	18,113	18,466	7408	NS
Specific Maltase activity	17.39	18.63	2.98	NS
Specific ALP activity	0.960	0.890	0.01	NS
Plasma T_3 (ng/mL)	3.41[b]	3.76[a]	0.09	NS
Plasma T_4 (ng/mL)	10.60[b]	12.75[a]	1.53	0.0151
Ratio T_3:T_4	0.344[a]	0.302[b]	0.001	0.0508

HP = poults from eggs incubated at high temperature profile.
LP = poults from eggs incubated at low temperature profile.

Table 6. Effects of different eggshell conductance values on anatomic and physiologic factors determining maturity of poults at hatching from large eggs.

	Low	Average	High	Overall SEM	P value
Body weight (g)	61.3	62.2	61.6	3.39	NS
BW without yolk (g)	50.9	53.8	53.2	2.63	NS
Yolk sac weight (g)	10.33[a]	8.44[b]	8.41[b]	0.60	0.0024
Jejunal length (cm)	14.64	14.34	14.06	0.66	NS
Jejunal weight (mg)	466	464	465	0.001	NS
Relative jejunum wt	0.93	0.86	0.88	0.0003	NS
Total maltase activity	416	387	398	80.47	NS
Total ALP activity	20,182[b]	26,590[a]	21,457[ab]	1,884	0.0019
Specific Maltase activity	16.61	15.26	16.18	2.30	NS
Specific ALP activity	0.799[b]	1,016[a]	0.844[ab]	0.01	0.0019
Plasma T_3 (ng/mL)	2.93	3.22	2.92	0.04	NS
Plasma T_4 (ng/mL)	10.40	10.95	9.46	0.88	0.0500
Ratio T_3:T_4	0.290	0.210	0.330	0.001	0.0007

Low = low eggshell conductance; Average = average eggshell conductance; High = high eggshell conductance.

PLATINUM BROODING

Stewart J. Ritchie

Canadian Poultry Consultants Ltd., Abbotsford, British Columbia

Managing poultry health in a constantly changing world is the theme of the 58th Western Poultry Disease Conference. The importance of optimum brood management cannot be overemphasized, not only in terms of allowing the bird to produce to its genetic potential, but also in terms of managing poultry health. For years poultry experts have provided information on getting chicks and poults off to a good start, however in the field this information is often overlooked.

In the field, problem solving exercises, often uncover deficiencies in brood management. In this constantly changing world, where enhanced automation, precision nutrition and precision genetic selection programs continue to change and where the specific requirements of neonates are numerous, the negative impact of a brooding deficiency is significant. Deficiencies in brood management can result in poor performance and may result in an increased incidence or severity of an infectious disease. As veterinarians, we have been very successful in providing our clients with an accurate and timely diagnosis. An accurate and timely diagnosis is important in order to initiate a cost effective treatment and to initiate and develop effective control and prevention strategies. More often than not, prevention strategies include recommendations for improvements to brood management.

In order to further emphasize and achieve a practical level of focus for commercial producers and service persons the Platinum Brooding Program is under development. The Platinum Brooding Program, at this time, consists of a one-day Platinum Brooding Class and a Platinum Brooding Service Field Call Protocol and Checklist. At this time, Platinum Brooding Distance Learning Modules are also being developed. This structured emphasis on brood management has developed, solely due to its current success in the field, where significant persistent performance and disease problems have been overcome by applying Platinum Brooding Protocols.

The Platinum Brooding Class takes place at a commercial broiler chicken farm and provides a thorough and comprehensive review of the critical brooding factors and an opportunity for hands-on experience. The Platinum Brooding Class begins with an emphasis and thorough review of biosecurity and biocontainment principles (1). Other agenda items include; an introduction and discussion of case reports, ready the barn section, chick delivery, barn equipment adjustments, completion of regulatory forms and chick necropsy and chick disease discussions (2,3). The Platinum Brooding Class notes consist of Integrated Poultry Health Management bulletins providing regionally accurate details on various topics or subsections, including for example; feed, light, litter, air, water, space, security and sanitation management.

The Platinum Brooding Service Checklist (attachment 1) is used to record important data. The Platinum Brooding Checklist is currently used as a guideline and provides the information necessary to identify critical deficiencies in brood management. The Platinum Brooding Checklist when accurately completed allows for an efficient, thorough and accurate review of current brood management practices. While Platinum Brooding Checklist targets vary on a regional basis, it has been accepted at this time for example; that **crop-fill** at 24 h post placement must be greater than 95%.

The identification of the **zone of comfort** has been considered one of the key components of the Platinum Brooding Program. Litter temperatures have been identified as a critical factor and have led to the development of litter temperature probes. Lighting intensity and distribution within the zone of comfort has also been identified as critical factors.

To date the Platinum Brooding Program has been used to provide critical information to farm managers and service-persons and used to solve various broiler performance and disease problems. The Platinum Brooding Program has also been used in the commercial layer industry, both in cages and on the floor brooding set-ups.

The Platinum Brooding Program simply provides a structured approach or system that is focused on brood management for use in the field, both for training and problem solving purposes. This presentation will discuss various field case reports where the Platinum Brooding Program has been used.

ACKNOWLEDGEMENTS

1. William Cox, British Columbia Ministry of Agriculture and Animal Health, Abbotsford, British Columbia.
2. Victoria Bowes, British Columbia Ministry of Agriculture and Animal Health, Abbotsford, British Columbia.
3. Mike McIlwee, Canadian Poultry Consultants Ltd., Abbotsford, British Columbia.

Figure 1. Platinum Brooding checklist.

Bulletin CPC-IPHM-102
Date: March 26, 2008

CANADIAN POULTRY CONSULTANTS LTD.

PLATINUM BROODING CHECKLIST
Integrated Poultry Health Management

Placement Date:		Premise Identification #:	
Farm Name:		# Birds Placed:	
Contact Name:		Date/Cycle:	
Farm Location:		CMS/PB#	
Feed Company:		Breed(s):	
Feed Representative:		Breeder ID:	
Hatchery:		Veterinarian Consulted:	
Hatchery Representitive:		Barn or Bin #:	
CHICKS		**SPACE**	
Rectal Temp. at Delivery	*Rectal Temperature Worksheet*	**Density in Brood Area**	
Weight @ Placement	*Placement Weight Worksheet*	**Density in Barn**	
Uniformity @ Placement	*Placement Weight Worksheet*		
Crop Fill @ 24 hours		**LITTER**	
Activity @ 24 hours		**Depth / area per unit**	
Distribution @ 24 hours		**Type of Litter**	
Navels	**Healed Unhealed**	**Brood Area**	
Hydration		**Temperature surface floor**	
FEED		**Moisture**	
Feeder Height		**Floor type**	Concrete Wood Other
Accessable	Yes No	**ZONE OF COMFORT**	
Distribution Comments		Infra-red Analysis	Yes No
Supplemental	Paper Trays #		
In Zone of Comfort	Yes No		
Feed Type	Mash Crumble		
Size of Crumble			
Feed Amount (grams/chick)			

Figure 2. Platinum Brooding checklist (cont.).

Bulletin CPC-IPHM-102
Date: March 26, 2008

CANADIAN POULTRY CONSULTANTS LTD.

PLATINUM BROODING CHECKLIST
Integrated Poultry Health Management

WATER		LIGHT	
Nipple Flow (ml/minute)		Intensity (Lux)	
Temperature		Light Type:	
pH		Brood Lights Zone of Comfort	Yes No
ORP		**AIR**	
Height (birds eye level) Uniform	Yes No	Temperature Sensor location	
Availability (#chicks/nipple in brood area)		Humidity (RH) Sensor location	
# chicks/nipple in barn		Static Pressure	
NVW		Ammonia	
Water Source	City Well Other	**SANITATION**	
Water Line Sanitation (describe program)		Litter Removed	Yes No
Product / concentration		Blown Out	Yes No
In Zone of Comfort	Yes No	Wash If yes:	Yes No Hot Water Cold Water
		Disinfectant / Insecticide Product/ concentration	
		Down Time (from manure out)	
		Mortality	Incinerate Compost Other
		Manure	Out Stored Distance

A NOVEL HATCHING METHOD ON APPLICATION TO AN EXISTING COMMERCIAL HATCHERY SYSTEM RESULTS IN A MARKED DECREASE IN LATE EMBRYO LOSS

R.W. Keirs[A], E.D. Peebles[A], and M.A. Dekich[B]

[A]Mississippi State University, Mississippi State, Mississippi, USA
[B]AviTech, LLC, Salisbury, Maryland, USA

ABSTRACT

Under typical field conditions is a common multistage incubation system, hatching eggs from a single 28 week broiler breeder flock was compared to itself on being transferred into two adjacent hatchers following inovo-injection on their 18th day of incubation. Novel hatching inserts were placed in all 90 trays of the test hatcher prior to transfer placing all eggs in the vertical position for the remaining incubation period. The first three vertical columns A, B, and C consisting of 45 trays in the test hatcher were compared to their respective negative controls located in the adjacent hatcher, vertical columns A, B, and C and whose 18 day transfer was conventional, whereby all eggs were removed from their setter flats and placed horizontally in a loose array in hatchery trays. Comparisons were made utilizing hatching eggs residues as outlined in the Hatching Efficiency Analysis System (HEAS), Keirs, *et al*. Results depicted a 44.8% reduction in late embryonic loss with a 2.74% increase in estimated hatching efficiency.

Total embryonic loss of broiler hatching eggs is approximately 7.00% divided between early embryonic loss (EEL), three through 12 days (D) and late embryonic loss (LEL), 16 through 21 D at 3.80%, Hatching Efficiency Analysis System (HEAS), Keirs *et al*. Estimated hatch of fertilized eggs or Estimated Hatching Efficiency (Est HE) was 92.50%. This information comes from a base line HEAS composite of over 5000/machine/flock records which included over 500,000 hatching egg residue tabulations. EEL was primarily due to bacterial contamination of 1.82%. HEAS doesn't note but does include the mid embryonic loss 13, 14 and 15 D which would account for no more than 0.20% of embryonic loss. LEL had the more erratic loss between hatch trays. With many dead 18 and 19 D appearing normal with the majority of the 21 D being alive.

A recent study utilizing a Latin Square experimental design, abstract #30258, International Poultry Scientific Forum, Keirs *et al*, resulted in nonconventional transfer of hatching eggs having a significant reduction in 18 D embryonic loss of 54% ($P = \leq 0.01\%$) and a 19 D numerical decrease of 60%.

The objective of that study was to discern variables other than ovo-injection impacting on 18 D embryonic loss. After ovo-injection at 18 D incubation eggs conventionally transferred from setter flats to a horizontal position on hatcher trays were compared to a nonconventional transfer whereby eggs in the vertical position remained in their respective setter flats and were transferred in total to hatcher trays to complete the hatching process.

The objective of this field study was to compare the impact of a novel 168 egg capacity hatching insert on hatching efficiency and specifically on 18 D embryonic loss in eggs from a young broiler breeder flock. Such an insert could obstensibly be used from set through completion of hatch with the eggs remaining equally spaced in the vertical position, air cell up. Eggs from a single 28 week old Cobb x Cobb breeder flock were hatched simultaneously in adjacent Jamesway Super J hatchers after a 18 D ovo-injection by AviTech's Intelliject. The entire 90 trays of one hatcher were the recipient of all 15,120 eggs from setter flats during a conventional transfer. The test hatcher had all trays receive the novel hatching tray inserts whereby after the ovo-injection, the eggs were deposited on the inserts, remaining in the vertical position throughout hatch. The HEAS method for tabulating hatch residue was conducted on each tray with emphasis on LEL and Est. H.E. The first three vertical columns (A, B, C) (15 trays/column) of the conventional transfer were compared to their respective columns of the test hatcher with inserts. This allowed for 15 trays of hatch residue per replicate and three replicates per treatment for a total of 45 trays of residue per each treatment.

Compared to the conventional method the novel insert transfer resulted in a reduction for 18 D, 19 D and LEL of 45%, 54% and 47% respectively. EST HE rose to 93.87% compared to 91.28% for the conventional method.

From a pragmatic perspective such changes would be economically dramatic, for a 1% increase of chicks produced in the annual US 9 billion broiler chicks would equate too nearly 25 million dollars in chick cost.

Further studies should be actively pursued.

Table 1. Conventional LEL Embryonic Loss vs. Novel Insert

Transfer Method		16D	18D	19D	21D	LEL	EST HE
Conventional 45 Trays	A	0.44	0.56	3.68	0.20	4.88	91.49
	B	0.28	0.48	3.65	0.32	4.72	91.33
	C	0.16	0.75	3.69	0.28	4.88	91.03
A B C		0.29	0.60	3.68	0.26	4.83	91.28
Novel Insert 45 Trays	A	0.04	0.20	1.87	0.63	2.74	93.44
	B	0.04	0.36	1.35	0.48	2.22	94.02
	C	0.08	0.44	1.83	0.40	2.74	94.16
A B C		0.05	0.33	1.68	0.50	2.57	93.87
Reduction %			45	54		47	2.84*

Transfer % *increase

COMPARISON OF TURKEY GROWER MANAGEMENT PRACTICES ON FLOCK HEALTH AND PERFORMANCE

Rosemary A. Marusak, Michael P. Martin, David Rives, David Ley,
J. Michael Day, and H. John Barnes

INTRODUCTION

When animals are reared in a confined environment with limitations on space and activity, additional management practices are necessary to ensure optimal health, growth, and well-being (1). Genetic selection for larger birds and rapid growth as well as economic and management factors lead to high bird density, creating potential for high pathogen loads and behavioral problems such as persecution. Although gold standard practices for bird management in the hatchery and grow-out facility are available (2), changes in bird genetics, microbial evolution, and altered resources and technology continually challenge current practices.

Herein, we attempt to identify some key management and welfare factors necessary for optimal turkey health and growth by comparing two sister flocks (SF) of Hybrid Converter (HC) turkeys reared in different settings: the Teaching Animal Unit (TAU) at North Carolina State University and a standard commercial grower (SCG) operation.

MANAGEMENT METHODS

Two thousand (2000) turkey poults were placed at the TAU in a single 5600 sq ft curtain-sided house that is used to grow one turkey and one chicken flock per year. The house is located next to a swine barn as well as nearby cattle, horse and small ruminant pastures, that are all part of the TAU. Five research animal units are in close proximity to the poultry TAU and may contain poultry intermittently but are run with separate staff and equipment. No commercial poultry are located near the TAU. Complete poultry house cleaning with a three month down time and fresh litter replacement is done between flocks. The house has three wall fans, two wall heaters, six air vents per N/S side and three ceiling fans for ventilation. The anteroom is equipped with a disinfectant footbath and sink. When birds are present, the house is evaluated four times per day for temperature, ventilation, and equipment function and birds are assessed for mortality, overall health, and comfort. Plasson drinkers were cleaned daily and litter was turned under drinkers and feeders as needed, but at least once per week. Caked litter was removed and replaced with dry or fresh litter in select areas as needed. Behavior and

medical issues were addressed during each walk-through. Birds were culled as needed.

Because of increased persecutions at eight weeks of age management practices were implemented to minimize bird losses. Damaged wing and tail blood feathers were removed and blood stains quickly cleaned with water. When additional hemostasis was indicated, a small amount of Clotisol® was applied. Birds with head and neck persecution were placed on ladder rungs approximately four ft off the ground and provided feed and water until recovery. All persecuted birds were left in the flock and no isolation pens or additional space was required. Enrichment items were added to the house to break cycles of persecution.

Seven thousand (7000) turkey poults were placed on the SCG operation; the birds were housed in one 16,000 sq ft curtain-sided barn. Grounds around the house were in good condition. The farm included five other adjacent barns (two for brooding and grow-out and three for grow-out only) with a total of 48,000 birds on the farm. The SCG birds shared the house with another flock until 5.5 wks at which time the other flock was moved to their own grow-out barn. SCG birds were under the management and supervision of an integrated turkey company and contract grower, and no attempts were made to influence those management practices. A daily walk through the house by the grower involved picking up dead and adjusting ventilation according to the recommended industry practice. Culling of birds by the grower was not carried out until three wks prior to processing. Due to the large number of birds on the farm and only one farm-hand, routine litter, feeder, and drinker maintenance was difficult. Water sanitation relied solely on chlorine additive. Standard barn clean-out practices with four wk downtime between flocks (three flocks/year) were followed. Greater than 30 large commercial poultry and swine houses are located within a two mile radius from the farm. Cattle graze in a lot adjacent to the barns.

For both the TAU and SCG operations, one half the poults were placed day of hatch (d0) and the other half placed day after hatch (d1). Birds were commingled on about day three and opened to full house (TAU) and ½-house (SCG) on day nine. Full house access for SCG birds was on about day 38. D0 and d1 birds were identified by unique toe trimming patterns. Both operations were delivered the same feed (turkey starter 1 with nitarsone, turkey starter 2 with nitarsone, turkey grower 1 with lasalocid, and turkey grower 2 with virginiamycin). SCG birds were given five additional medications during weeks 1-3, 5, and 7 to treat enteric disease.

Bird weights and fecal samples for culture and parasitology were collected at regular intervals until week 10 after which time TAU birds were processed. All TAU and select SCG mortalities were necropsied; intestines and viscera with gross lesions were submitted for bacterial analysis. Limited resources prevented *Salmonella* spp. identification. Select samples were submitted for histopathology. *Mycoplasma* testing was carried out on select abnormal joints. Darkling beetles were also collected from the SCG house and submitted for virus analysis. Uniformity index was calculated as (S.D.*100)/mean weight.

RESULTS

TAU birds. Cumulative mortality (10 wks) was 3.75%. Gross pathology diagnoses included neonatal/early brooding mortality associated with *E. coli* infection (21.3%), non-infectious neonatal/early brooding mortality (4%), *Staphylococcus* associated arthritis/musculoskeletal (9.3%), *M. Iowae* associated chondrodystrophy (17.3%), and cardiovascular (18.7%) diseases, pendulous crop (10.7%), trauma (14.7%), and persecution (9.3%). Uniformity index was generally <10% throughout the lifetime of the flock. *Salmonella* was not detected in feces of d0 birds on arrival, but was detected in the feces of d1 birds. D0 birds became *Salmonella* positive on day one. *Salmonella* was shed by both groups intermittently throughout the grow-out period. Overall mortality of d1 birds was 1.2 times that of d0 birds. At processing (10 wks), TAU birds averaged 5.73 kg, 0.42 kg heavier than the Hybrid Converter standard (5.69 kg); d0 birds were 0.177 kg heavier than d1 birds. Daily gain was 0.1827 and adjusted feed conversion for TAU birds was 2.00.

SCG birds. Cumulative mortality was 2.9% (10 wks) and 6.3% (13 wks). *Salmonella* shedding in feces of both d0 and d1 birds was detected in the earliest sampling (day three) and intermittently throughout the grow-out period. Birds showed flushing and decreased growth from wk one, and uniformity index was generally >10% throughout the life of the flock. A diagnosis of poult enteritis complex was made wk three with isolation of *C. perfringens* and *Campylobacter* from pooled feces. *Cochlosoma* was identified from intestines. SCG birds also had *M. iowae*-associated chondrodystrophy. Aggressive culling of "small birds" and an increase in mortality due to persecutions occurred in wks 10-13 accounting for 53% of the total mortalities. Mortality of d1 birds was 1.5 times that of d0 birds. SCG birds averaged 4.47 kg at 10 wk and d0 birds were 0.31 kg heavier than d1 birds. At processing (wk 13), feed conversion was 2.44 (farm average).

DISCUSSION

TAU and SCG birds differed markedly in their morbidity/mortality profiles and rates of growth. TAU birds had higher mortality from neonatal/early brooding diseases. In contrast to the TAU birds, SCG birds experienced gastrointestinal (GI) disease beginning in the first week. This resulted in a marked decreased growth rate and increased feed conversion. Birds placed day after hatch were affected to a greater extent than birds placed day of hatch.

The interaction between feed, gut health, immunity, and muscle growth is well-established (3). Immediate post-hatch feed accelerates GI and immune system maturation, establishes gut microflora, and positively influences muscle development (4,5). When a poult's GI tract is compromised at a very early age, immune system development is also affected. Rectal exposure to pathogens is met with decreased systemic and intestinal antibody responses and decreased lymphocyte populations in the bursa, ceca, and colon (3).

GI disease in SCG birds was most likely due to environmental challenges that promoted a higher load of pathogens combined with management issues. Lack of resources may have contributed to the management problems. The SCG house temperature and ventilation were not adjusted for optimal conditions throughout the growth of the birds, increasing potential stress that could lead to health and growth problems. As an example, during the first week of growth the in-house temperature reached 110^{o}F. Relatively high temperatures were sustained for at least the first two weeks of the birds' life, which could promote decreased appetite, jejunal atrophy, and decreased feed absorption (6). Litter quickly became caked, Plasson drinkers were not routinely cleaned, and darkling beetle infestation was not controlled. Further, sick birds were not regularly culled. The flock experienced continuous morbidity likely due to the environmental pathogen load. Medications were provided to the flock but it is unclear if they were efficacious as the causative factors for the morbidity were not diagnosed. It is also unclear if the medications may have altered the normal gut flora, thereby contributing to sustained illness and decreased growth. Once growth is inhibited, it is not possible for the birds to recover and regain weight up to the level and efficiency of an unaffected bird (4,5). This was observed in the study as the SCG flock uniformity index was >10% for most of the flock's life and TAU birds were on average 1.26 kg heavier than SCG birds at 10 wks.

Compounding the differences between the TAU and SCG flocks was the contribution of persecution to the morbidity and mortality. Simple and fast yet aggressive management of birds in the TAU flock decreased persecution mortalities over that seen in previous years. With no intervention, persecution remained an important cause of death in the SCG flock.

In conclusion, several factors contributed to major performance differences between the TAU and SCG sister flocks. Firstly, environmental conditions (e.g. area poultry density, number of houses on farm and number of flocks per year) led to increased pathogen exposure for the SCG flock. Secondly, the greater number of birds and houses on the SCG farm overwhelmed the resources available so that certain management practices were not performed. Thirdly, oversight of the TAU flock emphasized rapid responses to medical/behavioral issues such as bird persecution.

REFERENCES

1. Tabler, G.T. The Challenges Facing Turkey Growers. Avian Advice. 6 (1): Spring 2004. http://www.thepoultrysite.com/articles/264/the-challenges-facing-turkey-growers last accessed 1/15/09; Gross, W. B., P.B. Siegel. Why Some Get Sick. J. Applied Poultry Science pp. 453-460. 1997.

2. Schwartz, D.L. General Information and Fundamental Factors in Disease Prevention. In Poultry Health Handbook 4th ed. The Pennsylvania State University, University Park, PA. pp. 3-39. 1994.

3. Sklan, D. Early gut development: the interaction between feed, gut health and immunity in Interfacing Immunity, Gut Health and Performance. Tucker, LA and Taylor-Pickard, JA eds. Nottingham University Press. pp. 9-31. 2004.

4. Moore, D.T., P.R. Ferket, and P.E. Mozdziak. The effect of early nutrition on satellite cell dynamics in the young turkey. Poultry Science 84: 748-756. 2005.

5. Halevy, O., Y.Nadel, M. Barak, I. Rozenboim, and D. Sklan. Early posthatch feeding stimulates satellite cell proliferation and skeletal muscle growth in Turkey Poults. Halevy, O.J. Nutrition. pp. 1376-1382. 2003.

6. Azevedo, L.F. and J.J. Dibner. Maximizing gut health for peak performance. Inter. Poultry Production. 14: 17-20. 2006.

EVALUATION OF LITTER TREATMENT REGIMENS AT THREE USAGE LEVELS

J. P. Blake, J. B. Hess, and K. S. Macklin

Department of Poultry Science, Auburn University, Auburn, AL

SUMMARY

A series of experiments evaluated six litter treatment strategies in reducing ammonia volatilization during broiler production. Poultry Litter Treatment™ (PLT), granulated aluminum sulfate (Al-Clear™) (GA), Poultry Guard™ (PG), and Hydrated Lime (HL), liquid aluminum sulfate (A-7™) (LA), and concentrated sulfuric acid (SA) were applied at three levels each to evaluate the effect of application rate compared to a non-treated control. In each experiment, 70 mixed-sex broilers were placed in each of 16 pens (2.44 x 2.44 m), fed commercial diets, and weighed at 21, 42, and 49 days of age. Litter and ammonia were evaluated weekly. Results indicate that PLT, GA, LA, and SA were capable of reducing ammonia volatilization during broiler production. PG and HL treatments failed to support any reduction in ammonia volatilization.

INTRODUCTION

Interest in the use of litter treatments has increased over the last decade as growers and technical personnel alike recognize health and productivity benefits of improving the broiler house environment. It is known that high ammonia levels make birds more susceptible to respiratory diseases. Numerous laboratory and field studies have shown ammonia levels as low as 10 ppm affecting bird health and performance (1). Ammonia levels above 25 ppm in the poultry house can damage the bird's respiratory system and allow infectious agents to become established, leading to declining flock health and performance. Resistance to respiratory disease may be decreased and pathogenic bacteria can be significantly increased in the lungs, air sacs, and livers of birds exposed to ammonia because of damage that occurs to the tracheal cilia. In addition, growth and condemnation rate may be compromised in birds exposed to levels of ammonia exceeding 10 ppm. Built-up litter propagates higher in-house ammonia levels, which can adversely affect poultry health by making the birds more susceptible to respiratory diseases. Techniques to reduce ammonia levels and pathogenic microbes include changes in management practices and use of litter treatments.

Most litter treatments used in the broiler industry involve chemical reduction of litter pH so that bacteria associated with ammonia release are either inactivated, reduced in number, or both. Volatilization of ammonia has been attributed to microbial decomposition of nitrogenous compounds, principally uric acid, in poultry house litter. Once formed, free ammonia will be in one of two forms: as the uncharged form of NH_3 (ammonia) or the ammonium ion (NH_4), depending on litter pH. Ammonia volatilization remains low when litter pH is below 7.0, but can be substantial when above 8.0. Uric acid decomposition is most favored under alkaline (pH > 7) conditions. Uricase, the enzyme that catalyzes uric acid breakdown, has maximum activity at a pH of 9. As a result, uric acid breakdown decreases linearly for more acid than alkaline pH values. One principal ureolytic bacterium, *Bacillus pasteurii*, cannot grow at neutral pH, but thrives in litter above pH 8.5. Typically, litter pH in a broiler house ranges between 9-10. Gaseous emission of NH_3 can be inhibited if converted to NH_4^+ (ammonium); which can be accomplished by lowering litter pH. In general, an effective litter treatment results in the production of hydrogen ions (H^+) when it dissolves, and the hydrogen ions produced by this reaction will attach to ammonia to form ammonium, which further reacts with sulfate ions to form ammonium sulfate $(NH_4)_2SO_4$. Ammonium sulfate is a water-soluble fertilizer. As a result of these acid-based reactions, the amount of ammonia emitted from the litter will be reduced; which should increase the nitrogen (N) content of the litter.

The main goal in using a litter treatment is to effectively reduce ammonia emissions from poultry facilities, which will have a direct effect on improving litter management, nutrient enrichment, and reducing ammonia volatilization from poultry house litter. Unfortunately, most litter treatments are typically effective for only three to four weeks; whereas, broilers are housed for six or more weeks prior to slaughter. As a result, a series of experiments were conducted to evaluate six treatment regimens applied at three levels with a goal of quantifying the amount applied for its effectiveness in reducing ammonia volatilization as compared to an untreated control.

MATERIALS AND METHODS

Poultry Litter Treatment (PLT™), granulated aluminum sulfate (Al-Clear™) (GA), Poultry Guard™

(PG), and hydrated lime (HL), were applied at 24.4, 48.8, or 73.2 kg/100 m^2 (50, 100, or 150 lbs/1000 ft^2); a liquid acidified aluminum sulfate (A-7^{TM}) (LA), was applied at 81.4, 162.8, and 227.1 L/100m^2 (20, 40 or 60 gal/1000 ft^2); and concentrated sulfuric acid (98% H_2SO_4) (SA) was applied at 9.75, 19.50, and 29.26 kg/100m^2 (20, 40, or 60 lb/1000 ft^2) on new pine sawdust bedding and tested against a non-treated control (CON). In each experiment, 1120 commercial broiler chicks (Cobb X Ross) were obtained from a commercial hatchery and randomized with 70 birds placed in each of 16 pens (2.44 x 2.44 x 2.44 m; 8 x 8 x 8 ft). Birds were fed a corn-soybean meal starter (0.68 kg/bird; 22% CP, 3087 kcal/kg ME), grower (1.36 kg/bird; 20% CP, 3131 kcal/kg ME), finisher (1.81 kg/bird; 17.5% CP, 3197 kcal/kg ME) and withdrawal (c.a. 1.36 kg/bird; 16.5% CP, 3219 kcal/kg ME) to meet or exceed NRC (2) requirements. New pine shavings (54.42 kg; 120 lbs) were placed in each pen at the start of each experiment. Feed and water were provided *ad libitum* with 24 hr light. Birds and feed were weighed at 21, 42 and 49 d to determine growth and feed performance.

Litter and air quality samples were obtained initially and weekly through day 49. Ammonia measurements were conducted using a closed container of specified dimension (46 x 36 x 12 cm; 21 x 15.5 x 5 in) inverted over the litter bed and determined using a Drager CMS Analyzer equipped with a remote air sampling pump and appropriate ammonia sampling chip (0.2-5, 2-50, or 10-150 ppm). The tube from the sampling pump was located in the top center of the container. The sampling pump was evacuated (calibrated) for 60 seconds followed by a measurement period of up to 300 seconds. Most readings were usually achieved with 60 seconds following evacuation. Litter was collected weekly, starting the day prior to chick placement and continued through day 49. Collection was performed in each pen by using the grab sampling technique. Individual litter samples (3g) were mixed with 60 mL distilled water for pH measurement. Data was analyzed by analysis of variance using the General Linear Models procedure of the Statistical Analysis System (3). When significant ($P < 0.05$), means were separated by Tukey's HD multiple comparison procedure.

RESULTS AND DISCUSSION

There were no differences ($P > 0.05$) in growth performance in any experiment attributed to type or level of litter treatment. Initial litter pH was significantly lower ($P < 0.05$) for PLT, GA, PG, LA, and SA treated pens as compared to CON (ca 2.3 vs. 6.4) and was influenced by level of application. Results indicated that PLT, GA, and LA significantly ($P < 0.05$) reduced ammonia volatilization as compared to CON through day 42 at the intermediate and highest application rates. SA significantly ($P < 0.05$) reduced ammonia volatilization through day 35 at only the highest application rate as compared to CON. Although PG exhibited the ability to lower pH, it failed to elicit a significant ($P > 0.05$) reduction in ammonia. Conversely, HL elevated litter pH initially as compared to CON (12.8 vs. 6.3), but this effect disappeared after day 21. HL failed to support any reduction in ammonia volatilization. Litter analysis results did not indicate a significant ($P > 0.05$) increase in amount of nutrients retained due to treatment. Results indicate that PLT, GA, LA, and SA were capable of reducing ammonia volatilization during broiler production. Results show that higher levels of litter treatments can extend ammonia control and may contribute to improvements in bird health. In these trials, ammonia levels were often controlled at the intermediate and highest application levels for up to 42 days (starting with new pine shavings litter).

Originally, litter treatments were placed at a relatively low level (generally 50 lb/1000 ft^2) to give early ammonia control during the brooding period. More recently, higher levels have been suggested as the industry becomes more comfortable with performance benefits associated with improving air quality in the broiler house with litter treatment use. To maximize the effectiveness of any litter treatment, one must properly prepare and apply the litter treatment in addition to managing the house and litter. Prior to application of any litter treatment, the house needs to be de-caked or tilled. Afterwards, the litter treatment can be applied at the chosen level before birds are placed in the house. Spills or concentrated areas should be raked into the litter to prevent overexposure or consumption by the young birds. As with any litter treatment product, the rate selection for an individual's operation will be dependent on current management practices and needs based on such factors as ventilation control and litter moisture levels. Higher rates may be recommended when high ammonia conditions prevail.

Litter treatments have become a common means of improving the broiler house environment throughout much of the broiler industry. Although different litter treatments vary in their ability to control ammonia, each offers a unique set of characteristics that need to be considered in selecting the appropriate product to meet an individual's needs. The litter treatment that offers the best return on investment will depend on the user's ability to select the product that best meets application goals. It is likely that the use of these products will continue as growers manage reused litter to their best advantage.

REFERENCES

1. Carlile, F.S. Ammonia in Poultry Houses: A Literature Review. World's Poultry Science Journal. 40:99-113. 1984.

2. National Research Council (NRC). Nutrient Requirements of Poultry. 9th Rev. Edition. National Academy Press, Washington, DC. 1994.

3. SAS Institute. SAS/STAT User's Guide: Statistics, Version 6.12, SAS Institute, Inc., Cary, NC. 1997.

IMAPACT OF IN-HOUSE LITTER COMPOSTING ON COCCIDIOSIS, NECROTIC ENTERITIS, AND BROILER PERFORMANCE

Daniel A. Bautista[A], Jose Miguel Ruano[B], George W. Malone[B], Steve Fitz-Coy[C], Kathy Phillips[A], and Stephen L. Collier[A]

[A]University of Delaware-Lasher Laboratory, Carvel Research and Education Center, Georgetown, DE 19947
[B]University of Delaware, Department of Animal and Food Sciences, College of Agriculture and Natural Resources, Newark, DE
[C]Intervet/Schering-Plough Animal Health Millsboro, DE

INTRODUCTION

Coccidiosis remains one of the most expensive and common diseases in commercial broiler production. The disease can be mild or severe depending on the amount of ingested oocysts. The short direct life-cycle and high reproductive potential of coccidia in poultry often lead to severe outbreaks in the modern poultry house. Hence the need for young poultry to be on continuous medication with low-levels of anticoccidial drugs to reduce the infections to a low infection level (9). The protozoan parasites of the genus *Eimeria* multiply in the intestinal tract and cause tissue damage, with resulting interruption of feeding and digestive processes, dehydration, blood loss and increased susceptibility to other diseases. The tissue damage and changes in the intestinal tract function is a well-known predisposing factor leading to necrotic enteritis (NE). Studies have shown that coccidia species, such as *E. acervulina*, *E. maxima*, and *E. brunetti* are associated with NE (1,2,3,10).

Necrotic enteritis is an acute enterotoxemic condition of young chickens and turkeys, often associated with high sudden mortality for a short duration. The causative agent of necrotic enteritis is a gram positive, obligate, anaerobic spore-forming bacteria *Clostridium perfringens* (CP), with mortality ranging from 2 to 50%.

During the early years of NE control, in-feed antimicrobials were the preferred products for the control of NE. In recent years worldwide, there is pressure to remove or reduce the usage of in-feed antibiotics from poultry feeds. As a result, there has been a dramatic increase in the prevalence of necrotic enteritis in commercial poultry operations (10). Furthermore, the accumulation of cocci oocysts and CP in built-up old litter can place a heavier burden on anticoccidial and antimicrobial drugs, and cocci vaccines. In-house composting of litter has been shown to reduce a number of poultry pathogens and dramatically improve flock performance.

Recent studies indicated reduced incidence of necrotic enteritis (4) and gangrenous dermatitis (6). The goal of this study is to evaluate the effect of in-house litter composting conditions on coccidiosis and necrotic enteritis organisms in relation to the observed performance improvement through in-house litter composting.

MATERIALS AND METHODS

Inactivation of cocci oocysts and CP by composting temperature and gases. A mixture of broiler chicken-derived sporulated coccidia oocysts (*E. acervulina*, *E. maxima*, *E. tenella*, *E. mivati/mitis type*) suspended in potassium dichromate solution at 1 x 10^6 oocysts/mL was dispensed in six 14 mL polystyrene tubes containing 8 mL inoculum/tube. One set of tubes has the openings screw-capped and taped tightly then sealed with air-tight, heat-sealed plastic bags, and the other set's open ends covered with several layers of sterile gauze. The tubes are all taped around a wooden stick and placed in an upright position in different areas of the compost pile:

a) 1ft below the surface.

b) On the surface of the pile, covered by less than 1 inch litter.

c) Dirt floor along the sidewall of the broiler house at ambient summer conditions.

The tubes are placed on the day of the windrowing of the litter. Representative samples are taken out at three days and seven days respectively. The samples were gavaged into five-wk old SPF leghorns (n = 6). Each bird received 1 mL of the inoculum containing 1 x 10^6 oocysts. The birds were humanely euthanized by cervical dislocation and necropsied on day six dpi and evaluated by gross cocci lesion scoring by Johnson and Reid (5), oocyst counts, and histopathology. This experiment was repeated twice.

The effect of normal composting temperatures and ammonia levels on total *Clostridium perfringens* (CP) and spore counts (obtained by inactivating vegetative CP bacteria at 65°C/149°F for 30 min) was determined using separate water baths set at 130°F, 135°F, and 140°F to simulate common composting temperatures. Overnight CP broth cultures and spores are immersed in the water bath for 1, 3, 6, and 24 h and quantified using serial dilution and plating in Shahidi-Ferguson Perfringens (SFP) Agar. Ammonia concentrations of 2000 PPM was achieved in a covered litter compost pile at ambient temp (80°F). Tube samples with gauze caps were exposed to the ammonia for three and five days, and then plated into SFP Agar as described.

Inactivation of cocci oocysts in naturally contaminated litter. To approximate natural litter conditions, clean litter from bagged pine shavings were continuously contaminated with a mixture of broiler chicken-derived cocci oocysts (*E. acervulina*, *E. maxima*, *E. tenella*, *E. mivati/mitis type*). This is done using 150 seeder birds (five-wk old SPF leghorns) inoculated with 1 x 10^5 oocysts/mL via oral gavage. The birds were kept for four weeks to allow for enough cocci shedding in the litter to occur, then replaced with a new batch of inoculated birds. Three continuous batches of seeder birds were used. The litter was broken up, collected in a mound and mixed well by shovel. One half (control) was redistributed immediately in the original room in a floor pen four inches deep at ambient temperature and humidity to avoid inadvertent composting conditions. The other half of the pile was transferred to an adjacent cocci-free room with the same physical characteristics. This litter was piled up in a 3½ ft conical mound and allowed to go through the composting process for seven days, with the pile turned/mixed once at three days. Temperature (1ft. level) inside the compost pile surface was monitored at 4 h intervals up to day seven. The pile was broken up at the end of the composting period and was redistributed in a floor pen at four in. depth.

Experimental birds. Day-old straight-run commercial broiler chicks (n = 44 per group), were placed on the composted and uncomposted cocci-contaminated litter. An equal number of birds were placed on clean pine shavings without cocci (cocci-control group) to compare against the growth performance of birds placed in the other two groups. All the birds were raised up to six weeks of age. Average weekly body weights were taken. Necropsy and cocci evaluation was performed on dead birds. Representative birds (n = 6) were euthanized and examined for intestinal cocci lesions (Johnson and Reid method), oocyst counts, and intestinal segments were collected for histopathology evaluation on at 21 days of age. Freshly voided feces from the litter was collected and pooled for fecal flotation and quantification of cocci shedding.

RESULTS AND DISCUSSION

Inactivation of cocci oocysts and CP by composting temperature and gases. Internal windrow temperatures rose to 135°F in 48 h and peak to 150°F in 60 h, but typically, compost pile temperatures consistently peak at 140°F, depending on the litter moisture content (approx. 15 to 30%). Temperatures tend to decline gradually but elevate close to peak temperatures after the windrow is turned/aerated.

Birds inoculated with the cocci mixture placed one ft. underneath the compost pile (three and seven days, gauze capped and sealed tubes) were all negative for any detectable gross coccidiosis lesions, microscopic oocyst counts and histopathological lesions. In contrast, birds inoculated with cocci exposed to the surface of the litter windrow and dirt floor at ambient temperature, regardless of whether the tubes were sealed or gauze-capped, showed acute, moderate to severe lesions compatible with *E. acervulina*, *E. maxima*, *E. tenella*, and *E. mivati/mitis* type coccidiosis. This is accompanied by high counts of oocysts in the intestinal scrapings. The severity of cocci lesions are a function of the stage of the disease at necropsy. The results were consistent when the experiment was repeated. Exposure to composting temperatures alone (135° to 150°F) for at least three days can inactivate common poultry coccidia.

Inactivation of cocci oocysts in naturally contaminated litter. There was significantly higher mortality (6/30 birds) due to severe *E. maxima* and *E. tenella* coccidiosis and necrotic enteritis at days 17-28 of age in the uncomposted, cocci-contaminated litter (control group), compared to 1/30 birds on day 30 in the composted, cocci-contaminated litter (treatment group). The cocci and necrotic enteritis lesions were supported by high *E. maxima* and *E. tenella* oocyst counts and histopathology lesions. The control birds in

group 3 did not experience mortality until day 35. Six birds in this group died due to severe (+4) *E. tenella* lesions from day 35 to 42 (end of study), probably through cocci contamination by flies from the uncomposted litter group in the nearby separate room. It was speculated that flies could be responsible for the single mortality in the composted group, but putative premunity due to early low-level exposure to cocci oocysts may have prevented birds raised on composted cocci-contaminated litter group from developing severe acute cocci lesions compared to the immunologically naïve controls (see voided feces quantification data in the next paragraph below). The experiment was repeated (group 1 was composted again) with enhanced biosecurity to eliminate flies (regular pesticide fogging and fine mesh netting) in addition to the already established ban on stepping on the litter in the pens, and dedicated feed and equipment. The composted group and the control group did not have any cocci or necrotic enteritis-associated mortality up to the end of the study. The uncomposted litter group, on the other hand, had two cocci-related mortalities on day 25 (one bird with severe necrotic enteritis and high *E. maxima* cocci oocyst counts) and day 39 (one bird with severe bloody cecal contents and thickened lining (+4 *E. tenella* cecal lesions) with high (+4) counts of *E. tenella* oocysts.

Cocci lesions and microscopic scoring in live birds. Bird samples (6/6) grown on composted litter showed no gross cocci lesions and no oocysts in intestinal scrapings of the duodenum, jejunum and cecum. This was confirmed by histopathology results. Birds from the uncomposted litter showed (+2) moderate (1/6 *E. maxima*, 2/6 *E. tenella*) and severe (+3) gross cocci lesions (2/6 *E. maxima*, 3/6 *E. tenella*). Fecal flotation of pooled samples of freshly voided feces at day 21 revealed significant different between groups. There were no oocysts found in group 3. There is a very low (+1) oocyst count of *E. maxima* in group 2 compared to markedly higher (+3) counts of *E. maxima* and *E. tenella* oocysts, consistent with the cocci lesion and microscopic oocyst count scoring. The experiment was repeated again as mentioned in the previous paragraph. Birds from the control and the composted group (n = 10/group) are negative for any gross cocci lesions and microscopic counts. The uncomposted group had 2/10 birds with mild lesions (+1) associated with *E. maxima* and *E. tenella* respectively. The lesions are supported by low counts (+1) of *E. maxima* and *E. tenella* from scrapings taken from the respective areas of the lesion scores. At the end of the study (42 days of age), another cocci check was done on 15 birds per group. The control and the composted groups were negative for cocci lesions and oocysts. The uncomposted group on the other hand showed early cocci lesions scores: 4/15 (+1) and 3/15 (+2) *E. acervulina*-type lesions; and 4/15 (+1) *E. tenella* lesions. This is supported by the corresponding cocci oocyst counts: 1/15 (+1) and 2/15 (+2) *E. acervulina*-type; 5/15 (+1), 4/15 (+2), 7/15 (+3) *E. mivati* type; *E. maxima* lesions; 4/15 (+1), 5 (+2), 4/15 (+3) and 1/15 (+4) *E. tenella* oocyst counts. Fecal flotation and quantification of cocci (day 28) in voided feces showed *E. maxima* was being shed (5.4×10^5 per g feces) in the uncomposted litter group. This is compatible with the single mortality (necrotic enteritis) in this group on day 25 (see mortality). Fecal flotation to correlate with the cocci check at the end of the study was not done.

Effect on growth performance. There are no significant differences in body weights between the three groups from day of age to three weeks. However, the mean body weights of the birds raised on the uncomposted cocci-contaminated litter were significantly lower ($\alpha = 0.05$) than the birds in the composted cocci-contaminated group from weeks four to six (end of study). The mean body weight of the birds in the composted litter and the control group are not significantly different except at six weeks, where the mean weight of the composted group is significantly heavier ($\alpha = 0.05$) than birds in the controls and uncomposted litter group. This is a result of the controls inadvertently getting infected then subsequently succumbing to severe clinical signs of *E. tenella* coccidiosis starting at five wk. The mean weights of controls and the uncomposted litter groups which both experienced severe coccidiosis are not significantly different at wk six. The divergence in mean body weights is directly linked to the onset of above-mentioned clinical coccidiosis and concomitant necrotic enteritis. The results from the repeated experiment showed the same trend, without the previous experiment's cocci-initiated decreased weight gain at week six relative to the composted litter group (data not shown).

Both repeated experiments in contaminated litter showed that in-house composting of cocci-contaminated litter significant significantly reduced the incidence of clinical coccidiosis and necrotic enteritis and the amount of cocci oocysts being shed as evidenced in the oocyst counts and voided feces. Furthermore, the first experiment also suggested that reduced levels of cocci in the litter maybe responsible for beneficial premunity that alleviates cocci clinical signs on subsequent cocci infection, relative to a completely naïve flock. In a commercial farm setting, it is realistic to expect that most, but not all litter material is effectively removed, windrowed and composted. These can be reservoirs for cocci and other poultry pathogens. Be that as it may, the significant reduction of cocci levels in the litter as a result of composting may enhance the effectiveness of cocci vaccines and

anticoccidial medications by reducing the level of initial cocci field challenge. Reduction of cocci challenge will reduce the incidence of necrotic enteritis which is a common sequelae to coccidiosis.

Total *C. perfringens* counts (7.8 logs) were reduced by 2.4 logs in 1 h at 135°F and 140°F. There was a lesser degree of reduction (1.26 logs) at 130°F but showed comparable log reduction (2.35 logs) at 3 h, and similar log reduction at 6 h (2.65 logs) compared to 140°F (2.67 logs). Ammonia concentrations of 2000 PPM in a covered compost pile at ambient temp (80° to 85°F) did not decrease initial CP log counts. CP spore counts were not reduced by 130° to140°F temperatures and 2000 PPM ammonia levels indicating the durability of CP spores.

The reduced incidence of necrotic enteritis in a commercial broiler house using a tractor-attached aerator/windrowing equipment over conventional removal of caked litter between flocks have been documented (4). It is plausible, based on our findings, that this field observation can be explained by the reduction of coccidia oocyst levels (rather than CP spore levels) due to litter composting.

REFERENCES

1. Al-Sheikhly, F. and R.B. Truscott. The pathology of necrotic enteritis of chickens following infusion of broth cultures of *clostridium perfringens* in the duodenum. Avian Diseases, 21, 230-240. 1977.

2. Al-Sheikhly, F. and A. Al-Saieg. Role of coccidia in the occurance of necrotic enteritis of chickens. Avian Diseases, 24, 324-333. 1980.

3. Baba, E., A.L. Fuller, J.M. Gilbert, S.C. Thayer, and L.R. McDougald. Effect of *Eimeria brunetti* infection and dietary zinc on experimental induction of necrotic enteritis in broiler chickens. *Avian Dz* 36: 59-62. 1992.

4. Flory, G.A., R.W. Peer, B. Barlow, D. Hughes, G.W. Malone, and A.P. McElroy. Litter Reconditioning as an Alternative Litter Management Strategy within the Commercial Poultry Industry. www.deq.state.va.us/export/sites/default/vpa/pdf/Virginia_Litter_Reconditioning_Study.pdf

5. Johnson, J. and W.M. Reid. Anticoccidial drugs: Lesions scoring techniques in battery and floor-pen experiments with chickens. *Exp Parasitol* 28:30-36. 1970.

6. Malone, G.W. Management guidelines for In-house Composting. In 2008 *Delmarva Poultry Conference*, September 10, 2008. Ocean City, MD. http://www.mdchick.umd.edu/files/malonemanagement.pdf.

7. Macklin, K. In-House Windrow Composting. *In 2007 Delmarva Breeder, Hatchery, and Growout Conference*, September 12, 2007. Ocean City, MD. 2007. www.rec.udel.edu/Poultry/proceedings2007/Macklin_In-House%20Windrowing.pdf

8. McDevitt, R.M., J.D. Booker, T. Acamovic, and N.H.C. Sparks. Necrotic enteritis: A continuing challenge for poultry industry. *World's Poultry Science Journal*. 62: 221-247. 2006.

9. McDougald, L.R. and S.H. Fitz-Coy. Coccidiosis. *In Diseases of Poultry*.12th ed. p. 1070

10. Williams, R.B., R.N. Marshall, R.M. La Ragione, and J. Catchpole. Parasitol Res 90. 19-26. 2003.

PREVALENCE OF PARVOVIRUS INFECTION IN CHICKEN AND TURKEY FLOCKS IN THE UNITED STATES

Laszlo Zsak[A], Keith O. Strother, and J. Michael Day

Southeast Poultry Research Laboratory, USDA, Agricultural Research Service,
Athens, GA 30605
[A]Corresponding author, E-mail: laszlo.zsak@ars.usda.gov

SUMMARY

Enteric diseases of poultry cause significant economic losses because of decreased bird weight gain, increased morbidity and mortality and increased production costs from poor feed conversions and the cost of treatment. Pathogens involved in enteric disease include bacteria, protozoa and viruses. Currently, the role of these pathogens in the etiology of enteric disease of poultry is not completely understood.

Recently, we reported that using a random molecular screening method, novel parvoviruses were detected in intestinal samples of chickens and turkeys exhibiting characteristic signs of enteric disease (1). The technique is based on random amplification of particle-associated nucleic acids in clinical samples.

Using this method, we successfully identified parvoviruses in intestinal homogenates from affected birds and demonstrated that the chicken and turkey parvoviruses were closely related to each other and representative of a novel member of the *Parvoviridae* family.

Here, we describe the development and application of a conventional PCR assay to detect parvoviruses in commercial poultry flocks. In a nationwide survey a total of 138 field enteric samples from poultry flocks were tested for parvovirus presence by PCR. Of the tested chicken samples that were collected in 54 farms, 77% showed the presence of parvovirus while 78% of the turkey samples that were received from 29 farms were parvovirus positive. For the first time, our data clearly demonstrate that parvoviruses are widely distributed in commercial poultry flocks in the US. The high prevalence of parvovirus infection in young birds suggests a potential role of these viruses in the etiology of enteric disease of poultry.

Phylogenetic analyses comparing NS gene segments revealed a strong similarity between the chicken and turkey parvoviruses. It was also evident that most of the chicken and turkey parvovirus isolates formed distinct phylogenetic groups, suggesting that these viruses might have diverged from a common ancestor and subsequently went through a host specific adaptation.

(The full-length article will be published in *Avian Diseases*.)

ACKNOWLEDGMENTS

The authors thank Fenglan Li for her excellent technical assistance and the sequencing unit at SEPRL for outstanding support.

REFERENCES

1. Zsak, L., K.O. Strother, and J. Kisary. Partial genome sequence analysis of parvoviruses associated with enteric disease in poultry. Avian Pathol. 37:435-441. 2008.

ISOLATION AND DISTRIBUTION OF WEST NILE VIRUS IN EMBRYONATED CHICKEN EGGS

R. Crespo, H.L. Shivaprasad, and P.R. Woolcock

California Animal Health and Food Safety Laboratory System, University of California Davis, Fresno Branch, 2789 South Orange Avenue, Fresno, CA 93725, USA

Cell culture followed by indirect immuno-florescent antibody staining, reverse transcription-polymerase chain reaction (RT-PCR), or *in situ* enzyme immunoassay (EIA) are the only methods described in the literature for the isolation and identification of West Nile virus (WNV). RT-PCR directly from the tissue is the preferred method for WNV detection, because of the rapid turnaround time and lower biosafety risks. However, virus isolation may be requested as other viruses can be detected as well, including newly emerging viruses. Furthermore, many avian laboratories maintain a limited number of cell lines and most isolations are performed by inoculation of embryonated chicken eggs.

Eight psittacines (seven Rosellas, one Princess of Wales Parakeet) and one Red tail hawk submitted between 2004-08 to the California Animal Health and Food Safety Laboratory System were included in this study. The birds died suddenly or after a brief onset of loss of weight and occasionally neurologic signs. In one of the rosellas, Pacheco's disease was suspected. Histologically all birds had multifocal necrosis and lymphocytic infiltration in most organs, including heart, kidney, and liver. The red tail hawk also had non-suppurative perivascular cuffing of the brain.

Tissue pool consisting of heart, kidney and or liver alone were homogenized and inoculated into embryonating chicken eggs via the chorioallantoic membrane, chorioallantoic sac, and yolk sac. Virus particles in the range of 40 to 45 nm suggestive of flavivirus were identified from the allantoic fluid by negative staining electron microscopy, in all cases except in one Rosella. From this Rosella the liver inoculated into choriollantoic membrane was positive for WNV by RT-PCR. From all the cases, sections of embryo including various organs, their chorioallantoic membrane, and yolk sac were collected and processed for immunohistochemistry (IHC) for WNV. The tissues showing the most intense and widely distributed IHC staining was the chorioallantoic membrane (CAM). Furthermore, the CAM was more intensively stained if the first passage had been through the chorioallantoic

sac (CAS). No virus could be detected in the yolk sac by IHC. In the embryo, the WNV antigen was best detected in the muscles (both skeletal and smooth), followed by skin. Additionally, there were a few positive foci in the turbinates. WNV was not detected in the viscera of the embryo.

In conclusion, this study demonstrates that WNV may be isolated through inoculation of embryonated chicken egg embryos as occurs with other arboviruses. The best route of inoculation is CAS, and the virus can be best demonstrated by IHC in the CAM.

(The full-length article will be published in the *Journal of Veterinary Diagnostic Investigation*.)

ASSESSING THE FINANCIAL IMPACT OF DISEASES OF EGG PRODUCING FLOCKS

Simon M. Shane

Adjunct Professor, North Carolina State University, Durham, NC, 27713
Phone: (919) 806-8695, sshane@nc.rr.com

INTRODUCTION

The Proceedings of the 2008 U.S. Animal Health Association incorporate a report on the current disease status of egg-producing flocks. Based on a survey among members of the Association of Veterinarians in Egg Production, avian pathogenic *E. coli* (APEC) complex was cited as the most common condition impacting profitability. A structured approach is necessary to develop appropriate and cost-effective strategies to minimize the impact of disease and to implement nutritional and management programs which support optimal egg production, feed conversion, livability and product quality. The emergence of the APEC complex and specifically peritonitis can be used as a model to evaluate specific diseases and to calculate the cost-effectiveness of preventive approaches incorporating biosecurity and immunization.

Manifestations of APEC infection. Primary APEC peritonitis emerged among large in-line complexes in the Midwest during the mid 1990s. The condition can cause up to 15% losses in a flock after peak production. In addition flocks may show APEC peritonitis during molting and at the onset of the second cycle of production. Erosive mortality of under 2% over a few weeks is generally not diagnosed specifically as APEC peritonitis unless routine post-mortem examinations are performed. The pathogenesis of APEC peritonitis has not been determined but it is assumed that inhalation of dust contaminated with pathogenic bacteria results in introduction of the organism into the abdominal air sacs. Local infection extends bacteria to the adjacent peritoneal surfaces and the serosal membranes surrounding the intestines, liver and reproductive tract. This contributes to extensive peritonitis within a short time. During the acute phase of infection, mortality may increase from a normal rate of 0.1% per week to over 1.5% per week. Affected hens are usually well-fleshed indicating the rapid onset of infection and the development of acute septicemia which precedes peritonitis.

Secondary APEC airsacculitis occurs when susceptible flocks are exposed to respiratory infections including MG, ND, IB, LT and coryza in some areas. The severity of the primary infection is influenced by the effectiveness of the vaccination program and administration, immune status of the flock, ventilation, climatic stress and nutrition. Both pullets and mature flocks may show up to 10% mortality following an outbreak of a primary respiratory infection followed by secondary APEC airsacculitis.

Generally treatment of mature flocks with an antibiotic is unproductive especially if FDA Prudent Use Principles are applied in medication. Some success has been achieved in reducing mortality in both pullets and young hens by administering a mannanoligosaccharide feed supplement. Management changes which may ameliorate the clinical course of APEC infection include chlorination of drinking water to a level of 2 ppm, cessation of dust and cobweb removal using air blowers and rectifying deficiencies in ventilation.

Financial impact of APEC peritonitis. The financial losses associated with an episode of APEC peritonitis can be projected using realistic assumptions related to standard production and the mortality characteristic of field infection. Table 1 quantifies the assumptions applied to calculating losses in a caged flock. These include projections of egg production during the first and second cycles, standard mortality, nest-run average revenue of $1.00 per dozen and an

assumed production cost of $0.70 per dozen. In the specific example it is assumed that APEC mortality in the flock attains 5% by the 45th week of production. It is calculated that a flock of 100,000 hens would lose approximately five eggs per hen on average during the first cycle and seven eggs during the second cycle. Adjusting the contribution margin for feed not consumed by dead hens, the loss of one dozen eggs per hen spread over 100,000 hens started would amount to $27,000 over two cycles. If mortality due to APEC peritonitis occurred at the time of molting, the loss during a 30 week second cycle for 97,000 remaining hens would be $15,277 assuming mortality as shown in Table 2.

Infection of rearing pullets with APEC during the mid to second half of the growing cycle would result in cumulative mortality conservatively estimated at 2% due to airsacculitis. Assuming a pullet cost of $3.50, the loss for a flock of 100,000 chicks started would be $7,000. Generally flocks affected with respiratory disease and secondary APEC airsacculitis yields a proportion of pullets that are retarded in development. This may result in a delay in onset of production by as much as four weeks for up to 5% of the flock. This will reduce the production of affected hens by 12 eggs or the equivalent of 0.6 eggs per hen spread over the flock. This loss is calculated to be $1,500. If pullets which die during rearing cannot be replaced with surplus birds or available hens, the 2% mortality will result in a decrease in contribution margin as a result of transferring a flock with 2,000 fewer hens. The flock operator would carry the same fixed costs for the flock but would not bear the depreciation or cost of feed consumed. The loss of these hens would depress average flock yield by eight eggs on average over the total life of the flock representing a value of $18,366.

Determining the cost-effectiveness of vaccination. The value of effective vaccination can be determined in relation to a specific disease or complex by considering cost, protective efficiency and the magnitude of losses due to infection. In the specific case of APEC, alternatives include autogenous inactivated vaccine or modified live *E. coli* products. Inactivated vaccines are generally expensive to prepare, require individual administration by injection and have variable effectiveness given the spectrum of APEC strains to which flocks may be exposed. A modified live *E. coli* vaccine based on O78 strain *E. coli* modified to delete the *aroA* gene, necessary for metabolism of cyclic amino acids has been licensed by the USDA. Over the past two years, vaccines administered by the coarse spray route during the first three weeks, and then subsequently at 12 to 14 weeks of age have been shown to suppress APEC peritonitis and airsacculitis. Given an approximate cost of $10 per 1,000 doses an egg producer would invest $2,000 in vaccinating a flock of 100,000 pullets. If successive outbreaks of APEC peritonitis generate losses of $30,000 per flock, vaccination will provide a potential benefit to cost ratio of 15:1. Suppressing airsacculitis mortality in pullets, resulting in losses during rearing and during the subsequent laying cycle would provide a benefit to cost ratio of 8:1.

The bottom line. Given a structured evaluation of the impact of disease, environmental or nutritional factor on productivity of a flock, it is possible to evaluate alternative preventive or therapeutic strategies. Since factors specific to the operation in addition to industry costs and returns and macroeconomic factors influence cost and revenue at different times, an approach such as gross marginal analysis can be applied to evaluate strategies for treatment or prevention alone or in combination. Frequently it is necessary for a veterinarian to project benefit to cost ratios in order to convince a profit-accountable manager that a recommended expenditure on prevention is justified in anticipation of a disease challenge, given a reasonable estimate of the probability, costs of prevention and consequences of an infection.

Table 1. Assumptions applied to calculating losses from *E. coli* infection in caged flocks.

Assumptions Relating to Standard Caged Flock:

Achievable Egg Production*	1st Cycle	260 eggs20-65 weeks
	2nd Cycle	140 eggs70-100 weeks
Mortality*	1st Cycle	3%
	2nd Cycle	2%

ǂ Nest run average revenue	100c/dozen
Assumed production cost (45¢ feed + 25¢ other) =	70c/dozen
Contribution margin	30c/dozen

*Based on Breeder's Management guides for Leghorn hybrids
ǂ Based on prevailing industry costs & revenue

Table 2. Calculation of losses attributable to *E. coli* peritonitis.

Cost of *E. coli* Peritonitis Resulting in Mortality in 100,000 Hen Flock
5% mortality 45 weeks to 50 weeks

Total eggs lost per hen over entire flock — 1st Cycle = 5 eggs; 2nd Cycle = 7 eggs

Decreased margin allowing for feed adjustment for dead hens:
$0.27/dozen x 12/12 eggs x 100,000 = $27,000

Loss with occurrence due to stress of molting (70-100 weeks)
Eggs lost 7 in 2nd cycle
$0.27/dozen x 7/12 x 97,000 = $15,277

CONTROL OF AVIAN PATHOGENIC *E. COLI* IN COMMERCIAL POULTRY

D. Karunakaran, C. Kromm, G. Siragusa, and T. Rehberger

Agtech Products, Inc. Waukesha, WI

INTRODUCTION

Avian pathogenic *Escherichia coli* (APEC) comprise a specific subset of pathogenic *E. coli* that cause extraintestinal diseases of poultry (4). APEC consists mainly of enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) serovars (5). APEC is found in the intestinal microflora of healthy birds and infections are enhanced or initiated by secondary environmental and host predisposing factors. Colibacillosis is a common systemic infection caused by APEC, and occurs most commonly as acute septicemia or subacute airsacculitis and polyserositis in chickens, turkeys, and other avian species (6).

Recently, virulence factors associated with avian colibacillosis have been identified. These genes include the aerobactin iron sequestering system (*iuc*C), temperature-sensitive hemagglutinin (*tsh*), increased serum survival (*iss*), and the structural genes of the colicin ColV operon (*cva*C). The aerobactin iron

sequestering system allows bacteria to grow in low levels of iron (3) while the Tsh protein is an autotransporter that may contribute to the development of lesions within the air sacs of birds (4). The increased serum survival gene has been found to contribute to complement resistance of a human *E. coli* isolate and its presence is strongly correlated with *E. coli* isolated from birds with colibacillosis (7). The Col V plasmid codes for colicin V which causes membrane leakage in target cells (2).

The presence of these four genes identified through multiplex PCR can facilitate the identification of avian pathogenic *E. coli* isolates and when combined with randomly amplified polymorphic DNA (RAPD) PCR will be useful for examining the diversity of the avian pathogenic *E. coli*. This study reports the prevalence, distribution and diversity of APEC in commercial poultry. Understanding the distribution and diversity of APEC is the first step toward developing methods for controlling colibacillosis in poultry production.

MATERIALS AND METHODS

Three birds are collected from several commercial broilers and turkey flocks ranging from 17 to 77 days of age were collected. Whole intestinal tracts were removed, tied off at the esophageal and cecal ends, and immediately placed in whirl-pak bags containing enough sterile saline to cover the entire tract. Tracts were shipped overnight to Agtech Products, Inc. for analysis of pathogenic *E. coli*.

Three sections (upper, mid, and lower) were aseptically removed from each intestinal sample and pooled together for a composite sample for each bird. All three sections were rinsed with sterile phosphate buffer until all contents were washed out and the liquid ran clear. The sections were cut lengthwise to expose the epithelial lining and the sterile rinse was repeated. The three sections were weighed and combined in a sterile whirl-pak bag. Sterile 0.1% peptone water (99 mL) was added to each bag and the contents masticated for 60 seconds. All samples were plated on CHROMagar (CHROMagar Paris, France) for the enumeration of *E. coli*. Spiral plating techniques were used at 10^{-2} and 10^{-4} dilutions on the Autoplate 4000 (Spiral Biotech, Inc., Norwalk, MA). Plates were incubated at 37°C for 24 h before counting on the Q-Count system (Spiral Biotech). The counts were recorded and the plates with *E. coli* colonies were saved for further analysis.

Five colonies were picked from each sample unless there were less than five present. Colonies were grown in Trypticase Soy Broth (TSB) at 37°C for 24 h. DNA was isolated using a High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany). A multiplex PCR procedure (8) was used to determine the virulence genes present in each isolate. The PCR mixture contained: 5µL 10X buffer without $MgCl_2$, 2 µL 10mM dNTP mix, 0.3 µL 5U/µL Platinum Taq Polymerase (Invitrogen, Carlsbad, CA), 4 µL 50mM $MgCl_2$, 0.5 µL *iss* upper and lower primer, 0.3µL *tsh*, *iuc*C, *cva*C upper and lower primers, 31 µL sterile dH_2O, and 5 µL genomic DNA to give a final volume of 50 µL. Amplification for the multiplex analysis was performed in a GeneAmp PCR System Thermal Cycler (Applied Biosystems, Foster City, CA). The PCR program began with a pre-incubation of 95°C for 5 min.; then nine cycles of: 95°C for 59 s, 55°C for 30 s, 72°C for 59 s; 28 cycles of: 94°C for 30 s, 55°C for 30 s, 72°C for 30 s; and finished with a seven min. incubation at 72°C. PCR products were identified by electrophoresis on a 3% Nu-Sieve agarose gel (BioWhittaker, Rockland, ME) and visualized by UV transillumination after staining in ethidium bromide solution. Gel images were captured using the Syngene BioImaging System.

Images from the RAPD PCR gels were incorporated into a dendrogram using the Bionumerics software program (Applied Maths, Belgium). The molecular weights of the banding patterns were analyzed and both primers used band matching at 2.00% tolerance. Dice comparisons were created for each primer separately before composing a composite data set. Both primers were weighted equally and by combining the Dice comparisons a composite dendrogram was created. *E. coli* was enumerated using a spiral plating technique on CHROMagar and counted on the Q-Count system.

A multiplex PCR procedure was used to determine the pathogenicity of the *E. coli* isolates. In this procedure, four virulence genes were identified as markers for pathogenicity. A positive control was used to indicate where the four genes migrated on the gel. The gels were analyzed visually for bands at the appropriate molecular weight.

As stated by Skyberg, *et al*. (8), the possession of two or more of these genes is a strong predictor of virulence. While the use of a chick embryo lethality assay may be the best way to determine virulence of avian *E. coli*, a multiplex procedure could allow for rapid screening of possible pathogenic *E. coli*.

RAPD PCR is performed to examine the diversity between the *E. coli* isolates. The dendrogram constructed from the RAPD PCR analysis shows the relative relatedness of the isolates to one another. The virulence genes present for each isolate along with the ages of the birds are included on the dendrogram to show the distribution of the pathogenic *E. coli*. A line is drawn at an 80% similarity coefficient results in clusters or family groups.

CONCLUSIONS AND APPLICATION

- All commercial poultry farms show varying prevalence levels of *E. coli* within sites.
- Subclinical levels of APEC compromise bird health and performance.
- The molecular tools discussed help understand the diversity of APEC and the distribution of virulence factors.
- *E. coli* isolates with two or more virulence factors indicating a possibility of being pathogenic isolates.
- Application of Direct Fed Microbial products in reducing APEC levels will be discussed.

REFERENCES

1. Altekruse, S.F., F. Elvinger, C. DebRoy, F.W. Pierson, J.D. Eifert, and N. Sriranganathan. Pathogenic and fecal *Escherichia coli* strains from turkeys in a commercial operation. Avian Diseases 46:562-569. 2002.
2. Brock, T.D., M.T. Madigan, J.M. Martinko, J. Palmer. Biology of microorganisms. Seventh Edition. pp 264-265. 1994.
3. Dho-Moulin, M., and J.M. Fairbrother. Avian Pathogenic *Escherchia coli* (APEC). Vet. Res. 30:299-316. 1999.
4. Dozois, C.M., M. Dho-Moulin, A. Bree, J.M. Fairbrother, C. Desautels, and R. Curtiss III. Relationship between the Tsh autotransporter and pathogenicity of avian *Escherichia coli* and localization and analysis of the *tsh* genetic region. Infection and Immunity. 68:4145-4154. 2000.
5. La Ragione, R.M., A.R. Sayers, and M.J. Woodward. The role of fimbriae and flagella in the colonization, invasion and persistence of *Escherchia coli* 078:K80 in the day-old-chick model. Epidemiol. Infect. 124:352-363. 2000.
6. Lee, M.D. and L.H. Arp. Colibacillosis. A laboratory manual for the isolation and identification of avian pathogens. Fourth Edition. pp 14-16. 1998.
7. Pfaff-McDonough, S.J., S.M. Horne, C.W. Giddings, J.O. Ebert, C. Doetkott, M.H. Smith, and L.K. Nolan. Complement resistance-related traits among *Escherchia coli* isolates from apparently healthy birds and birds with colibacillosis. Avian Diseases. 44:23-33. 2000.
8. Skyberg, J.A., S.M. Horne, C.W. Giddings, C. Doetkott, R.E. Wooley, P.S. Gibbs, and L.K. Nolan. Development of a multiplex PCR protocol to discern virulent from avirulent avian *Escherchia coli*. American Association of Avian Pathologists and American Veterinary Medical Association Meeting, Nashville, Tennessee. Poster 14, p 40. 2002.

CROSSPROTECTION STUDY OF A MODIFIED LIVE *E. COLI* VACCINE AGAINST THREE HETEROLOGOUS APEC SEROTYPES IN COMMERCIAL BROILER CHICKENS

Kalen Cookson[A], Steve Davis[B], and Lisa Nolan[C]

[A]Fort Dodge Animal Health, Overland Park, Kansas
[B]Colorado Quality Research, Wellington, Colorado
[C]Dept. of Vet Microbiology & Preventive Medicine, Iowa State University

INTRODUCTION

E. coli induced chronic respiratory disease is a leading cause of broiler mortality and condemnations. Recent *E. coli* field surveys have revealed two interesting findings: 1) there is tremendous diversity in O serotypes within even a limited geographic region (4) and 2) most *E. coli* isolates coming from diseased flocks contain a preponderance of virulence genes, as measured by PCR analysis, while commensal fecal isolates tend to contain very few (4,5). We have reported previously on a live *E. coli* vaccine's ability to protect commercial broilers against different O78 avian pathogenic *E. coli*, or APEC (1,3). Because of the diversity of APEC serotypes in the field, this study was conducted to measure that live *E. coli* vaccine's ability to cross-protect against three of the more common, heterologous (non-O78) APEC isolates.

MATERIALS AND METHODS

A pilot challenge study was first performed using ten broiler APEC isolates from a total of six different serotypes (2). All ten APECs caused significant levels of airsacculitis (30 to 83%) and varied in their ability to cause mortality (0 to 50%). Three of these isolates (#1, #9 and #8) were chosen for this study based on their serotypes (O1, O2 and O18, respectively) and their ability to cause a high incidence of respiratory disease.

At day of hatch 1,280 commercial, straight-run broilers were divided equally into two treatments. All birds were coarse sprayed with a modified live Newcastle/infectious bronchitis vaccine while half also received Poulvac® E. coli. After 30 minutes the two vaccine treatments (No Vax and D1 Vax) were allocated to a total of 64 pens of 20 birds each. Birds were raised on wood shavings and fed a typical broiler ration *ad libitum* throughout the course of the study. At 18 days of age, half of each of the day one treatments were vaccinated by coarse spray with Poulvac E. coli, resulting in four *E. coli* vaccine treatment groups: 1) No Vax, 2) D18, 3) D1 and 4) D1&18. At 42 days of age all four vaccine treatments were pared down equally to 18 birds per pen and further divided into four different challenge groups: a) No challenge, b) APEC O1, c) APEC O2 and d) APEC O18. Challenge inocula were administered intratracheally (IT) at a dose of 2.0, 2.0 and 2.1 x 10^7 CFU/bird, respectively. Dead and moribund birds were removed daily until termination of the study at 47 days, when all birds were weighed and necropsied. Lesions of colibacillosis were recorded and airsacculitis was scored using the following scale: 0) none, 1) mild suds, 2) moderate suds or multifocal exudate and 3) heavy suds or severe, profuse exudate.

RESULTS

Control Groups. None of the unchallenged vaccine treatments suffered from mortality from 42-47 days of age (see Table) and they had similar, low levels of airsacculitis (rate of 4.2-9.9% and mean scores ranging from 0.25 to 0.43). This was considered within normal limits considering the time of year of the study (March). In contrast, the unvaccinated challenge controls had mortality ranging from 0.0 to 13.9% and significant levels of airsacculitis (rate of 80.6-93.1% and mean scores ranging from 2.17 to 2.54). Similar to the pilot challenge study, APECs O1 and O2 were similar in virulence while the O18 isolate was capable of causing significantly higher mortality (13.9%).

Vaccination/Challenge Groups. Except against the O18 challenge in the Day 18 vaccine treatment, all other vaccine treatments had significantly lower mean airsac lesion scores than the challenge controls. In fact, the Day 1&18 treatment mean lesions after O1 and O2 challenge were not different from the negative controls. All vaccine treatment groups had a significantly lower percentage of challenged birds showing airsacculitis. Again, the Day 1&18 treatment saw the greatest reductions, with no significant differences versus the negative controls against O1 and O2 challenge. None of the vaccine treatments was able to significantly reduce mortality from the O18 challenge; however, the rate in the Day 1&18 group was low enough that it was not statistically different from the negative controls.

DISCUSSION

Recent field surveys of diseased broiler flocks have supported previous findings of Lisa Nolan and her associates (4,5). While there is tremendous diversity and no apparent pattern in O serotypes within a given geographic location, a common thread is that the vast majority of these isolates are not commensal, avian fecal *E. coli* (AFEC) but isolates loaded with virulence genes and capable of causing a high level of disease and mortality. Because Poulvac E. coli types as an O78 serotype, the goal of this study was to see if this vaccine would also protect against heterologous *E. coli* serotypes. In case the standard broiler application (day of age, coarse spray) would not provide adequate cross-protection, two other vaccination regimens were employed. As it turned out, the order of protection in the day-of-age treatments was comparable to protection levels seen against O78 APECs (1,3).

While all three vaccination strategies gave significant protection against the three serotypes tested, there were at least subtle differences between them. The two-time vaccination program resulted in numerically greater reductions in airsac lesion scores over the Day 1 strategy, regardless of the challenge isolate, and was significantly more successful than Day 18 vaccination. The Day 18 vaccination program performed about as well as the Day 1 program in this (late-challenge) model, but it seems plausible that there may be advantages to hatchery vaccination—in uniformity of application and immunizing chicks prior to a potentially early field exposure—in a real-life setting. The additional level of reductions in the two-time vaccination group, especially against mortality from the more virulent O18 isolate, suggests that certain high-challenge situations might further benefit from a booster vaccination.

REFERENCES

1. Cookson, K. and S. Davis. *E. coli* challenge study in commercial broilers by either respiratory or skin route of exposure and the effect of prior vaccination with a live attenuated (aro-A) *E. coli*.

Abstract 4457. 144th AVMA Annual Convention, Washington, D.C. July 2007.

2. Cookson, K., S. Davis and L. Nolan. Comparison of ten avian pathogenic E. coli strains in commercial broiler chickens. Proceedings of the 57th Western Poultry Disease Conference. Puerto Vallarta, Mexico. pp 180-182. April 2008.

3. Cookson, K., K. Macklin and J. Giambrone. The efficacy of a novel live E. coli vaccine using a broiler skin challenge model. Abstract 1568. Proceedings of the 23rd World's Poultry Congress. Brisbane, Australia. July 2008.

4. Cookson, K., L. Nolan and C. Gustafson. The characterization of several avian pathogenic E. coli (APEC) strains from commercial broilers using PCR analysis of key virulence genotypes. Abstract 6027. 145th AVMA Annual Convention, New Orleans, La. July 2008.

5. Rodriguez-Siek, K.E., C.W. Giddings, C. Doetkott, T.J. Johnson, and L.K. Nolan. Characterizing the APEC pathotype. Vet Res. 36: 241-256. 2005.

Table 1. Summary of mortality and *E. coli* lesions related to each APEC challenge isolate.

***E. coli* Vaccine Treatment**	**APEC Challenge Treatment**	**Mean Airsac Lesion Score**		**% Airsacculitis >1**		**% Mortality**	
		Value	Statistic *	Value	Statistic	Value	Statistic
None	None	0.43	A	6.94	A	0.00	A
	O1	2.17	F	80.56	E	0.00	A
	O2	2.20	F	82.86	E	5.71	ABC
	O18	2.54	F	93.06	E	13.89	C
Day 18	None	0.31	A	9.86	A	0.00	A
	O1	1.32	CDE	44.44	BCD	0.00	A
	O2	1.57	DE	51.39	D	1.39	AB
	O18	1.68	EF	54.17	D	9.72	BC
Day 1	None	0.31	A	4.23	A	0.00	A
	O1	1.17	BCDE	40.28	BCD	0.00	A
	O2	1.31	CDE	46.48	CD	0.00	A
	O18	1.23	CDE	42.25	BCD	9.86	BC
Day 1&18	None	0.25	A	7.04	A	0.00	A
	O1	0.66	AB	23.29	AB	1.37	AB
	O2	0.80	ABC	26.76	ABC	2.82	AB
	O18	1.04	BCD	36.11	BCD	5.56	ABC

*Groups sharing a letter are not statistically different, based on Tukey's test (HSD; $P < 0.05$).

EXPERIMENTAL DUAL CHALLENGE WITH *ORNITHOBACTERIUM RHINOTRACHEALE* AND *MYCOPLASMA SYNOVIAE* IN BROILERS

R. Cerdá[A,B], J. Uriarte[A], J. Origlia[A], and M. Petruccelli[A]

[A]Laboratory of Diagnosis of Avian and Piliferou Diseases, Faculty of Veterinary Medicine, National University of La Plata, CC296, 1900, La Plata, Argentina
[B]ECO Animal Health, London, UK

SUMMARY

Ornithobacterium rhinotracheale (ORT) and *Mycoplasma synoviae* (MS) have been recognized as primary respiratory pathogens in broiler chickens. However, their possible pathogenic synergism has not been demonstrated using a dual infection study in broiler chickens. In the present study, three week old broiler chickens were inoculated either with a standard ORT strain (intratracheally), a MS field strain (aerosol) or both pathogens at the same time. At seven and 14 days post challenge, chickens from each group were euthanatized and analyzed for thoracic and abdominal air sac lesions, lung and trachea lesions. Significant differences in lesion scores were observed in the ORT/MS group compared to either the uninoculated, control group or the MS group. Infections were confirmed by strain re-isolation, PCR, and serology. This is the first report of an ORT/MS dual infection study in chickens.

RESUMEN

Desafío dual experimental de *Ornithobacterium rhinotracheale* y *Mycoplasma synoviae* en pollos.

Ornithobacterium rhinotracheale (ORT) y *Mycoplasma synoviae* (MS) han sido reconocidos como patógenos respiratorios primarios en pollos. Sin embargo, su sinergismo patogénico no ha sido aun demostrado experimentalmente en este tipo de aves. En el presente estudio, pollos de tres semanas fueron desafiados ya sea con una cepa estándar de ORT (vía intratraqueal), una cepa de campo de MS (vía aerosol), o ambos agentes conjuntamente. A los 7 y 14 días postinoculación se sacrificaron pollos de cada grupo para la evaluación de lesiones en sacos aéreos torácicos y abdominales así como también de lesiones en pulmón y tráquea. Se observaron diferencias significativas en el score de lesiones entre el grupo ORT/MS y el grupo control sin inocular como así también con el grupo desafiado con MS. La confirmación de las infecciones experimentales se llevaron a cabo mediante reaislamiento de los agentes descargados, PCR y pruebas serológicas. Este es el primer reporte de una infección dual experimental de ORT y MS en pollos.

INTRODUCTION

Ornithobacterium rhinotracheale (ORT) and *Mycoplasma synoviae* (MS) have been recognized as primary respiratory pathogens in broiler chickens (6). Both bacterial species have a worldwide distribution and are associated with respiratory disease and high economic losses (3,4). Despite the known multi-factorial etiology of the respiratory complex in poultry, only a few experimental trials have been done to establish possible interactions between bacteria and viruses with ORT (1). Apart from one field report regarding dual ORT and MS infection in turkeys (7), there are no reports on natural or experimental ORT and MS interactions in broiler chickens. The present study was undertaken to evaluate the possible synergy between ORT and MS in broiler chickens.

MATERIALS AND METHODS

Experimental animals. Forty commercial Ross broiler chicks, free of mycoplasma and *Salmonella*, were hatched and later housed in isolators. Food and water was available *ad libitum*.

***Ornithobacterium rhinotracheale* inoculum.** The ORT challenge inoculum was prepared from strain ATCC51463. The bacterium was cultured in 5% sheep blood agar media supplemented with 10 ug/mL gentamicin in a 5% CO_2 atmosphere. After 48 h incubation, ten bacterial colonies were transferred into 5 mL of PBS and a challenge inoculum containing 10^9 colony forming units (CFU)/mL was prepared.

***Mycoplasma synoviae* inoculum.** The MS strain used was a recent field isolate from an outbreak of infectious synovitis in a laying hen farm in Buenos Aires province, Argentina. The strain was cloned and prepared using Frey (Gibco) broth supplemented with 12% pig serum and had an organism density of 10^8 color changing units/mL (CCU/mL).

Experimental design. Forty, one-day old, chicks were randomly allocated to four experimental groups

such that each group contained 10 chicks. At two weeks of age the birds were shown to be free of maternally- derived antibodies to ORT and MS using a commercial enzyme-linked immunosorbent assay (ELISA)(IDEXX) and a rapid serum agglutination test (RSA) (Intervet Schering Plough, The Netherlands), respectively. At this time all the chickens received a ND-IB (La Sota-Massachusetts) vaccination via eye drop. At three weeks of age the different groups received the following treatments: (ORT/MS) challenge with ORT and MS cultures, (MS) MS culture, (ORT) ORT culture and an uninoculated control group.

Lesion scores. Postmortem gross lesions were scored at seven and 14 days post-inoculation (PI) using five chickens from each group. Thoracic and abdominal air sacs, lung and trachea lesions were scored. Air sacs were examined and given a score according to the amount of cheesy exudate contained within the air sacs as follows: 0 = no visible exudate; 1 = 25% or less of the air sac contained exudate; 2 = 25 to 50% of the air sac contained exudate; 3 = more than 50% of the air sac contained exudate. The maximum air sac score per bird was 6; lungs, 0 = no abnormalities, 1 = unilateral pneumonia, 2 = bilateral pneumonia. The maximum lung score per bird was 2; trachea, 0 = no abnormalities, 1 = some exudate in the tracheal lumen, 2 = lumen of the trachea filled with exudate. The maximum trachea score per bird was 2.

Confirmation of infection. A pool of tracheal swabs, for re-isolation of bacteria and PCR, in addition to blood samples for serological tests (RSA for MS and ELISA for ORT), were taken at the end of the trial in order to try to confirm the infection by ORT and MS.

Statistical analysis. The statistical analysis of the lesion scores was done using the Kruskal-Wallis one-way analysis of variance.

RESULTS

The lesion score results are shown in the table. Significant differences in score lesions ($P < 0.05$) were observed between group ORT/MS compared to group MS in air sac and lung lesions at seven days PI and between group ORT/MS compared to the uninoculated control group in all the organs evaluated at both times of necropsy. Infections were confirmed by bacterial re-isolation (100% for MS and 30% for ORT), PCR (100% for both agents) and serology (100% for both agents).

DISCUSSION

Although no interactions were observed between ORT and MS in turkeys under field conditions (7), a high synergism between both pathogens has been seen using the experimental conditions of the present study, in broilers. The use of the ND-IB vaccine and the pathogenicity of the MS field strain could be reasons for the results. The Argentinean MS field strain used has shown to be highly pathogenic for boilers (2). Further studies should be carried with different MS strains and different management conditions in order to determine possible results, according to the different scenarios, in the field.

REFERENCES

1. Marien, M., A. Decostere, A. Martel, K. Chiers, R. Froyman, and H. Nauwynck. Synergy between avian pneumovirus and *Ornithobacterium rhinotracheale* in turkeys. Avian Pathol. 34 (3), 204-211. 2005.

2. Meghan, M., S. Kleven, and D. Brown. Sialidase Activity in *Mycoplasma synoviae*. Avian Dis. 51:829–833. 2007.

3. Kleven, S. *Mycoplasma synoviae* infection. In: Diseases of Poultry (Calnek, B. W., Ed.) 10th edition, Iowa State University Press, Ames, Iowa, pp. 220–225. 1997.

4. van Empel, P. and H. Hafez. *Ornithobacterium rhinotracheale*: a review. Avian Pathol. 28. 217-227. 1999.

5. van Veen, L. Country report on The Netherlands. In: Aerosols, Newsl. World Vet. Poult. Assoc. p. 12. 1999.

6. van Veen, L., P. van Empel, and T. Fabri. *Ornithobacterium rhinotracheale*, a primary pathogen in broilers. Avian Dis. 44. 896-900. 2000.

7. Zorman-Rojs, O., I. Zdovc, D. Bencina, and I. Mrzel. Infection of Turkeys with *Ornithobacterium rhinotracheale* and *Mycoplasma synoviae*. Avian Dis. 44:1017-1022. 2000.

ACKNOWLEDGMENTS

We thank Adrian Mocket from ECO Animal Health for critical reading and comments on the manuscript. We also thank Andrew Pridmore for supplying the ORT strain and Fernando Marino and Daniel Gornatti for laboratory assistance. This study was supported by ECO Animal Health, UK.

Table 1: Postmortem lesion scores after seven and 14 days post-inoculation of ORT and MS.

Group	7 days PI			14 days PI		
	Air sacs	Lung	Trachea	Air sacs	Lung	Trachea
ORT/MS	5.4 (90.0)[b]	1.8 (90)[b]	1.8 (90)[b]	3.6 (60.0)[b]	1.4 (70)[b]	1.4 (70)[b]
MS	2 (33.3)[a]	0.2 (10)[a]	1 (50)[ab]	2.4 (40.0)[ab]	0.4 (20)[ab]	1.2 (60)[b]
ORT	2.2 (36.7)[ab]	0.6 (30)[ab]	0.8 (40)[ab]	2.2 (36.7)[ab]	0.6 (30)[ab]	1 (50)[ab]
Uninoculated	0.2 (3.3)[a]	0.2 (10)[a]	0.2 (10)[a]	0.4 (6.7)[a]	0.2 (10)[a]	0.2 (10)[a]

Scores are given as the maximum possible lesion scores in the group (percentage in brackets). Within columns, values with different lowercase superscripts are significantly different ($P < 0.05$).

APPLICATION OF *MYCOPLASMA SYNOVIAE* VACCINE (MS-H) IN LAYERS

Teruaki Ouchi[A], Yasuhisa Munakata[B], and Hiroshi Sakamoto[A]

[A]Nippon Biologicals, Inc., Kita Aoyama 1-5-12/5F, Minato-ku, Tokyo 107-0061 Japan
[B]Nippon Zenyaku Kogyo Co., Ltd., 1-1 Tairanoue, Sasagawa, Asaka-machi, Koriyama, Fukushima 963-0196 Japan

Significant advances in the control of mycoplasmosis have been realized since the introduction of attenuated live MG vaccines (e.g., ts-11). Until recently, the disease induced by *Mycoplasma synoviae* (MS) has remained problematic due to the lack of an effective vaccine. Additionally, MS may be contributory to other disease syndromes. Recent studies have shown that MS, aside from causing production losses in its own right, it can also be a significant factor in initiating *E. coli* peritonitis, a major cause of mortality in layers. At the 2004 American Association of Avian Pathologists meeting, Dr. Kenton Kreager reported that field evidence suggested synergy between the two pathogens to be a major problem for US layer flocks (1). Furthermore, an experimental study has supported the postulation that a virulent MS strain to be a primary factor in this syndrome (2).

NBI Technology Committee conducted two field studies in large multiple-age commercial layer farms to determine whether MS live, MS-H strain vaccination could cost-effectively increase production and decrease mortality (3).

Layer flocks vaccinated with MS-H showed significant improvements compared to non-vaccinated flocks previously placed on the same farm. In the field studies, reduced eggshell top cone abnormalities from 2 to 4% to 0% was seen in flocks vaccinated with MS-H. This is an important observation since MS is suspected to cause eggshell top cone abnormalities (4). In the first study, the cumulative egg weight per hen was 795 g greater at 57 wks. In the second study, the cumulative egg weight average was 787 g greater at 50 wks. Additionally, there was a reduction of mortality from *E. coli* peritonitis. From these results, NBI Technology Committee concluded MS-H vaccination to be effective and economic.

FIELD STUDY 1

MS-vaccinated group. 82,000 Hy-Line Gray layers (four subgroups placed March-October 2006)

Control group. 184,000 Hy-Line Gray layers (Nine subgroups placed 2000 to 2005)

***Vaccination programs**. Identical for both groups, except the MS-H live vaccine administration on day 31 via eye drop to layers in MS-H vaccinated group.

1 (d)	Marek's
7	IB (H120) + ND (VG/GA)
15 & 26	IBD
31	MG (ts 11) + IB (H120) + ND (VG/GA) **+[MS-H]***
50	Pox +ILT
63	ND (VG/GA) + IB (C-78)
70	AE
83	ND/IB2/Coryza (A&C) + SE

Historically, pullets on this farm were grown MS free for 120 days before being transferred to the laying house. By 150 days of age almost 100% of birds tested MS positive by serum agglutination testing. However, almost 100% of pullets vaccinated with MS-H tested

MS positive by six wks post vaccination. This is an expected result from the MS-H vaccination.

RESULTS

Comparison 1. Table 1 compares the **MS-H vaccinated group,** consisting of four subgroups placed into production March - Oct. 2006 to the Control group (all nine subgroups placed 2000 to 2005).

MS-H vaccinated group: Significant Improvements.

1) Age at 50% egg production was 4.7 days earlier.
2) Peak egg production rate was 1.5% higher.
3) HD egg production rate was 6.4, 2.8 and 2.2% greater for Stage 1, 2 and 3, respectively.
4) HH egg production rate was 6.5, 3.7 and 2.9% greater for Stage 1, 2 and 3, respectively.
5) Mortality (compared with the control group):
Stage 1) 0.042%/wk lower (0.68%/15 wks),
Stage 2) 0.029% lower (0.44%/15 wks),
Stage 3) no significant difference.
6) Cumulative egg wt. at 57 wks was 795 g greater
7) Overall FCR was 0.13% better.
8) Feed intake: Stage 1) 2.3 g greater,
Stage 2) 3.3 g less,
Stage 3) 3.6 g less.

Comparison 2. Table 2 compares MS-H vaccinated group to the Control group consisting only the most recently placed subgroups (the four subgroups placed 2004 to 2005) to minimize impact of differences of time and conditions.

MS-H vaccinated group: Significant Positive Results.

1) The time to 50% egg production was 9 days earlier.
2) Peak egg production rate was 1.6% higher.
3) HD egg production rate was 9.9, 2.1 and 1.4% greater for Stage 1, 2 and 3, respectively.
4) HH egg production rate was 9.8, 2.7 and 1.8% greater for Stage 1, 2 and 3, respectively.
5) Mortality (compared with the control group):
Stage 1) 0.036%/wk lower (0.57%/15 wks),
Stage 2) 0.013% lower (0.2%/15 wks),
Stage 3) 0.033% lower (0.5%/7 wks).

Data analysis suggests the lower mortality is attributable to fewer cases of E. coli peritonitis.

6) Cumulative egg wt at 57 wks was 787 g greater
7) FCR was 0.1% lower during Stage 1.
The overall difference was 0.07% (not significant).
8) Feed intake: Stage 1) 5.6 g greater, Stage 2) no difference, and Stage 3) 3.5 g less.

Comparison 3. Table 3 provides data related to forced molting**.** The mean time for forced molting in the MS-H vaccinated group was 5.2 wks later than that in the Control group (all subgroups combined 2000 to 2005) and 3.8 wks later than that in the most recently placed Control group (four subgroups 2004 to 2005). Even though forced molting in the MS-H vaccinated group was four wks later, the egg production was similar at the time of molting for both groups. The MS-H vaccinated group sustained a longer period of good egg production.

SUMMARY

The MS-H vaccinated layers tended to increase egg weight rapidly during the early egg production stage, while reducing it during the late egg production stage due to improved laying persistency. The benefits of the vaccine are as follows:

1) Prevents delay of egg production
2) Decreases mortality
3) Improves egg production rate
4) Improves laying persistency
5) Reduces under-grade eggs in the late production
6) Increases cumulative egg production
7) Improves FCR

ECONOMIC BENEFITS

HH cumulative egg production is considered the best indicator of the economic benefit gained from use of the vaccine. The HH cumulative egg production out to 57 wks was approximately 800 g (13 eggs) greater in the vaccine group, which is estimated to represent a net profit of about 100 yen ($0.94)*. This is a conservative estimate. This extra income comfortably covers the cost of the MS-H vaccinations. Feed conversion ratio was better in the MS-H vaccinated group by approximately 7%, compared to all nine flocks of Control group. This savings amounts to a 120-yen ($1.13)* reduction in the cost of feed per hen (assuming that the hens are fed up to 78 wks).

FIELD STUDY 2

MS-H vaccinated group. 315,000 Lohmann (Julia-LSL)
(nine subgroups) placed April 2006 to Feb. 2007.

Control group. 245,000 Lohmann (Julia-LSL) layers

(seven subgroups) placed prior to the above period.

Vaccination programs. are identical to Field Study 1.

The study was conducted at a farm that historically maintains an egg production rate greater than 90% for approximately 20 wks, followed by a period of relatively good and stable egg production. Pullets are raised MS-free but within 30 days after transfer to layer house, all hens became sero-positive for MS. The farmer suspected that MS was negatively influencing the duration of the 90% egg-production rate and increasing the mortality.

RESULTS

MS-H vaccinated layers showed significant improvements compared to MS non-vaccinated layers. When performance comparisons were made, the flocks vaccinated with MS-H laid ≥ 90% rate for approximately 10 wks longer. By 50 wks, this resulted in a 5% increase in egg production (additional seven eggs per hen) compared with the non-vaccinated flocks. (Data available on request.) The other benefits gained from administration of MS-H vaccine were similar to results of the Field Study 1.

CONCLUSION

In these two large-scale field studies, flocks vaccinated with the MS-H consistently performed better than previously placed, non-vaccinated flocks. The actual benefits of using MS-H vaccine in other commercial settings in the future might vary from farm to farm, depending upon such factors as management practices, concurrent disease, and severity of wild MS field challenge. However, these current studies provide strong evidence that MS-H vaccine will easily prove to be economically justified in today's competitive layer industry.

REFERENCES

1. Kenton Kreager. Symposium on Emerging & Re-emerging Diseases. AAAP meeting, July 25, 2004, Philadelphia, PA. 2004.
2. Ziv Raviv, N. Ferguson-Noel, V. Laidinis, R Wooten, and S.H. Kleven. Role of *Mycoplasma synoviae* in commercial layer *Escherichia coli* peritonitis syndrome. Avian Diseases 51:685-690, 2007.
3. MS-H: Vaxsafe® MS (strain MS-H)*, Bioproperties, Australia
4. Ferberwee A., J. de Wit, and W. Landman. *Mycoplasma synoviae* associated eggshell apex abnormalities. The 15th Congress & Exhibition of the World Veterinary Poultry Association, Sept. 10-15, 2007: Beijing China, pg. 234. 2007.

* Exchange rate: 2005: 1 USD=106 Yen

** Registered in Australia, Mexico, Japan, S. Africa, Columbia, Brazil, and currently pending registration USA.

Table 1. MS-H vaccinated group compared to control group (all nine subgroups 2000-2005).

Difference (I–N)			**+2.2**	**+2.9**	**–**	**+2.6%**	**+0.795 kg**	**–0.2 g**	**–3.6 g**	**–0.11**

Table 2. MS-H vaccinated group compared control group (four subgroups most recently placed).

(Nov. 29, 2007, T. Ouchi)	Age at 50% Egg Prod.	Peak Egg Prod. (%)	HD Egg Prod. (%)	HH Egg Prod. (%)	Mortality%/wk	Rate of Normal Eggs (%)	Cumulative Egg Weight (kg/hen)	Mean Egg Weight (g)	Feed Intake (g/hen/day)	FCR
Stage 1 (21-35 wks)										
MS-H vaccinated	148.8	94.7	85.5	84.8	0.098	97.3	5.225	57.7	102.1	1.91
Control group (N)	157.8	93.1	75.6	74.9	0.134	97.8	4.650	57.3	96.4	2.01
Difference (I–N)	**–9.0**	**+1.6**	**+9.9**	**+9.8**	**–**	**–0.5%**	**+.575 kg**	**+0.5 g**	**+5.6 g**	**–0.10**
Stage 2 (36-50 wks)										
MS-H vaccinated			87.5	85.4	0.163	97.7	10.913	63.4	104.4	1.88
Control group (N)			85.4	82.7	0.175	97.6	10.150	63.7	104.5	1.93
Difference (I–N)			**+2.1**	**+2.7**	**–**	**+0.1%**	**+.763 kg**	**–0.3 g**	**–0.2 g**	**–0.04**
Stage 3 (51-57 wks)										
MS-H vaccinated			80.8	76.7	0.256	98.5	13.362	64.3	105.9	2.04
Control group (N)			79.4	74.8	0.289	95.2	12.575	64.8	109.4	2.12
Difference (I–N)			**+1.4**	**+1.8**	**–**	**+3.3%**	**+0.787 kg**	**–0.5 g**	**–3.5 g**	**–0.08**

Table 3: Forced Molting

(Dec 2, 2007, T. Ouchi)	Subgroup No.	Age at Forced Molting (wks)	Egg Production Rate at Forced molting (%)	Mean Egg Prod. Rate Before Forced molting (%) **	Substandard Eggs (%)*
Control group No vaccine	00531	65	78.7	86.1	0.9
	20907	56	79.6	83.4	1.5
	30405	57	76.6	81.8	1.9
	30619	62	77.4	82.5	3.7
	30827	60	74.2	83.3	2.6
	40610	58	75.5	82.5	2.7
	41030	66	78.7	84.7	6.0
	50323	62	75.3	82.5	10.0
	50604	64	78.3	85.0	12.9
Mean (2000-2005)		**61.1**	**77.1**	**83.5**	**4.7**
Mean (2004-2005)		**62.5**	**77.0**	**83.7**	**7.9**
MS-H vaccinated Group	60321	64	72.3	83.0	2.4
	60531	67	79.4	86.7	2.3
	60812	68	79	86.3	2.7
	61021	(not molted)	Not included in molting data comparisons		
Mean (March-Oct 2006)		**66.3**	**76.9**	**85.3**	**2.5**

* % are taken before grade and packaging.

** Average egg production rate from 50% of flock producing eggs until just before molting.

A STUDY ON PRODUCTION STATUS OF *MYCOPLASMA GALLISEPTICUM*-FREE AND MG-INFECTED COMMERCIAL LAYER FLOCKS IN TABRIZ REGION OF IRAN

A. Azizpour[A], M.H. Bozorgmehri Fard[B], S. Charkhkar[C], N. Shikhi[C], A. Feizi[D], A .Zakeri[E], and S. Farashi Bonab[F]

[A]Post Graduate Student of Poultry Diseases, Faculty of Specialized Veterinary Sciences, Islamic Azad University, Science and Research Campus, Tehran and Member of Young Researchers Club (Ardabil branch), Islamic Azad University, Ardabil branch, Ardabil, Iran
[B]Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[C]Department of Clinical Sciences, Faculty of Specialized Veterinary Sciences, Islamic Azad University, Science and Research Campus, Tehran, Iran
[D]Department of Clinical Sciences, Faculty of Veterinary Medicine, Islamic Azad University, Tabriz Branch, Tabriz, Iran
[E]Department of Animal Sciences, Agriculture Faculty, Islamic Azad University, Tabriz branch, Tabriz, Iran
[F]Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
Corresponding author's email: Aidin_azizpour@yahoo.com

ABSTRACT

Mycoplasma gallisepticum (MG) is the most pathogenic and economically significant mycoplasma of poultry and has a worldwide distribution. The aim of this study was to determine the effects of MG on performance of commercial layer flocks in Tabriz, one-day old, Hyline W-36-strain eight female flocks of commercial layer chickens from a breeding company. Four MG-free flocks and four MG-infected flocks were compared for production factors during rearing and production periods. The production factors data were analyzed by using analysis of variance (one-way ANOVA models) and in cases of significant difference, Tukey' test was used. In rearing period, livability and body weight at the age of 17 weeks in MG-infected flocks were significantly lower than MG-free flocks ($P < 0.05$). The most total feed intake in rearing period was in MG-infected group; however, difference between this group and MG-Free group wasn't

significant. At production period (18-80 weeks), livability, hen housed egg production to 80 weeks, and peak production in MG-infected group were less than MG-Free group; whereas total feed intake was higher in MG-infected group; however, these factors weren't significantly different between MG-infected and MG-Free groups. According to the results of this research peak production of MG-infected group was 4.73% less than MG-Free group, and MG-infected group produced 8.5 eggs per hen fewer than MG-free group in the laying cycle. This investigation indicated that overall, MG-infected group had lower economic performance.

INTRODUCTION

Avian mycoplasmosis is an important disease in poultry industry of many countries and causing economic loss (1,6). The disease can be caused by several species of genus *Mycoplasma* (class Mollicutes, order Mycoplasmatales, family Mycoplasmataceae), such as *Mycoplasma gallisepticum*, *Mycoplasma iowae*, *Mycoplasma meleagridis*, and *Mycoplasma synoviae* (1,7,14). It mainly occurs in chickens and turkeys but many other domestic and wild birds may be infected (10,15). Mycoplasmas are transmitted both horizontally, through infectious aerosols, contaminated feed and water, close contact between birds, and vertically in laid eggs (transovarian transmission), and can be remain in the flock constantly as subclinical form (5,10,14). *M. gallisepticum* (MG) is the most important mycoplasmal pathogen in poultry and is responsible for significant economic losses in the poultry industry worldwide (3,5). This organism is the etiologic agent of chronic respiratory disease (CRD) in chickens and infectious sinusitis in turkeys. Clinical symptoms of MG infections in these avian species include rales, coughing, nasal discharge, sinusitis, and severe air sac lesions (6,10,14). The mortality rate is low unless a secondary microorganism infection is present (6,11). The aim of this study was to determine the effects of MG on performance of commercial layer flocks during the rearing and production periods in Tabriz.

MATERIALS AND METHODS

Flocks. For this study, one-day old Hyline W-36-strain eight female flocks of commercial layer chickens from a breeding company in Tabriz-Iran were chosen. Four flocks were MG-free (capacity overall 40000) and four flocks were infected with MG (capacity overall 40000). The flocks were reared in nearly the same conditions and were compared for production factors during rearing and production periods from February 2006 to November 2007.

Preparation of sera samples. On day two, 20 chicks were randomly chosen per flock and blood samples were obtained aseptically from the selected birds. The blood was allowed to clot and kept for 1-2 h at room temperature. After clotting, sera were separated, centrifuged at 1500 g for 6 min, poured into sterile vials, individually labeled, and stored at 4ºC.

Serum plate agglutination (SPA) test. The SPA test was carried out with crystal violet stained MG antigen (Nobilis®, Intervet International, Holland) for detection of MG antibodies in collected sera to determine the infection. Briefly, 20 µL of antigen and 20 µL of chicken sera were mixed and followed by gentle rocking. Results were read within two min. In positive cases granules formed slowly which was seen during rocking, but in negative case no such granules formed within two min. All serum samples were first tested at a dilution of 1:2. Subsequently, positive samples were serially diluted from 1:4 to 1:32 in saline and re-tested. Serum samples that showed agglutination at a dilution of 1:16 or higher were considered positive. The flock was considered MG-infected if 10% or more serum samples were positive in SPA test (4,7,14).

Measurements. Performance factors that were investigated in the MG-infected and MG-free flocks during growing period (1-17 weeks) included livability (%), body weight at 17 weeks (g), and total feed intake (kg). During the production period (18-80weeks), livability (%), total feed intake (kg), hen housed egg production to 80 weeks, and peak production (%) were evaluated.

Statistical analysis. The data of production factors were analyzed by using analysis of variance (one-way ANOVA models) and in cases of significant difference, Tukey' test was used. Results are expressed as mean±SD and differences were considered significant at $P < 0.05$.

RESULTS

The results of production factors analysis of MG-free and MG-infected flocks are shown in Table 1. At the rearing period, livability and body weight at the age of 17 weeks in MG-infected flocks were significantly lower than MG-free flocks ($P < 0.05$). The most total feed intake in rearing period was in MG-infected group; however, difference between this group and MG-free group wasn't significant. At production period (18-80 weeks), livability, hen housed egg production to 80 weeks, and peak production in MG-infected group were less than MG-free group; whereas total feed intake was higher in MG-infected group; however, these factors weren't significantly different between MG-infected and MG-free groups.

DISCUSSION

Due to the substantial performance and production losses, MG has been described as the most economically important pathogenic *Mycoplasma* species affecting poultry (3,6). Production losses between 10 and 20% have been reported in layers and broiler breeder chicken flocks infected with MG (1). The annual economic impact of MG infections in the United States was estimated at between $118 and $150 million for the layer industry alone in 1994 (12). The economic losses is due to prevention and treatment costs, mortality, decreases egg production and hatchability, and reduced feed conversion efficiency and weight gain (2,3,6,10,13,15). In a study, layers maintained free from infection with MG laid on the average 15.7 more eggs/hen housed than the layers infected with MG (2). In another study using data collected from 366 commercial layer flocks in California, an MG-infected flock produced 12 and five fewer eggs per hen than an uninfected flock during first and second cycles, respectively (9). Similar to previous studies, this investigation indicated that MG-infected group in general had lower economic performance. Livability and body weight at the age of 17 weeks in MG-infected flocks were significantly lower than MG-free flocks ($P < 0.05$). Peak production of MG-infected group was 4.73% less than MG-free group, and MG-infected group produced 8.5 eggs per hen fewer than MG-free group in a laying cycle, which was about 60 weeks in duration. Based on this information we can assume that MG infection in 1000 hens might causes 8500 fewer eggs production costing about $625. It is necessary to mention that this calculation of egg production is without the livability of birds on laying point.

REFERENCES

1. Bradbury, J.M. Avian Mycoplasmosis. In: Frank Jordan *et al*. (eds) Poultry Diseases. 5th edn. W.B. Sanders Company, Lowa. pp: 178-193. 2001.

2. Carpenter, T.R., E.T. Mallinson, K.F. Miller, R.F. Gentry, and L.D. Schwartz. Vaccination with F stirian *Mycoplasma gallisepticum* to reduce production losses in layer chickens. Avian Dis 25: 404-409. 1981.

3. Evans, J.D., S.A. Leigh, S.L. Branton, S.D. Collier, G.T. Pharr, and S.M.D. Bearson. *Mycoplasma gallisepticum*: Current and Developing Means to Control the Avian Pathogen. J. Appl. Poult. Res 14, 757–763. 2005.

4. Feberwee, A., D.R. Mekkes, J.J. De Wit, E.G. Hartman, and A. Pijpers. Comparison of culture, PCR and different serologic tests for detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infections. Avian Dis 49, 260-268. 2005.

5. Feberwee, A., D.R. Mekkes, D. Klinkenberg, J.C.M. Vernooij, A.L.J. Gielkens, and J.A. Stegeman. An experimental model to quantify horizontal transmission of *Mycoplasma gallisepticum*. Avian Pathology 34 (4), 355-361. 2005.

6. Levisohn, S. and S.H. Kleven. Avian mycoplasmosis. Rev Sci Tech 19 (2): 425-442. 2000.

7. Lierz, M., N. Hagen, D. Lueschow, and H.M. Hafez. Use of polymerase chain reactions to detect *Mycoplasma gallisepticum*, *Mycoplasma imitans*, *Mycoplasma iowae*, *Mycoplasma meleagridis* and *Mycoplasma synoviae* in birds of prey. Avian Pathology 37(5), 471-476. 2008.

8. Mushi, E.Z., M.G. Binta, R.G. Chabao, M. Mathaio, and R.T. Ndebele. Detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* antibodies in the sera of indigenous chicken by Rapid Plate agglutination test at Mnopane, Gabornone, Botswana, Onderstepeort. J. Vet. Res 66: 333-334. 1999.

9. Mohammad, H.O., T.E. Carpenter, and R. Yamamoto. Economic impact of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in commercial layer flocks. Avian Dis 31 (3): 478-482. 1987.

10. Nascimento, E.R., V.L.A. Pereira, M.G.F. Nascimento, and M.L. Barreto. Avian Mycoplasmosis Update. Brazilian Journal of Poultry Science 7 (1), 1-9. 2005.

11. Pakpinyo, S., P. Pitayachamrat, S. Saccavadit, T. Santaswang, A. Tawatsin, and J. Sasipreeyajan. Laboratory Diagnosis of *Mycoplasma gallisepticum* (MG) Infection in Experimental Layer Chicken Receiving MG Vaccines and MG Organisms. TJVM 36(2): 29-37.

12. Patterson, P.H. Coping with *Mycoplasma gallisepticum*. Internews 7:1–3. 1994.

13. Roussan, D.A., E.A. Abu-Basha, and R.R. Haddad. Control of *Mycoplasma gallisepticum* Infection in Commercial Broiler Breeder Chicken Flocks Using Tilmicosin (Provitil Powder®) Oral Formulation. International Journal of Poultry Science 5 (10): 949-954. 2006.

14. Ley, D.H. *Mycoplasma Gallisepticum* Infection. In:Saif, Y.M. *et al*. (eds). Disease of poultry 12th edn. Blackwel Publishing Company. Iowa State Press University, Chapter 21, pp: 807-833. 2008.

15. Sarkar, S.K., M.B. Rahman, M. Rahman, K.M.R. Amin, M.F.R. Khan, and M.M. Rahman. Sero-Prevalence of *Mycoplasma gallisepticum* Infection of Chickens in Model Breeder Poultry Farms of Bangladesh. International Journal of Poultry Science 4 (1): 32-35. 2005.

Table 1. Performance factors of MG-free and MG-infected groups during rearing period (1-17 weeks) and production period (18-80 weeks).

Rearing period				
Groups	Livability (%)	Body weight at the age of 17 weeks (g)	Total feed intake (kg)	
MG-Free	97.18 ± 0.17a*	1190 ± 0.002 a	5.40 ± 0.12	
MG-infected	96.30 ± 0.18b	1180 ± 0.006 b	5.54 ± 0.18	
Production period				
Groups	Livability (%)	Total feed intake (kg)	Hen housed egg to 80 weeks	Peak production (%)
MG-Free	92.12 ± 1.54	42.78 ± 0.80	313.75 ± 6.74	89.25 ± 2.20
MG-infected	90.30 ± 1.50	44.80 ± 1.86	305.25 ± 4.10	84.52 ± 3.92

*a.b: Means having different superscript in a column differ significantly ($P < 0.05$).

EFFECTIVE CALORIC VALUE OF BIRD HEALTH AND MANAGEMENT

R. G. Teeter[A], A. Beker[A], C. Brown[A], C.Broussard[B], S.Fitz-Coy[B], J.Radu[B], and L. Newman[B]

[A]Department of Animal Science, Oklahoma State University, Stillwater, OK, USA
[B]Schering-Plough Animal Health, Summit, NJ
Phone: 405-880-1170; Fax: 405-744-7390; E-mail: teeterbob1@hotmail.com

ABSTRACT

Experiments were conducted to quantify the impact of coccidiosis upon dietary effective caloric value (ECV) and other energetic costs in broilers. Five 6 - day regions spaced throughout a 48 day growth curve were examined. Challenge consisted of an oral dose of sterile saline or a mixture of three *Eimeria* species administered as oocysts at 14, 21, 28, 35, and 42 days. Variables examined, six days post challenge, included gross and microscopic lesion scores, live weight gain and gain composition, FE and heat production (Kcal/h). Lesion scores for coccidiosis non-challenged (CNC) birds did not differ from zero ($P > 0.10$) throughout the testing period. Coccidiosis challenged (CC) scores were inversely correlated ($P < 0.01$) with live weight, weight gain, FE, energy consumption and retained energy. Effective caloric value declined markedly as lesion score increased and especially so as birds matured. Indeed the lesion score 1 ECV fell from a high of 3200 ± 4, matching the dietary MEn value with no lesion score, to just 3075, 2719 and 2520 at 20, 34 and 48 days, respectively. The ECV consequence of even low level lesion scores far exceeds husbandry considerations as lighting program and feed form. In summary results indicate that coccidiosis consequence in growing broilers is age dependent with deleterious impact being more pronounced in immunologically naïve birds late in the growth curve.

INTRODUCTION

Feeding equal quantities of MEn energy as carbohydrate, protein and lipid can result in marked differences in actual energy gain. This occurs as the heat increment associated with substrate utilization varies with the substrate type and metabolic fate. Numerous nonnutritive factors have also been documented to impact bird performance. For example, managerial issues as ventilation, stocking density, lighting program and feed processing have received

considerable study for calorific impact upon broiler production. As a result, the metabolizable energy system may lead to unanticipated varying cellular energy/nutrient ratios. This is especially true when the aforementioned factors are coupled with coccidiosis. Coccidiosis is well known to adversely impact production and increase feed costs to achieve desired weights. The amount of dietary ME_n available to promote BWT and FCR has been defined as the effective caloric value (ECV; McKinney and Teeter, 2004) of dietary ME_n. Consequently, ECV transforms performance variability into dietary energy costs. The objective of the following study was to quantify the impact of coccidiosis upon ECV and measures of bird energy metabolism.

MATERIALS AND METHODS

Experiments using 1,200 Cobb X Cobb broilers were conducted to quantify coccidiosis impact upon bird energy balance. Five 6 - day regions, spaced throughout a 48 day growth curve, were examined. General bird management in floor pens and metabolic chambers as well as dietary ration specifications and ECV calculations have been previously described (1, 2). Challenge consisted of an oral dose of sterile saline or a mixture of three *Eimeria* species as E. *maxima*, *E. acervulina*, and *E. tenella* initially at 20,000; 50,000; and 30,000 oocysts per bird and increasing to 55,000; 105,000; and 50,000 oocysts per bird, respectively at 42 days to mimic production environments. Challenges were administered at 14, 21, 28, 35, and 42 days. Variables examined six days post challenge included gross and microscopic lesion scores live weight, FE, (upper small intestine: USI; mid small intestine: MSI; ceca: C), and microscopic lesion scores (*E. maxima*; *E. tenella*; and *E. acervulina*) with scores as 0 = none and 4 = high. Bird heat production (Kcal/h) was measured continuously by indirect calorimetry and body composition via x-ray analysis. Effective caloric value was estimated according to McKinney and Teeter, 2004.

RESULTS AND DISCUSSION

Study results are displayed in Table 1 for three of the five evaluation intervals. As the 21 and 35 day data were generally intermediate to the 14, 28 and 42 day results, they were not shown to conserve writing space. Coccidiosis challenge adversely impacted bird live weights, feed efficiency and MEn consumption. Reduced MEn consumption itself would be expected to worsen feed efficiency as maintenance energy costs become a higher percentage of energy consumption. However, with coccidiosis the maintenance energy expenditure itself increased significantly. In this study the determination of bird heat production and protein and lipid gain were determined enabling quantification of maintenance energy cost of all treatments as Kcal/day (2,7). Data indicated that bird maintenance cost increases with bird size, classically so for CNC birds, while the cost for CC birds increased linearly with lesion score. If CC birds exhibited higher body weights, then elevated maintenance would be expected. But, the live weight and FCR of CC birds was reduced ($P < .01$). Indeed, the elevated maintenance cost occurred with birds consuming less energy. Calories lost in excreta, measured by energy balance, further exacerbate coccidiosis consequence. In this study, CNC birds were within 95% of complete energy accounting throughout the growth curve. This deviation averaged 12% for score 2 birds on day 20 while exceeding 26% for the lesion score 2 birds at 48 days. As a result, with coccidiosis the combination of lowered appetite coupled with elevated maintenance and increased calories lost in excreta markedly impact bird performance.

Clearly, the combined actions of CC on bird appetite, maintenance and excreta energy loss impact bird performance. Effective caloric value expresses the combined impact of these variables as a dietary equivalence that would be needed to achieve the same action. Converting mash ration to 100% pellets has been valued at +187 Kcal/kg diet and lighting programs to +115 Kcal/kg ration. Conversely, since rations are not altered, the ECV provides an expression of ration caloric density loss due coccidiosis. In the reported study, the ECV of CNC birds differed little from the calculated energy value adding credence to the methodology. In contrast the ECV was sharply impacted by coccidiosis, falling as much as 71% (-2282 Kcal/kg ration) for lesion score 2 birds at 48 days while score 2 birds at 20 days only declined 18% (-601 Kcal/kg ration) 1 Lesion score 1 birds at 20 days declined just 4% (-121 Kcal/kg ration) while the score 1 bird ECV at 48 days declined over 21% (-685 Kcal/kg ration). The combination of lowered energy consumption coupled with elevated maintenance cost and excreta energy loss, makes the coccidiosis challenge critical to avoid. If some coccidiosis challenge is to occur, the data strongly suggests that early exposure will have less overall consequence to energy utilization.

ACKNOWLEDGEMENTS

The author wishes to thank Cobb-Vantress, Inc. Siloam Springs, AR, USA for research support and Schering Plough for their research and travel support.

This work has been approved for publication by the Director, Oklahoma Agricultural Experiment Station, Stillwater, OK 74087, USA.

REFERENCES

1. Belay, T., and R.G. Teeter. Virginiamycin effects on performance and salable carcass of broilers. J. Appl. Poult. Res. 3:111-116. 1994.

2. Brown, C. 2007. Evaluation of coccivac-B® and sacox 60® for control of 3 strains of *Eimeria* in broilers. MS. diss., Oklahoma State University, 2007.

3. McKinney, L.J., and R.G. Teeter. Predicting effective caloric value of nonnutritive factors: I. Pellet quality and II. Prediction of consequential formulation dead zones. Poult. Sci. 2004.

Table 1. Coccidiosis mediated lesion score effects upon production and energetic criteria at standardized weights[1].

Variable		Lesion Score		
Average Daily Gain		0	1	2
(Age, days)	Initial Live Wt. (g)			
14-20,	904	76.5	60.6	40.7
28-34	2096	92.6	54.3	27.3
42-48	3398	97.3	32.7	-7.0
Gain/Feed				
14-20,	904	0.64	0.60	0.38
28-34	2096	0.54	0.37	-0.04
42-48	3398	0.43	0.10	-0.49
ME_n Consumption/Day (Kcal)				
14-20,	904	386	342	300
28-34	2096	562	477	420
42-48	3398	701	570	482
Maintenance Cost (Kcal/Day)				
14-20,	904	124	151	281
28-34	2096	187	218	308
42-48	3398	281	308	315
Added Excreta (Kcal/Day)				
14-20,	904	16	22	35
28-34	2096	24	57	86
42-48	3398	38	94	130
Retained Energy (Kcal/Day)				
14-20,	904	188	149	100
28-34	2096	274	162	87
42-48	3398	305	110	-0.9
Effective Caloric Value (Kcal/kg feed)				
14-20,	904	3202	3075	2601
28-34	2096	3214	2719	2502
42-48	3398	3205	2520	923

[1]Values created using predictive models ($R^2 > 0.95$) and standardized initial weights.
[2]Homogenous arrays of lesion scores were applied.

ORGANIC ACIDS AND ESSENTIAL OILS: A VIABLE ALTERNATIVE TO ANTIBIOTIC GROWTH PROMOTERS IN POULTRY PRODUCTION

R. Gauthier

Jefo Nutrition Inc., 5020 Avenue Jefo, St-Hyacinthe, Quebec, Canada, J2S 7B6
rgauthier@jefo.ca

SUMMARY

A better understanding of the mode of action of organic acids and essential oils on bacteria and the discovery of a strong synergy between those two families of compounds, coupled with an appropriate processing technology adapted to the anatomy and physiology of poultry, has given rise to a new and more efficacious alternative to antibiotic growth promoters. In order to evaluate the real efficacy of this strategy, *in vitro* and *in vivo* experiments were conducted showing that by applying the microencapsulation technology, the dosage of both organic acids and essential oils could be reduced substantially.

INTRODUCTION

In the late 80s and early 90s, strong regulatory actions have removed most of the antibiotic growth promoters from the European Union market; the last ones have been withdrawn in January 2006. The adjustments following the withdrawal of these products in animal production have been difficult at times, and many replacement solutions have been proposed by the feed additive industry. It is not easy to replace products that have proven to be generally efficacious for the last 50 years. A consensus seems to develop among the scientific community concerned by this subject (9); and one approach is definitely standing out for its efficacy, technological, and economical feasibility: the organic acids. Another option is, under the generic name of "botanicals", essential oils (plant extracts or related compounds).

ORGANIC ACIDS

Organic acids have been used successfully in pig production for more than 30 years and continue to be the alternative of choice. Even if much less work has been done in poultry (1), we can now confirm that the organic acids are very efficacious provided their use is adapted to the physiology and anatomy of poultry. Organic acids (C1-C7) are widely distributed in nature as normal constituents of plants or animal tissues. They are also formed through microbial fermentation of carbohydrates mainly in the large intestine (6). They are also found in their sodium, potassium, or calcium form. Over the years, it was thought that a pH reduction of the gastrointestinal tract content was the mode of action; research has proven differently. The key basic principle on the mode of action of organic acids on bacteria is that non-dissociated (non-ionized) organic acids can penetrate the bacterial cell wall and disrupt the normal physiology of certain types of bacteria that are called "pH sensitive" meaning that they cannot tolerate a wide internal and external pH gradient. Among those bacteria we have *E. coli*, *Salmonella* spp., *C. perfringens*, *Listeria monocytogenes*, and *Campylobacter* spp.

Upon passive diffusion of organic acids into the bacteria where the pH is near of above neutrality, the acids will dissociate and lower the bacteria internal pH, triggering mechanisms that will impair or stop the growth of bacteria. On the other hand, the anionic part of the organic acids that cannot escape the bacteria in its dissociated form will accumulate within the bacteria and disrupt many metabolic functions and lead to osmotic pressure increase, incompatible with the survival of the bacteria. It has been well demonstrated that the state of the organic acids (undissociated or dissociated) is extremely important to define their capacity to inhibit the growth of bacteria. As a general rule, we need more than ten to one hundred times the level of dissociated acids to reach the same inhibition capacity of bacteria, compared to undissociated acids (8).

Too often, *in vitro* assays showing the antibacterial capacity of organic acids are done at a low pH, to avoid the dissociation of the acids. At a pH below 3.0 to 3.5, almost all organic acids are very efficacious in controlling bacteria growth. This does not reflect at all what is happening in the gastrointestinal tract of poultry. Logically, organic acids added to feeds, should be protected to avoid their dissociation in the crop and in the intestine (high pH segments) and reach far into the gastrointestinal tract, where the bulk of the bacteria population is located. More likely, the organic acids in poultry might play a direct role on the intestinal bacteria population, reducing the level of some pathogenic bacteria (ex. *C.*

perfringens) and mainly controlling the population of certain types of bacteria that compete with the birds for nutrients (3).

ESSENTIAL OILS

Essential oils are any of a class of volatile oils obtained from plants, possessing the odor and other characteristic properties of the plant, used chiefly in the manufacture of perfumes, flavors, and pharmaceuticals (extracts after hydro-distillation).

Essential oils or plant extracts can be used as appetite stimulant, aroma, stimulant of saliva production, gastric and pancreatic juices production enhancer, and antioxidant. However there is no clear demonstration of the importance of these factors on the chicken performance.

Plants contain hundreds of substances having different properties but essential oils composed mainly of nine groups (and many sub-groups) of molecules are of interest to us. There are many chemical constituents but no two oils are alike in their structure and effect. One must make a difference between non purified plant extracts containing numerous different molecules interacting and pure active compounds, either extracted from plants or synthesized (nature identical). According to the plant chosen, one or more active compounds are dominant and the quantity found will differ according to factors like plant variety, soil, moisture, climate, time of harvest, etc. Almost all essential oils (EO) are based on isoprene (5C) frame.

Nutritionally, metabolically, and toxicologically, we have a clear interest in using as low as possible levels of essential oils in animal nutrition. Essential oils are extremely potent substances; they can lead to feed intake reduction, gastrointestinal tract microflora disturbance, accumulation in animal tissues and products.

Most essential oils are GRAS (generally recognized as safe) but they must be used cautiously because they can be toxic (allergens) and potent sensitizers and their odor/taste may contribute to feed refusal (2,5). They are also very volatile and will evaporate (sublimate) rapidly, leading to a large variation in concentration in the finished feeds. Encapsulation of essential oils could solve the problem (5).

It is extremely difficult to generalize on the mode of action of essential oils on bacteria and yeasts because each essential oil has different properties and each type of microorganism has a different sensitivity. Generally, gram-positive bacteria are considered more sensitive to essential oils than gram-negative bacteria (2) because of their less complex membrane structure.

The consensus on the mode of action of essential oils on bacteria is now that these compounds influence the biological membranes of bacteria. The cytoplasmic membrane of bacteria has two principal functions (10); a barrier function and energy transduction, which allow the membrane to form ion gradients that can be used to drive various processes and the formation of a matrix for membrane-embedded proteins influencing the ATP-synthase complex.

A VIABLE ALTERNATIVE

In our own experiments with organic acids, we have experienced very consistent results, both under research station and field conditions; our rate of positive response exceeded 90% for weight gain and feed conversion using a blend of protected organic acids. Not only protected organic acids can act as growth promoter but also play a role in the prevention of necrotic enteritis and in the reduction of intestinal *Salmonella* spp. It appears that the amplitude of the response is often related to the level of contamination or intestinal disease challenge in the flock.

More and more, the concept of combining essential oils and organic acids is proving to be efficacious (13) because there appears to be a synergy between the two concepts (11,12). Our own experiments in field trials, or when using a chicken necrotic enteritis challenge model, have shown a strong synergy between essential oils and organic acids.

TECHNICAL PROBLEMS WITH ORGANIC ACIDS AND ESSENTIAL OILS

The use of organic acids and essential oils in the feed industry is often a source of problems, such as corrosion, worker's safety, handling, vitamin stability in premixes, environmental concern, and stability of products.

It has been demonstrated that when both organic acids and essential oils are protected in a triglyceride matrix, the quantity required to achieve maximum performance in poultry can be reduced drastically. The active ingredients can be delivered into the intestine, directly where the bulk of gastrointestinal bacteria are located (7). Without protection, organic acids are readily dissociated in the first part of the chicken gastrointestinal tract and are rendered useless (1). Essential oils are very rapidly absorbed in the duodenum and cannot interact with the microflora (4).

CONCLUSION

There is a general consensus on the efficacy of organic acids as the best alternative to antibiotic growth promoters.

Essential oils have a limited effect as a replacement of antibiotic growth promoters but they

can act in synergy with organic acids both for their growth promoting effect and prevention of specific intestinal diseases. Now there is an encapsulation technology that enhances the efficacy of organic acids and essential oils, at a low inclusion level.

REFERENCES

1. Dibner, J.J. and P. Butin. *Journal of Applied Poultry Research*. 11:453-463. 2002.
2. Lambert, R.J.W., P.N. Skandamis, P.J. Coote, and G.J.E. Nychas. *Journal of Applied Microbiology*, 91:453-462. 2001.
3. Lee, M.D. Molecular basis for AGP effects in animals. *Antimicrobial Growth Promoters: Worldwide Ban on the Horizon*. Noordwijk aan Zee, The Netherlands, p. 37-38. 2005.
4. Lee, K.W., H. Everts, and A.C. Beynen. *International Journal of Poultry Science*. 3:738-752. 2004.
5. Lis-Balchin, M. Feed additives as alternatives to antibiotic growth promoters: botanicals. *Proceedings of the 9th International Symposium on Digestive Physiology in Pigs*. University of Alberta, Canada, publisher. 1:333-352. 2003.
6. Partanen, K.H. and Z. Mroz. *Nutrition Research Reviews* 12:117-145. 1999.
7. Piva, A. and M. Tedeschi. Composition for use in animal nutrition comprising a controlled release lipid matrix, method for preparing the composition and the method for the treatment of monogastric animals. United States Patent Application Publication. Pub. No.: US 2004/0009206 A1; EU patent EP1391155B1. 2004.
8. Presser, K.A., D.A. Ratkowsky, and T. Ross. *Applied & Environmental Microbiology*. 63:2335-2360. 1997.
9. Rosen, G. Setting and meeting standards for the efficient replacement of pronutrients antibiotics in poultry and pig nutrition. *Antimicrobial Growth Promoters: Worldwide Ban on the Horizon*. Noordwijk aan Zee, The Netherlands, p. 66. 2005.
10. Ultee A., E.P.W. Kets, and E.J. Smid. *Applied and Environmental Microbiology* 65:4606-4610. 1999.
11. van Dam, J.T.P., M.A.M. Vente-Spreeuwenberg, and H.P.T. Kleuskens. Combination of medium chain fatty acid and organic acids provides a cost-effective alternative to AGP in pig nutrition. *Antimicrobial Growth Promoters: Worldwide Ban on the Horizon*. Noordwijk aan Zee, The Netherlands, p. 5. 2005.
12. van Kol, E.M.R. Organic acids and essential oils in AGP free diets. *Antimicrobial Growth Promoters: Worldwide Ban on the Horizon*. Noordwijk aan Zee, The Netherlands, p. 7. 2005.
13. van Wesel, A.A.M., H.B. Perdok, and D.J. Langhout. Phasing out antimicrobial growth promoters. *II Congresso Latino Americano De Suinicultura*, Foz do Iguaçu, Brasil, 20-22 Outubro, p. 141-144. 2004.

Table 1. Effect of essential oils and essential oils-organic acid combination on production parameters, no challenge farm trial, broiler chickens, 28 days of age, $P < 0.05$ (Jefo Nutrition internal data).

	# of chickens	Body weight (g)	DWG (g)	Feed conversion	% mortality
Negative control	1198	1.406[a]	48.70[a]	1.605[a]	3.83
Essential oils (2)	1200	1.367[b]	47.31[b]	1.621[a]	3.00
EO-OA (1)	1198	1.436[c]	49.79[c]	1.557[b]	2.59
EO-OA (2)	1200	1.467[d]	50.90[d]	1.560[b]	3.67

IT'S THE FEED! VITAMIN E DEFICIENCY IN TURKEYS

T. R. Olson

Moroni Feed Company, Moroni Utah

SUMMARY

Moroni Feed Company, a turkey cooperative located in the mountain valley of Sanpete County Utah, places approximately 5.8 million turkey poults annually. Throughout the 2007 production year, turkey performance was significantly less than expected. Most flocks had poor feed conversion and failed to reach market weights. Increased flock mortality was also considerable with mortalities reaching at best 6% and at worst 25% at five weeks of age. This mortality was attributed to poor starts, a sharp increase in poult enteritis, and chronic colibacillosis.

Unprecedented changes had occurred within the Moroni Feed Company production system just prior to the 2007 production year. Changes included: a switch from >90% Orlopp strain poults to all Nicholas/Hybrid strains, a company owned hatchery that moved from multiple stage to single stage incubation using new setters and hatchers, the purchasing of poults from an outside hatchery that required considerable shipping distances, and the addition of a new nutrition consulting firm with a concomitant change to a "hotter" diet with significant increased fat levels. To further complicate matters, a move was made by many member growers to upgrade their production facilities with new equipment. The most significant change was a move to radiant heat brooder stoves. Other changes included new ventilation equipment and a move to automatic nipple water systems.

Needless to say there were fingers pointed in just about every direction to place blame on the poor performance. Rule outs and areas of concern included poor poult quality, introduction of a new enteric and/or respiratory virus or viruses into production units, something "wrong" with the feed (bacterial contamination, mycotoxins or mixing errors). All this was complicated by the unfamiliarity of a new breed and the many changes in management strategies both good and bad.

All rule outs were examined thoroughly and some changes were made that resulted in some measure of success. However, it wasn't until late fall that a major break-through occurred. A submission was made to the Moroni Feed Company Veterinary Diagnostic Laboratory of some 21-day-old tom poults with the chief complaint of lateral recumbence and paddling. Lesions included a unilateral hyperemic wing and a cherry-red cerebellum, pathognomonic for nutritional encephalomalacia due to vitamin E deficiency. Tissue samples were submitted to the Fresno Branch of the California Animal Health & Food Safety Laboratory System where the diagnosis of encephalomalacia was confirmed.

During the next several months, great effort was made to find the cause of the vitamin E deficiency. It was later determined that the correct level of vitamin E, as part of a premix, was being added to all diets. However, fat and meat meal additions to the diet had not been protected with an antioxidant. Therefore as diets went through varying stages of rancidity, vitamin E acting as an antioxidant was oxidized rendering it inactive.

During the months that followed, as poults consumed feed with adequate levels of vitamin E, flock performance and mortality returned to normal levels.

SAFETY OF FEEDING RACTOPAMINE TO FINISHING TURKEYS

D.J. Ivers, C.T. Herr, D.H. Mowrey, and J. D. Trites

Elanco Animal Health, Eli Lilly and Company, Greenfield 46140

Separate tom and hen turkey studies were conducted to evaluate the safety of ractopamine (RAC) when fed at various doses in the finisher phase. Each gender-specific study utilized 48 pens (eight birds per pen) with the pen being the experimental unit. Treatment design consisted of 0, 13, and 130 ppm of ractopamine added to finishing feed rations for 14 days. Health observations were observed twice daily and blood chemistries were taken on day 0 and day 14. Parameters measured included blood, coagulation, and hematology variables, as well as bone strength. Two birds per pen were necropsied on day 14 and selected tissues were evaluated microscopically. These studies demonstrate that feeding finishing tom and hen turkeys diets containing up to 130 ppm RAC for 14 days is safe to the birds.

EFFICACY OF DIFFERENT DOSES OF PAROMOMYCIN IN THE FEED AGAINST *HISTOMONAS MELEAGRIDIS* IN EXPERIMENTALLY CHALLENGED TURKEY POULTS

H. M. Hafez[A], A. Lotfi[A], R. Hauck[A], and K. De Gussem[B]

[A]Institute of Poultry Diseases, Faculty of Veterinary Medicine, Free University Berlin,
Königsweg 63, 14163 Berlin, Germany
E-mail: hafez@vetmed.fu-berlin.de
[B]Huvepharma, Uitbreidingstraat 80, 2600 Antwerp, Belgium
E-mail: Koen.degussem@huvepharma.com

SUMMARY

The protozoan parasite *Histomonas meleagridis* is capable of causing an enterohepatitis in gallinaceous birds. In the last years outbreaks of histomoniasis in turkey flocks have caused considerable economic losses, since currently no prophylactic or therapeutic drugs against the parasite are licensed in the European Union due to food safety concerns (1).

Paromomycin is an aminoglycoside antibiotic with activity against most gram-negative and many gram-positive bacteria, some protozoa and many cestodes. Currently, paromomycin is registered for all food producing animal species, but not for use in animals from which milk or eggs are produced for human consumption.

According to Lindquist (3) the application of 1000 to 2000 ppm paromomycin sulfate in the feed reduced mortality after experimental infection with *H. meleagridis* significantly (3). *In vitro* paromomycin sulfate reduced the growth of parasites at 10 µg/mL, and was completely effective at 100 µg/mL. However, 200 or 400 ppm paromomycin sulfate given in the feed starting one day before infection did not reduce lesions in ceca and livers of experimentally infected chickens (2).

In the present study we evaluated the efficacy of different doses of paromomycin in the feed against *H. meleagridis* in experimentally challenged turkey poults.

To that end 130 male one-day-old turkey poults were divided in four groups of 30 birds each (groups IC, T100, T200, T400) and an additional group (NC) consisting of 10 birds. Groups NC (non-infected-non-treated, negative control) and IC (infected non-treated, positive control) received feed without anti-histomonal drugs. Groups T100, T200, and T400 were given feed with 100, 200 and 400 ppm paromomycin (histoBloc®, Huvepharma, Antwerp, Belgium) respectively starting on day one through day 42 (end of the experiment). On day 21, each bird in groups IC, T100, T200 and T400 received 1 mL Dwyer's medium containing 150,000 histomonads intracloacally. After the challenge, the birds were observed for three weeks post infection, and daily mortality, feed and water consumption, as well as the feed conversion rate were recorded. After three weeks the surviving birds were euthanized. All birds were examined for pathological changes. Lesions in ceca and liver were scored.

Before the challenge there was no significant difference between untreated and treated groups in regard to daily weight gain, feed and water consumption, as well as feed conversion rate.

There was no mortality in group NC during the entire experiment. In the infected groups mortality started around day nine post infection and ended between day 15 and 20 post-infection. Mortality was 80% in group IC (infected non-treated). In the treated groups the mortality rate was: 73.3% in T100, 43.3% in T200, and 20% in T400 group. In all treated groups mortality was significantly less than in group IC.

The mean lesion scores in liver and ceca were significantly lower in groups T200 and T400 than in groups IC and T100. No histomonal DNA was found in ceca and livers of the surviving birds.

The mean weight of the surviving birds was not significantly different from the birds of the uninfected control. The feed conversion rate as well as European production efficiency factor was significantly better in groups T200 and T 400 than in groups T100 and IC.

In conclusion, a prophylactic application of histoBloc in the feed was effective against the challenge with *H. meleagridis*. In the field it might prevent the losses of whole turkey flocks due to outbreaks of histomoniasis.

REFERENCES

1. Hafez, H.M., R. Hauck, D. Lüschow, and L.R. McDougald. Comparison of the specificity and sensitivity of PCR, nested PCR, and real-time PCR for the diagnosis of histomoniasis. Avian Dis.49:366–370. 2005.

2. Hu, J. and L.R. McDougald. The efficacy of some drugs with known antiprotozoal activity against *Histomonas meleagridis* in chickens. Vet Parasitol 121:233-238. 2004.

3. Lindquist, W.D. Some effects of paromomycin sulfate on blackhead in turkeys. Am J Vet Res 23:1053-1056. 1962.

HEMATOBIOCHEMICAL CHANGES OF LAYER CHICKENS IN EXPERIMENTAL AFLATOXICOSIS

N. K. Thapa[A], M. Muralimanohar [B], C. Balachandran[C], and G. Sarathchandra[D]

[A]Dy.cheif Veterinary officer, NCAH, Serbithang, Thimphu post box 155, Bhutan
Nkthapa08@hotmail.com
[B]Director, Centre for animal health studies, TANUVAS, Chennai-600 051
[C]Professor & Head, Department of veterinary Pathology, Madras Veterinary College, Chennai-600 007 India
[D]Associate professor and Head, CFFRAL(Central feed and food residue analytical laboratory), TANUVAS, Chennai-600 051, India

SUMMARY

Twenty seven-week old laying chicken were randomly distributed to three groups of 10 birds each and were fed with diets containing 0, 1 and 2 ppm AF for 42 days. The mean (±SE) PCV values were 27.60 ± 0.92, 25.60 ± 0.20 and 21.40 ± 1.16% for 0, 1, and 2 ppm respectively, indicating significant ($P < 0.05$) decrease in the PCV value in aflatoxin (AF) treated birds compared to the control. No significant differences were observed in hemoglobin (Hb) and total erythrocyte count (TEC) values. The means (±SE) of serum glucose values were 131.31 ± 11.11, 185.30 ± 1.11 and 177.71 ± 11.11 mg/dL. Significant increase ($P < 0.05$) in serum glucose values were observed in the AF fed layer chicken. However, no significant differences were observed in serum cholesterol, total protein, and albumin values. The means (±SE) of alkaline phosphatase (ALP) were 970.53 ± 128.04, 805.93 ± 128.04 and 1264.21 ± 128.04 U/L for 0, 1 and 2 ppm AF fed layer birds respectively indicating significant ($P < 0.05$) increase in the level of ALP in 2 ppm AF fed layer birds when compared to the 0 and 1 ppm AF fed birds. However, no significant differences were observed between the control and AF treated layer birds for alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Mean (±SE) values of blood urea nitrogen (BUN) were 12.90 ± 1.30, 10.33 ± 1.30 and 7.41 ± 1.30 mg/dL for 0, 1 and 2 ppm AF fed layer birds respectively which revealed significant ($P < 0.05$) decrease in the levels of BUN in AF treated birds and variable results were observed for serum creatinine when compared to the control. Mean (±SE) values of serum sodium were 165.19 ± 8.93, 166.99 ± 8.42 and 130.14 ± 8.42 mmol/L for 0, 1 and 2 ppm AF fed layer birds respectively. The values decreased significantly ($P < 0.05$) in 2 ppm AF fed layer chicken. Serum calcium, phosphorous and potassium values did not differ significantly between the control and AF treated layer chicken.

INTRODUCTION

Contamination of feed with mycotoxin is a global problem. Among mycotoxins, aflatoxins (AFs) are of the most important mycotoxins encountered in the feeds world wide (Williams *et al*., 2004). AFs are difurocoumarin derivatives produced by toxigenic fungi of *Aspergillus* species. Poultry industry suffers greater economic losses due to greater susceptibility of the species compared to other animals to the toxin and also due to continuing intermittent occurrences in feeds (Fraga *et al*., 2007).

Compared to the broilers the literature with regards to poultry layers are very limited, hence the study was conducted.

MATERIALS AND METHODS

Aflatoxin (AF) was produced on rice (7). Thirty numbers of 27 weeks old White Leghorn Forsgate layer chickens were randomly divided into three groups of 10 birds each and were fed with control and treatment diets containing 1 and 2 ppm AF for six weeks. Blood samples were collected after 42 days of trial by cardiac puncture in a vial containing Heller and Paul double oxalate mixture. Hb, PCV and TEC were estimated. Serum total protein, albumin, cholesterol, ALT, AST, ALP, BUN, creatinine gamma glutamyl transferase (GGT), calcium, phosphorous, sodium, potassium, and uric acid were analyzed using the reagents/kits (Agappe and Accurex).

Data were subjected to one/two way analysis of variance (ANOVA) using SPSS software version 10.00.

RESULTS AND DISCUSSION

The mean (±SE) PCV values were 27.60 ± 0.92, 25.60 ± 0.20 and 21.40 ± 1.16% Significant decrease in PCV was observed in 2 ppm AF fed layer chicken Compared to the compared to control. While Hb and TEC values did not differ from the control. Gounalan *et al*. (3) also reported significant reduction in PCV and Hb values in layer chicken fed with 0.5 ppm of AF from 0 to 12 weeks age. However, Fernandez *et al*. (1) reported increase in TEC and hematocrit in layer chicken by feeding 2.5 and 5 mg/kg for 32 days.

Overall means (±SE) of serum glucose values were 131.31 ± 11.11, 185.30 ± 1.11 and 177.71 ± 11.11 mg/dL. The serum glucose levels showed significant ($P < 0.05$) increase in AF fed birds when compared to the control. No significant differences for cholesterol, total protein and albumin. Similarly, no significant changes were reported in serum protein in layers fed up to 5 ppm or more AF for 28 or 32 days (2,4). However, hypoproteinemia and hypoalbuminemia were reported in laying hens fed up to 5 ppm (3,8).

There was numerical decrease in GGT and ALP in 1 ppm AF treated birds. Earlier workers reported elevated serum ALT, AST and GGT in birds fed 500 ppb AF from two days onwards (3,5,6).

Mean (±SE) values of BUN were 12.90 ± 1.30, 10.33 ± 1.30 and 7.41 ± 1.30 mg/dL and serum creatinine were 0.42 ± 0.02, 0.47 ± 0.02 and 0.35 ± 0.02 mg/dL for 0, 1 and 2 ppm respectively. There was significant ($P < 0.05$) decrease in the level of BUN and creatinine in 2 ppm level when compared to the control. In AF fed birds, no significant changes were observed in serum uric acid. Increase in uric acid and creatinine were reported by feeding 0.5 ppm AF to layer chicken up to 12 weeks of age (3).

Feeding 1 and 2 ppm of AF to layers for 42 days resulted in significant decrease in serum sodium level in 2 ppm fed birds when compared to the control groups. Serum calcium, phosphorous and potassium levels did not show any differences between the control and toxin treated birds. However, significant reduction in serum calcium and phosphorus were reported by feeding AF up to 5 ppm (2,5,8,9).

Feeding of 1 and 2 ppm aflatoxin for 27 weeks in layer chicken caused significant ($P < 0.05$) decrease in the PCV value and significant increase ($P < 0.05$) in serum glucose values when compared to the control birds. No significant differences were observed between the control and AF treated layer birds for ALT and AST. There was significant ($P < 0.05$) increase in the level of ALP in 2 ppm AF fed layer birds when compared to the 0 and 1 ppm AF fed birds decrease in the levels of BUN. Variable results were observed for serum creatinine. Serum sodium values decreased significantly ($P < 0.05$) in 2 ppm AF fed layer chicken when compared to the control and 1 ppm AF fed layer birds. Serum calcium, phosphorous and potassium values did not differ significantly between the control and AF treated layer chicken.

REFERENCES

1. Fernandez, A., M.T. Verda, J. Gomez, M. Gascom, and J.J. Ramos. Research in Veterinary Science, 58 (2): 119-122. 1995.
2. Fernandez, A., M.T. Verda, M. Gascon, J. Ramos, J. Gomez, D.F. Luco, and G. Chavez. Avian Pathology, 23: 37-47. 1994.
3. Gounalan, S., C. Balachandran, and B. Murali Manohar. 2006. International conference on advance Veterinary practice in medicine and surgery augmenting health and production, Chennai, 21-25 June, 2006.
4. Iqbal, Q.K., P.V. Rao, and S.J. Reddy. Indian Journal of Animal Sciences, 53: 1277-1280.1983.
5. Kim J.G., L.W. Lee, P.G. Kim, W.S. Roh, and H. Shintani. Journal of Food Protection, 66 (5): 866–873. 2003.
6. Rao, V.N. and H.C. Joshi. Indian Veterinary Journal, 70: 344–347. 1993.
7. Shotwell, O.L., C.W. Hesseltine, R.D. Stubblefield, and W.G. Sorenson. Applied Microbiology, 111 (3): 425 – 428. 1966.
8. Stanley, V.G., M. Winsman, C. Dunkley, T. Ogunleye, M. Daley, W.F. Krueger, A.E. Sefton, and A. Hintom. Journal of Applied Poultry Research, 13: 533-539. 2004.
9. Umesh, D., V.N. Rao, and H.C. Joshi. Indian Journal of Veterinary Medicine, 15(1): 32-34. 1995.

Table 1. Hematobiochemical values of AF fed layer chicken.

Hematobiochemical parameters	Aflatoxin level (ppm)		
	0	1	2
Hematological values			
PCV (%)	27.60a±0.92	25.60ab±0.21	21.40b±1.16
Hb (g/dL)	10.60±0.06	11.32±0.07	10.56±0.04
TEC (millions/mm^3)	2.35±0.02	2.11±0.02	1.84±0.01
Serum Metabolites			
Glucose	131.31a ±11.11	185.30b±11.11	177.71b±11.11
Cholesterol	124.57± 11.81	111.83 ± 11.81	137.66± 11.81
Total protein	4.88±0.20	4.49±0.20	4.44±0.20
Albumin	1.94±0.10	2.00±0.10	2.06±0.10
Serum enzymes (U/L)			
ALT	4.74±0.74	5.26±0.81	3.56±0.70
AST	164.89 ±6.49	173.58 ±6.49	168.38±6.49
GGT	16.68ab±1.32	13.08a ±1.32	16.16b±1.32
ALP	970.53ab±128.04	805.93a±128.04	1264.21b±128.04
BUN, creatinine & uric acid values		(mg/dL)	
BUN	12.90a±1.30	10.33ab±1.30	7.41b±1.30
serum creatinine	0.42ab±0.02	0.47a±0.02	0.35b±0.02
uric acid values	4.50±0.52	4.46±0.52	4.95±0.52
Serum mineral and electrolytes			
Calcium (mg/dL)	10.63±0.21	11.02±0.21	11.18±0.21
Phosphorous (mg/dL)	5.70±0.57	7.43±0.57	6.87±0.57
Sodium (mmol/L)	165.191a±8.93	166.99a±8.42	130.14b±8.42
Potassium (mmol/L)	8.02±1.43	8.07±1.43	9.52±1.43

Means with same superscript within a column do not differ significantly ($P > 0.05$).

MOLECULAR TYPING OF INFECTIOUS BRONCHITIS VIRUS

H. M. Hafez, V. L. deQuadros, and D. Lüschow

Institute of Poultry Diseases, Faculty of Veterinary Medicine
Freie Universität Berlin, Königsweg 63, 14163 Berlin, Germany

SUMMARY

Infectious bronchitis (IB) is an acute, rapidly spreading disease of chickens characterized by respiratory signs, drop in egg production, and poor egg quality or nephritis/nephrosis. The causative agent, infectious bronchitis virus (IBV) is a member of the genus *Coronavirus*, within the family *Coronaviridae*. The genome of IBV consists of single-stranded positive sense RNA coding for four structural proteins: the nucleocapsid protein – N, the membrane protein – M, the small membrane protein – E, and the spike protein – S which consists of the two subunits S1 and S2. The high genetic variation within the S1 protein is responsible for multiple serotypes and variant strains which have been identified throughout the world. The occurrence of IBV serotypes and strains vary from country to country as well as from region to region.

In the last years, molecular biological methods like polymerase chain reaction (PCR) systems have become increasingly important for a fast and sensitive diagnosis of IB. The use of IBV universal primers

allows the general diagnosis of the infection, the various serotypes and variant strains can be differentiated by IBV type specific primers or rather by restriction enzyme analysis of PCR products or sequence analysis of selected genome regions. The objective of the present study was the development of a molecular system for detection and typing of IBV field strains circulating in German poultry flocks. For this reason a system composed of three different steps was established:

1. The general diagnosis of IBV infection is carried out by a RT-PCR using a universal primer pair which amplified a fragment within the conserved N protein gene of IBV (3) and which is able to detect all circulating serotypes and variant strains (Fig. 1). In addition, IBV positive samples are investigated by a subtype specific RT-PCR for detection of subtype 4/91 IBV with primers located within the S1 gene region (3) and which allowed an exclusive hybridization with Subtype 4/91 IBV RNA (Fig. 1).

2. For further molecular typing of almost all serotypes and variant strains of IBV a RT-PCR system combined with restriction enzyme analysis (REA) or sequence analysis of PCR products was established. For this purpose a primer pair was chosen, which was localised on the one hand within relative conserved parts of the S1 gene and enclosed on the other hand the S1 gene hypervariable region (4,6). The subsequent restriction enzyme analysis of amplified PCR products using at least two different enzymes should allow an easy and fast differentiation. Initial investigations revealed that selected primers were able to amplify a fragment of the expected size from all available reference and variant strains. These strains were differentiated by the use of two different restriction enzymes (Fig. 2).

However, analysis of IBV universal PCR positive field samples showed with the used primer pair in some cases only very weak or negative results. So, in consequence of the high sequence variation of some IBV strains the system was expanded by a further primer pair and a set of nested primers for amplification of some field strains (1,2) and subsequent sequence analysis of the amplified fragment. Up to now different viruses of the Massachusetts type as well as 4/91, D1466, V1397, and QX-like IBV were detected and differentiated with the established system.

3. Recent investigations reported about the incidence of the new subtype QX-like IBV in Europe (5). In this view we have developed a subtype specific RT-PCR for fast and sensitive detection of QX-like IBV. The designed primers which amplified a fragment of about 220 bp within the S1 gene region revealed only positive results for this subtype (Fig. 3).

Using this PCR a retrospective study from 2004 to 2008 of IBV positive samples showed a dramatic increase of QX-like IBV in German poultry flocks. In 2008 QX-like IBV was detected in more than 60% of IBV positive samples, mainly in samples derived from broiler flocks.

Altogether, the established system composed of three different steps has shown to be a reliable method for diagnosis and monitoring of IBV infection.

REFERENCES

1. Bochkov, Y.A., G. Tosi, P. Massi, and V.V. Drygin. Phylogenetic analysis of partial S1 and N gene sequences of infectious bronchitis virus isolates from Italy revealed genetic diversity and recombination. Virus Genes 35:65-71. 2007.

2. Dolz, R., J. Pujols, G. Ordonez, R. Porta, and N. Majo. Antigenic and molecular characterization of isolates of the Italy 02 infectious bronchitis virus genotype. Avian Pathology 35:77-85. 2006.

3. Handberg, K.J., O.L. Nielsen, M.W. Pedersen, and P.H. Jorgensen. Detection and strain differentiation of infectious bronchitis virus in tracheal tissues from experimentally infected chickens by reverse transcription-polymerase chain reaction. Comparison with an immunohistochemical technique. Avian Pathology 28:327– 335. 1999.

4. Kwon, H.M., M.W. Jackwood, and J. Gelb, Jr. Differentiation of infectious bronchitis virus serotypes using polymerase chain reaction and restriction fragment length polymorphism analysis. Avian Diseases 37:194-202. 1993.

5. Worthington, K.J., R.J. Currie, and R.C. Jones. A reverse transcriptase-polymerase chain reaction survey of infectious bronchitis virus genotypes in Western Europe from 2002 to 2006. Avian Pathology 37:247-257. 2008.

6. Yu, L., Z. Wang, Y. Jiang, S. Low, and J. Kwang. Molecular epidemiology of infectious bronchitis virus isolates from China and Southeast Asia. Avian Diseases 45:201-209. 2001.

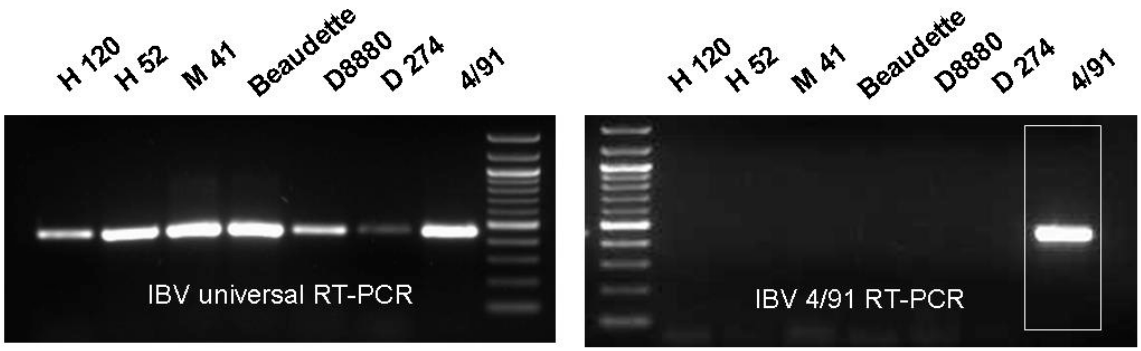


Fig. 1 Specificity of selected primer pairs of the IBV universal and IBV 4/91 RT-PCR

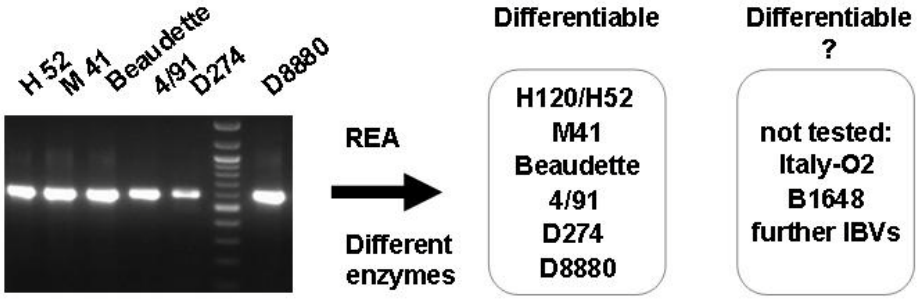


Fig. 2 Differentiation of IBV reference strains by REA of PCR products

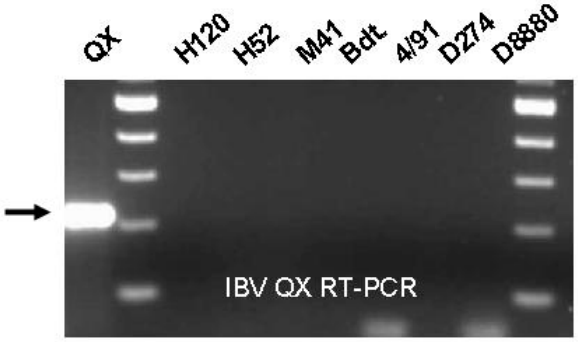


Fig. 3 Specificity of IBV QX RT-PCR

PHENOTYPE AND FUNCTIONS OF T CELLS ISOLATED FROM MUCOSAL LININGS OF TURKEYS EXPOSED TO AVIAN METAPNEUMOVIRUS SUBTYPE C (AMPV/C)

Ra Mi Cha[A], Mahesh Khatri[B], and Jagdev Sharma[C]

[A]Department of Veterinary and Biomedical Sciences, University of Minnesota, 1971 Commonwealth Avenue, St. Paul, MN 55108,
[B]Current address: Department of Food Animal Health Research Program, Ohio Agricultural Research and Development Center, The Ohio State University, 1680 Madison Avenue, Wooster, OH 44691
[C]Current address: The Biodesign Institute, Arizona State University, 1001 S. McAllister Ave., Tempe, AZ 85287

SUMMARY

aMPV/C causes an acute, immunosuppressive respiratory tract infection in turkeys. The virus mainly replicates in the upper respiratory tract (URT). Two-week-old aMPV/C-free commercial turkeys were inoculated oculonasally with live aMPV/C. At five and seven days post inoculation (DPI), lymphocytes infiltrating the mucosal linings of the URT were isolated by enzymatic treatment. The proportions of T cell phenotype (CD4/CD8) in the turbinate cells were tested by FACS analysis. aMPV/C exposure increased the proportion of CD8+ cells but not of CD4+ cells. In addition, the gene expression of CD8 but not of CD4, was increased in the URT. Lymphoid cells isolated from the URT of virus-exposed turkeys were deficient in the proliferative response to a T cell mitogen. Cytokine gene expression in the URT was examined by quantitative RT-PCR. At five and seven DPI, RNA extracted from turbinate tissue had upregulated expression of genes of IL-10 and IFN-γ. These results indicated that aMPV/C stimulated mucosal cellular immunity in the URT.

THE COMPATIBILITY OF HVT RECOMBINANTS WITH OTHER MAREK'S DISEASE VACCINES

G. Slacum, R. Hein, and P. Lynch

Intervet/Schering-Plough Animal Health, Millsboro, DE 19966

Recently, several recombinant turkey herpesvirus (rHVT) vaccines have been introduced in the US. These rHVT constructs are based on the insertion of immunogenic genes from Newcastle disease virus (NDV), infectious larygotracheitis virus (ILTV), or infectious bursal disease virus (IBDV). The HVT used in these recombinants belong to serotype 3 of Marek's disease virus (MDV). The common vaccines used to control MD are derived from three different serotypes of MDV, serotype 1, (eg. Rispens CVI 988), serotype 2 (eg. SB1) and serotype 3 (eg. HVT). In several cases, the poultry industry uses bivalent (HVT+SB1 or HVT+CVI988) or trivalent (HVT+SB1+CVI988) combinations based on the virulence of the MDV field challenge.

In situations where there is virulent MDV, producers will possibly combine the rHVT vaccines with other MD vaccine serotypes (Rispens CVI 988 and/or SB1), as is currently done with conventional HVT vaccines. Several trials have been carried out to investigate the potential interference that serotype 1 (Rispens CVI988) and serotype 2 (SB1) vaccines might have on the rHVT vaccines. A rHVT vaccine has been licensed in combination with SB1 (eg. INNOVAX-ND-SB). As expected, a synergistic affect was shown similar to that observed with the conventional HVT vaccine when combined with SB1. Data from our trials will be presented which show that Rispens CVI988 vaccine does not interfere with the rHVT vaccines, similar to the conventional HVT vaccine. In contrast, interference occurs when rHVT vaccines are simultaneous administrated with a conventional HVT vaccine. The interference is expressed as a decrease in the immunity against the virus from which the donor genes in the rHVT vaccine are derived.

PROTECTION AGAINST VARIANT STRAINS CONFERRED BY THE RECOMBINANT HVT-IBDV VACCINE VAXXITEK®

Francisco Perozo[A], Pedro Villegas[A], Rafael Fernandez[B], and Julio Cruz[B]

[A]Poultry Diagnostic and Research Center, Athens, GA., USA
[B]Merial Select, Inc. Gainesville, GA, USA

SUMMARY

The use of viral vectors for transgenic expression of immunogenic proteins is a current trend in poultry disease control. The objective of this work was to assess the protection against variant E strain challenge conferred by day-one vaccination using VAXXITEK®, a recombinant herpesvirus of turkey expressing the immunogenic viral protein 2 from a classical IBDV. Specific pathogen free (SPF) and commercial one-day old birds were vaccinated with VAXXITEK by the subcutaneous route and challenged with the variant E strain at 18 or 28 days of age. The protection criteria included: bursa/bodyweight ratio and bursal histopathology scores. Adequate protection was demonstrated. Bursal indexes in vaccinated SPF and broiler groups were significantly higher than in the challenged controls. Vaccination protected against bursal damage as indicated by significantly lower bursal lesion scores in the vaccinated birds at both challenge points. These results indicate that single dose recombinant HVT-IBDV vaccination protects chickens against classical and variant strains.

®VAXXITEK is a registered trademark of Merial in the United States of America and elsewhere.

EXPERIMENTAL DESIGN

One hundred twenty SPF birds and 120 broilers were divided in six groups. On day one and for each type of birds, two groups were vaccinated by the subcutaneous route with 0.1 mL containing 3000 plaque forming units of the recombinant vHVT013-19 (VAXXITEK). At 18 and 28 days, one vaccinated and one unvaccinated group was challenged by the intraocular route with $10^{3.2}$ EID_{50}/0.03 mL of variant E; a third group remained as unchallenged control. Bursa/bodyweight ratio and bursal histopathology were assessed as protection criteria.

RESULTS AND DISCUSSION

The results indicate an adequate protection in SPF and broilers: the relative bursa:body weight was significantly higher ($P < 0.05$) and the bursal lesion scores significantly lower in the vaccinate birds when compared with the unvaccinated-challenged controls regardless of the age at challenge (Table 1). The variant strains isolated and genotyped in the United States and in other countries exhibit antigenic drift affecting neutralizing epítopes in the capsid protein VP2 (1,2). The VP2 gene expressed by the recombinant HVT-IBDV comes from a classical strain (52/70 Faragher strain); hence variability in the protection against the neutralizing epitopes present in the variant strains VP2 is possible. The levels of protection observed in this trial suggest that under experimental conditions and against the variant E strain, VAXXITEK provides adequate protection. Similar levels of protection produced by another HVT-vectored IBDV vaccine have been previously reported (3).

REFERENCES

1. Letzel, T., F. Coulibaly, F.A. Rey, B. Delmas, E. Jagt, A.A. van Loon, and E. Mundt. Molecular and structural bases for the antigenicity of VP2 of infectious bursal disease virus. J Virol 81:12827-12835. 2007.

2. Snyder, D.B. Changes in the field status of infectious bursal disease virus. Avian Pathol 19:419-423. 1990.

3. Tsukamoto, K., S. Saito, S. Saeki, T. Sato, N. Tanimura, T. Isobe, M. Mase, T. Imada, N. Yuasa, and S. Yamaguchi. Complete, long-lasting protection against lethal infectious bursal disease virus challenge by a single vaccination with an avian herpesvirus vector expressing VP2 antigens. J. Virol. 76:5637-5645. 2002.

Table 1. Bursa:body weight ratio and the bursal lesion scores of SPF and broiler birds challenged with variant E strain.

		Early Challenge (18 days)*				Late Challenge (28 days)			
Treatment ***	**Challenge**	Bursa: Body** Weight		Busal Lesion Scores		Bursa: Body Weight		Busal Lesion Scores	
		SPF	Broiler	SPF	Broiler	SPF	Broiler	SPF	Broiler
VAXXITEK®	Variant E	4.68[a]	2.53[a]	2.43[b]	1.75[b]	5.49[a]	1.69[a]	2.1[b]	1.44[b]
Unvaccinated (+) control	Variant E	1.76[b]	0.85[b]	4.00[a]	3.95[a]	1.64[b]	0.80[b]	4[b]	4[a]
Unvaccinated (-) control	No challenge	6.03[a]	1.78[a]	2.00[b]	2.00[b]	5.46[a]	1.98[a]	1.7[b]	1.85[b]

* Each value represents the average of 20 birds per treatment.

** Bursa/body weight ratios calculated using the following formula: Bursa weight/body weight x 100.

*** Means with the same letter within column are not significantly different by the SNK test ($P < 0.05$).

TIME-SCALED PHYLOGENIES OF BOTH GENOME SEGMENTS OF INFECTIOUS BURSAL DISEASE VIRUS SUGGEST MULTIPLE RE-ASSORTMENT EVENTS IN ITS EVOLUTIONARY HISTORY

Chi-Wai Yip, Chung-Chau Hon, Tsan-Yuk Lam, Meng Shi,
Fanya Zeng, and Frederick Chi-Ching Leung

School of Biological Sciences, The University of Hong Kong, Hong Kong SAR, China

ABSTRACT

Infectious bursal disease virus (IBDV) causes an immunosuppressive disease in chickens, resulting in serious economic impacts in the worldwide poultry industries. The evolutionary history of IBDV was puzzled by the phylogenetic discordance between the two genome segments for years. In this study, phylogenies of segments A and B were reconstructed to estimate the earlier divergence events in the evolutionary histories of both segments. The evolutionary rates and the time of most recent common ancestor (tMRCA) results of individual lineage in each segment were estimated independently. These evolutionary rates and tMRCA results were implemented as prior timestamps for the reconstruction of the phylogenies of the two segments. The earlier divergence events of various genotypes from the two phylogenies jointly suggest multiple re-assortment events in the evolutionary history of IBDV. This study is expected to provide a better understanding on the evolutionary history of IBDV and highlight the multiple occurrences of reassortment events which shaped the phylogeny of IBDV nowadays.

CASE REPORT DOCUMENTING AN OUTBREAK OF VERY VIRULENT INFECTIOUS BURSAL DISEASE IN NORTHERN CALIFORNIA PULLETS

Simone T. Stoute,[A,E] Peter R. Woolcock,[B] Daral J. Jackwood,[D] Susan E. Sommer-Wagner,[D] George L. Cooper,[A] Gabriel C. Senties-Cue,[A] Arthur A. Bickford, [A] Mark L. Anderson,[C] and Bruce R. Charlton[A]

[A]California Animal Health and Food Safety Laboratory System, Turlock Branch,
[B]Fresno Branch, [C]Davis Branch, University of California, Davis, 1550 N. Soderquist Road, Turlock, CA 95381
[D]Food Animal Health Research Program, Department of Veterinary Preventative Medicine, Ohio Agricultural Research and Development Center, The Ohio State University, 1680 Madison Avenue, Wooster, OH 44691

SUMMARY

Very virulent infectious bursal disease (vvIBDV) is antigenically similar to the classical strains of infectious bursal disease (Gumboro) but is characterized by high mortality and the ability to overcome previously protective levels of maternal immunity. In December 2008, 11 and 14 week old pullets from two ranches were submitted to the California Animal Health and Food Safety Laboratory (CAHFS), Turlock branch due to spiking mortality. Affected birds had been spray vaccinated with an intermediate infectious bursal disease vaccine at 10, 20, and 28 days of age. Post mortem lesions included enlarged edematous bursa of Fabricius, hemorrhages on the breast and thigh muscles, as well as hemorrhage at the junction of the gizzard and proventriculus. Histologically, there was severe lymphoid depletion, necrosis and inflammation of bursas. Necrotic lesions were also observed in the thymus, spleen, cecal tonsils, and livers. Diagnosis of vvIBDV was based on necropsy lesions, molecular detection of virus synonymous with vvIBDV strains and high mortality in SPF birds inoculated with virus. The mortality in affected, submitted flocks peaked at 26% and 34% before rapidly returning to normal. vvIBDV was first isolated from broilers in the 1980s and rapidly spread across Asia, Africa, and more recently, South America. This virus is relatively stable in the environment, highly contagious, and immunosuppressive. Establishment of vvIBDV in commercial flocks in the United States has the potential to cause substantial economical losses to the broiler and layer industry. This case is the first official documented report of very virulent infectious bursal disease in the United States. Details of the pathological findings will be presented at conference.

BC POULTRY INDUSTRY BIOSECURITY INITIATIVE: THE EVOLUTION OF A "NECESSARY EVIL"

Bill Cox

Canadian Animal Health Management Services Ltd

INTRODUCTION

The poultry industry in British Columbia is as one of the most densely populated in Canada, in spite of its serving a relatively small local market. Compounding the density is the mixed population of hatching egg, broiler, turkey, and layer flocks, each of which is, for the most part, a small independently owned farm. With proximity, common service activity, and common social activity considered, the industry is extremely vulnerable to the incursion and spread of a serious infectious disease. In February, 2004, the inevitable happened and the Fraser Valley poultry industry was hit with highly pathogenic avian influenza H7N3.

BACKGROUND

In Canada, the conventional poultry industry, including broiler breeders, broiler chicken, layers, and turkeys, operates under a supply managed system. Under the oversight of federal boards, a marketing board for each commodity functions in each province to adjust product supply based upon expected local demand. Price is also regulated on a cost-of-production

basis. As a consequence of this system, poultry production matches population closely for each province and there is minimal export. Concentrations of production, therefore, are located where it is most advantageous within each province.

In British Columbia, the poultry industry is concentrated in the Fraser Valley, east of Vancouver. The Fraser Valley is located in the southwest corner of British Columbia and agriculture extends from the eastern margins of the Metro Vancouver area eastward to the transition of the Fraser Valley to the Fraser Canyon, a distance of little more than 100 km (60 miles). The north-south scope of the area is no more than 20 km at its widest point, bounded on the north by the Coast Mountains and on the south by the Canada-U.S. border. The greatest concentration of poultry production is contained within 20 km of the city of Abbotsford. This situation has evolved over the last 100 years and has resulted in a number of serious challenges including carrying capacity of the land, urban encroachment, the presence of the major Pacific flyway for wild waterfowl, and, of course, biosecurity.

On February 18, the first case of avian influenza in the 2004 BC outbreak was identified and control measures were activated on February 19. Following depopulation and cleaning and disinfection of the premises, the Emergency Operations Centre was shut down on March 3, 15 days after identification of the index case. The situation was considered to be under control. Six days later, however, a second case was suspected, and confirmed the following day, to be AI on a farm 1.5 km (1 mile) away from the index farm. Emergency operations were recommenced on this confirmation. Thirty-one days after the index case, the third case was identified and by 12 days following that, five commercial premises within the 5 km High Risk Region (HRR) were declared to be positive. At the request of the industry, on April 5, day 48 of the event, the entire Fraser Valley was declared a Control Area. By day 55, 25 commercial flocks were now positive for H7 AI. Emergency activities continued to expand as new High Risk Regions were declared within the Control Area and it was not until May 18, 92 days since the index case was identified, that the 42nd and last premises was detected. Cleaning and disinfection was complete on the last infected premises on June 18, day 123, and repopulation of layer and breeder flocks was allowed to commence on July 9 (day 143). The response effort was officially concluded on August 18, 182 days after the index case and after over 90% of commercial poultry barns in the Fraser Valley were emptied.

POST EVENT REVIEW

In October, 2004, an industry forum was held to review the entire outbreak event. Gathering together leaders from all sectors of the poultry industry, veterinarians, Canadian Food Inspection Agency (CFIA) and British Columbia Ministry of Agriculture and Lands (BCMAL), a detailed assessment was conducted. From that process, a number of gaps were identified and recommendations made to move forward. It was recognized that, at least initially, the response was not well organized and many mistakes occurred, in handling destruction on infected premises. There was no formal industry emergency response plan in place. Biosecurity breaches, both within industry and responder groups, significantly enabled the spread of the disease. Lack of readily available information, especially farm locations and population details, seriously hampered sampling and eradication efforts. These observations, among others, led to a series of recommendations for action 1.

Recommendations emerging from the event review included measures to address public health, biosecurity, emergency response, and recovery. A list of action items was written with the commitment of all parties to work together to ensure their successful implementation. Among the 15 specific recommendations were the proposals for the industry to lead in implementing mandatory biosecurity standards and an Emergency Response Plan, with technical assistance from government agencies. Within the context of an Emergency Response Plan was the identified need for a premises identification program.

EVOLUTION OF BIOSECRUITY INITIATIVE

The industry group designated to lead the biosecurity initiative was the BC Poultry Association (BCPA), representing the four regulated commodities (hatching egg, chicken, table egg, and turkey) and the non-regulated specialty bird group. The BCPA was the formalized successor to its more loosely structured predecessor, simply referred to as the Poultry Association. The first task of the BCPA was to spearhead the compilation of a biosecurity manual beginning in the fall of 2004. With Dr. Victoria Bowes as lead author, Angela Ryder from industry as editor, and with input from other veterinarians, industry experts, and leading producers, work began on preparing a manual for voluntary compliance by producers. Bound in a red 3-ring binder, the first Biosecurity Manual became commonly known simply as the “Red Binder”2 and was launched in February, 2005. Analysing the various risks encountered during normal farm operation, specific mitigating procedures that would address each risk were listed. For example,

vehicles, especially those that visit multiple farms, are recognized as a potential platform for pathogen spread. So, procedures were described that either prevent vehicle entry or require a cleaning and disinfection procedure prior to entry. These intuitive steps expanded into a set of 27 standards. Within the manual was an accompanying self-assessment guide for producers. This allowed each farmer to evaluate his or her own biosecurity practices against the standards and develop a framework for improvement. The Red Binder ultimately served as the framework for the formal BC Poultry Industry Biosecurity Program and its Mandatory Biosecurity Standards.

With the trilateral (industry, provincial government, federal government) agreement to enable the development and delivery of an industry-led biosecurity program, a pool of money was committed by Agriculture and Agrifood Canada (AAFC) and BCMAL to help pay for the program and its delivery. The money was administered through the Investment Agriculture Foundation, a not-for-profit organization that manages and distributes government funds in support of innovative projects that benefit agriculture in BC. While the supply managed system and its regulations allow for enforcement of programs and their auditing for compliance, a system of encouragement was recognized as being more likely to succeed. Consequently, financial assistance was made available, allowing producers to not only comply but also make improvements that they might not otherwise do, provided they related to the basic biosecurity standards or enhanced standards. Additionally, success with their farm plan was recognized through certification that could be promoted by the farm.

The development of a set of mandatory biosecurity standards was the first technical challenge to be tackled. The supply managed system of marketing chicken, turkey, and eggs present in Canada provided the mechanism through which mainstream commercial production could be subjected to mandatory biosecurity standards. Once written into the board orders, biosecurity plans detailing implementation of mandatory standards became a requirement for all supply managed poultry farms. A Biosecurity Committee led by the newly-formed BC Poultry Association was convened to steer the process and, from that, a technical subcommittee struck to develop, organize, and detail the program. Following a selection process from a "Request for Proposals," a contractor was hired to coordinate the writing of the standards and their guidelines, develop the auditing process, and deliver the program.

Development of the Biosecurity Program started in summer of 2005, more than a year after the start of the AI outbreak. The first program goal was to compile a set of practical but effective standards that would be consistent with existing plans, including the On Farm Food Safety Programs and the BC Environmental Farm Plan3 (EFP). The On Farm Food Safety Programs were initiated among the four feather groups – hatching eggs, broiler chicken, table eggs, and turkeys – beginning as a national initiative. The programs were implemented on a province by province basis. Structured on a HACCP model, the food safety programs focus on biosecurity as it relates to the transmission of food-borne contaminants such as *Salmonella*, with little emphasis on bird health. The Environmental Farm Plan concept initiated in 2003 and the program launched to farmers in 2005. Improved land stewardship and environmental sustainability were the ultimate goals of this program. Delivery of the program is through the BC Agriculture Council. Each farm's individual plan was based on a set of auditable standards contained in the EFP. It was the structure of the EFP upon which the BC Poultry Biosecurity Program was modelled.

From the outset, it was recognized that the mandatory standards to be developed would have to be practical, effective, and auditable. Using the Red Binder as the foundation, its 27 biosecurity standards or procedures were closely reviewed and revised by the Biosecurity Technical Subcommittee. All principles were retained, but some were combined into a single standard, while others became steps needed to accomplish other standards. The final 18 mandatory standards were listed in 2006 (Table 1) and organization into a written manual began in earnest. The 18 standards were grouped into four broad categories: Farm Access (5 standards), Barn Access (4 standards), Flock Health Management (2 standards), and Farm Management (7 standards). Standards covered elements from procedures for entering and leaving a premises or its barns to required flock observations and responses when anomalies were seen. The Biosecurity Standards can be reviewed in detail at the website given in the References 5.

It was recognized during the HPAI 2004 response that the poultry industry had a key collaborative role in an avian Foreign Animal Disease response and this highlighted the need for an industry-specific plan. The Poultry Industry Emergency Response Plan (ERP) 4 was developed in a manner similar to the Biosecurity Program, with an Emergency Response Committee to steer the process and ad hoc subcommittees to deal with specific details. The ERP was designed to be entirely complementary with the Biosecurity Plan. A response triggered by flock health observations, as detailed by procedures outlined in the Biosecurity Plan, would potentially activate the Industry Emergency Response Plan. As a complementary project to the Biosecurity Standards and the Emergency Response Plan, a Premises Identification Program was initiated.

The goal of this program was to have the capability of rapidly locating all farms and collate real-time population details to aid any emergency response effort by identifying all farms within a high risk zone and predicting surveillance needs or, in the event of infected premises, for predicting euthanasia and disposal needs for those farms. This program was to be an important part of the Industry Emergency Response Plan.

Interaction of the Biosecurity Program with the Emergency Response Plan was essential, and was covered through prescribed procedures activated under specific circumstances. For example, flock health standards require that a Standard Operating Procedure is in place that prescribes situations in which veterinary or laboratory diagnoses are required. This improves the chances of picking up a serious disease problem early in its course. A self quarantine guideline is provided to assist the producer in following appropriate steps should a serious infectious disease be suspected. The self quarantine procedures outline steps that will increase the ability of the farmer to contain a disease. A farm log is required that tracks all visitors and activity, facilitating any trace-back or trace-forward that might be required for an infectious disease response. These activities would potentially trigger the Industry Emergency Response Plan and activate the first steps required of all industry in the event of an animal disease emergency.

The first edition of the Biosecurity Program was released in early 2007 and consisted of a Planning Guide and a Reference Guide. The actual delivery of the program to the farms was the next challenge. Following the lead of the Environmental Farm Plan, a team of planning advisors and auditors was trained in biosecurity in general and the Biosecurity Standards in particular. Each farmer was responsible for developing their own farm's specific plan and the planning advisor's role was to meet personally with farmers and assist in its development. It was the farmer's responsibility to implement the plan. Once the plan was in place and the farmer was ready, an audit was requested. An auditor attended the farm and reviewed the producer's plan, checking for the specific critical points to assure that compliance was realized. The farmer was left with a checklist that detailed the audit results and the elements that required corrective action. Identified deficiencies were corrected by the farmer prior to a follow-up audit. Once complete to the satisfaction of the auditor, the farm was certified under the BC Poultry Industry Biosecurity Program.

The goal was to have the plan rolled out to all farms by September 30, 2008, first audits complete by October 31, 2008, and certification complete on all farms by December 30, 2008. The plan has now been successfully implemented on 99% of the regulated poultry farms in British Columbia. Only a handful of farms having difficulty meeting some standards or refusing to cooperate remain to be certified.

CHALLENGES

Many challenges were encountered throughout the life of the Biosecurity Program. Most were resolved, though not necessarily to everyone's satisfaction. Some, however, remain contentious. A number of identified weaknesses among contemporary practices meant that some cultural changes were required. Among these were details such as increased documentation, tighter control over people entering the premises, and prescribed procedures for entering a premises and its barns.

There was a certain amount of industry push-back on the whole process, with most producers feeling that they had sufficient biosecurity to keep themselves from risk. The most acute division was between those producers in the zone that was depopulated during AI 2004 and those outside the zone. The trauma of experiencing depopulation was apparently a major motivator for the implementation of biosecurity. Educating farmers about the benefits of biosecurity when they have not, in fact, experienced a significant disease event is a difficult but important part of implementing an industry-wide program.

One of the greatest challenges met during the development of the standards was the issue of wording. While auditable standards were required, the need for flexibility was highlighted by the variation in farm types, not just among commodity groups but also among farms within each commodity group. This resulted in a great deal of time spent debating over the wording of standards and requirements to meet those standards. As a simple example, appropriate use of the words "will," "must," or "should" was argued extensively. To deal with this problem at least to some degree requires that the technical committee has a good understanding of the structure and variation within the industry for which the program is being designed. A "cookie-cutter" approach will not work and a program for one area cannot be easily fit into another unrelated area.

To help deal with the issues of variance from a fixed standard, a risk analysis procedure was put into place. This procedure allowed for a variance from the standard provided a valid reason was given and a plan was provided to reduce the risk that might be associated with the variance. The requirement for gates to be closed was a hotly contested standard; while people, equipment, and vehicles were recognized as important vectors for disease agents, the standard requiring gates to be kept closed was challenged on many fronts. Several good arguments were brought

forward in an attempt to soften this standard, including matters of safety and practicality. The risk analysis procedure helped to answer some of these issues on a case-by-case basis, but the controversy still rages.

The use of an anteroom as a transition area from the Controlled Access Zone to the Restricted Access Zone (the bird housing area) created significant controversy as this meant a major cultural change for some producers. Funding was available to assist in making changes where required in certain circumstances and, eventually, the matter was resolved. This, however, did not occur without some foot-dragging in some quarters.

While the conventional poultry sectors in BC was well engaged in the biosecurity process, there was a large fifth player that could not be as readily included – the unregulated specialty bird producers. This group included principally producers of waterfowl and game birds. Because they did not fall under a supply managed system, there was no way for costs to be built in to their prices to account for the changes that would be required to bring their farms into compliance. Many of such producers viewed the efforts that would be required by them to be of benefit only to the conventional poultry sectors. Furthermore, without oversight by any board, there was no way that auditable standards could be enforced. The specialty bird sector remains to be brought under the biosecurity umbrella, and solutions to some of the issues are still being sought.

No plan, particularly one promoted by government or regulatory agencies, is without its fierce opponents. A handful of producers resisted the implementation of a plan and auditing of their premises. These holdouts eventually saw value in their participation in the program when it became clear that penalties in the form of lost quota would be dealt to those that were not certified by the deadline of December 31, 2008.

CONCLUSION

While most farmers would view anything that is mandatory is an evil thing, the necessity of mandatory standards was recognized after the costly avian influenza outbreak of 2004. Once that recognition was clear, the road to a concrete biosecurity plan was open, but it was definitely not without its bumps and detours. After much debate and many challenges, the BC Poultry Biosecurity Program became a reality and raised the bar significantly for a very vulnerable industry. With this plan in place, poultry production in BC should be just a bit more biosecure.

REFERENCES

1. Report on the Canadian Poultry Industry Forum, http://www.bcac.bc.ca/documents/CPIF-Dec15withlinks.pdf.
2. The BC Poultry Industry Biosecurity Initiative, http://www.bcac.bc.ca/documents/BC%20Poultry%20Association%20Biosecurity%20Initiative%20Version%201.0%2005.02.16.pdf.
3. The Canada – British Columbia Environmental Farm Plan Program, http://www.bcac.bc.ca/EFP_pages/documents/index.html.
4. The BC Poultry Industry Emergency Response Plan, http://www.bcac.bc.ca/bio_emergency.htm
5. The BC Poultry Biosecurity Program, http://www.bcac.bc.ca/bio_program.htm.

Table 1. The BC Poultry Industry Biosecurity Standards.

Standard #	Standard
Farm Access Standards	
1	A secure barrier that restricts vehicle entry must be present at all primary and secondary accesses to the Controlled Access Zone.
2	Approved biosecurity signage must be clearly displayed at all primary and secondary accesses.
3	All primary accesses to the Controlled Access Zone must be constructed of hard surface or gravel that prevents any persistent accumulation of pooled water.
4	All primary accesses to the Controlled Access Zone must have an approved Cleaning and Decontamination site for vehicles and personnel.
5	The Controlled Access Zone must be maintained clean and free of organic debris at all times.
Barn Access Standards	
6	All poultry barn entrances shall remain locked at all times that the barn is unoccupied by farm personnel.
7	Approved restricted access signs shall be posted at all barn entrances.
8	All poultry barns must have an anteroom at all primary entrances that allow personnel to comply with the farm biosecurity procedures during entry and exit.
9	Barn entryways and anterooms must be maintained clean and free of debris at all times.
Flock Health Management Standards	
10	Individual flock health records must be maintained
11	Poultry mortalities and cull eggs must be handled and disposed of in an approved manner.
Farm Management Standards	
12	An effective pest control program must be in place.
13	A management program that prevents the contamination of feed and water sources must be in place.
14	All equipment and materials related to the production of poultry that enter or leave the Controlled Access Zone, regardless of size or use, must be clean and decontaminated.
15	All farms must have a documented manure management strategy.
16	On-farm biosecurity training is required for all producers and farm employees.
17	Standard operating procedures (SOP) for on-farm biosecurity must be available.
18	An activity log book for the premises that records visitors and daily on-farm activities relevant to the biosecurity standard operating procedures must be maintained.

INTERESTING DIAGNOSTIC CASES FROM PENNSYLVANIA

P. A. Dunn[A], E. A. Wallner-Pendleton[A], S. E. Myers[A], S. Kariyawasam[A], H. Lu[A], D. J. Hampson[B], A. Murdoch[C] , and C. DebRoy[A]

[A]Animal Diagnostic Laboratory, Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA 16802
[B]Murdoch University Veterinary School, Murdoch, WA, Australia
[C]Spirogene Pty Ltd, Murdoch University, Murdoch, WA, Australia

Descriptions of several cases of different poultry species and production types submitted to the Pennsylvania State University Animal Diagnostic Laboratory of the Pennsylvania Animal Diagnostic Laboratory System (PADLS) will be presented.

CASE REPORTS

Broiler: A series of clinically similar cases were submitted from one broiler company during the fall and winter of 2008. The company consists of feed mill and grow-out operations, but does not include breeder, hatchery or processing plant facilities. Most chicks are from one hatchery source. Part of the production is raised without antibiotics and part is on conventional feed and medication programs, with a different genetic strain of bird for each. The common complaint was increased culls due to lameness. Typically the lameness was first noticed in a small percentage of the birds at 14 to 16 days, and weight loss, stunting and dehydration of affected birds would follow. Low level incidence would continue throughout the grow-out, even with consistent culling. Poor culling efforts in some flocks resulted in large numbers of birds left by the catch crew. In one example, ~400 birds in a ~30,000 bird flock were left in the house to be killed by the grower. Flock livability rates were typically decreased by 2 to 4% when compared to normal baselines for this company. Performance at the processing plant was not reported as significantly different in most cases. Flocks in both antibiotic free (abf) and conventional programs were affected. *In ovo* administration of antibiotics at the hatchery was used in the conventional program, but had recently been discontinued in the abf program. Progeny from one breeder flock raised on the abf program were more often affected, but there was no specific breeder flock association identified for the other strain on the conventional program. The occurrence of the problem in a flock did not appear to correlate with first week mortality rates, type of house, litter source, feed, lights, geography, flock supervisor, feed deliveries or other traffic. Routine flock monitoring during this time period for "gut health" and bursa scores revealed adequate (better than usual) coccidiosis control and normal bursas.

Bird ages in the submitted cases ranged from 18 to 42 days. The most consistent lesions were osteochondritis, most often in the proximal tibiotarsus, and synovitis of various leg joints. Other lesions included pericarditis, splenomegaly, and hepatomegaly. The most consistent bacteria isolated by routine culture of swabs from the lesions (bone, joint, spleen, liver, pericardium/heart) were gram positive cocci that were positive for the bile esculin reaction, but negative for growth in the presence of 6.5% sodium chloride. In every case, the isolates were identified as *Streptococcus suis* by the automated identification system routinely used in our laboratory. However, results of DNA sequencing of the 16S ribosomal RNA gene of six representative isolates matched 97 to 99% with *Enterococcus cecorum*. The results showed that the isolates were not related to *Streptococcus* spp. including *Streptococcus suis*. Attempts at isolating this bacterium from different sources/inputs for this company, including one- and two-day-old chicks (yolk sac, liver, pericardium, joint, brain), litter, feed truck, and feed, were negative. During the course of this investigation in 2008, *Enterococcus cecorum* infection was confirmed by culture in submissions from seven different farms, and was suspected in seven others. Retrospectively, "*Streptococcus suis*" was isolated from bone, joint, liver, and heart from two similar cases from this company in 2005. Two of the involved growers (one from a 2005 case and one from a 2008 case) also raise swine, and the initial identification of the bacterium led to speculation that transmission of a specific swine pathogen to chickens may have occurred. Identification at the genetic level suggests otherwise. An underlying cause (change in bacteria, host or environment) for this problem has not been determined.

Broiler Breeder: Cases from three broiler breeder flocks of 22 to 28 weeks of age from one company were submitted. Increased culls that were underweight and not maturing into breeding condition compared to flock mates were evident within a few weeks after placement in the breeder houses. Both females and males were affected. The manager

suspected that some of the affected birds were blind. Gross eye lesions consisting of bilateral cloudy areas centrally and/or at the margins of the iris were present in several birds, and bilateral buphthalmos was present in a few. Corneal ulcers were not present. Cataracts and anterior uveitis of variable severity were confirmed microscopically as the main lesions. After affected breeders were culled, the flocks achieved acceptable production statistics for the rest of the lay period. The problem was not seen in any other breeder flocks in this company.

History revealed that these birds were from pullet/cockerel flocks in which a confirmed diagnosis of hypoglycemia-spiking mortality syndrome (HSMS) was made at about nine to 14 days of age, and that other causes of chick mortality and neurologic disease had been ruled out at that time. Although cataract formation has not previously been reported as a sequela to HSMS in chickens, both hypoglycemia and hyperglycemia are known initiators of lenticular degeneration in other species. Most confirmed cases of HSMS have been in broilers that have insufficient life span for detection of slowly developing lesions.

Layer: Several cases of layers were submitted for increased numbers of eggs with stained shells, wet droppings, soiled vents, and in a few instances, mildly decreased egg production. In the most extreme case, 6 to 8% of the eggs were sorted out as "dirties." A large layer company reported consistent economic losses from downgraded eggs partly due to "dirties." For example, a 1.2 million bird complex had 3 to 4% stained eggs compared to 1% or less on other farms. At the time, this represented a loss of $0.30 per dozen or $5500 per week on this complex. In most of the cases, feed, water, equipment and management factors had been ruled out as contributors. The most common finding was detection of spirochete-type bacteria in the cecal mucosa, and lack of detection of other enteric and urinary tract pathogens. PCR tests on some of these samples most often identified the spirochete as *Brachyspira intermedia* (*BI*). In a few cases, a presumptive identification of *Brachyspira pilosicoli* (*BP*) was made by histopathology based on the colonization pattern considered typical for this species (mats of spirochetes aligned in parallel to each other and perpendicular to the cecal mucosal epithelium). Small challenge studies in our lab with a *BI* isolate from a field case were not successful at reproducing the condition reported in the field, but bird numbers were quite low and other confounders were present.

In the summer and fall of 2008, in collaboration with veterinary scientists in Australia, a survey was done to determine the presence of certain *Brachypira* spp. in Pennsylvania (PA) layer flocks and possible correlation with production problems. A series of fecal sample collections and flock manager questionnaires from 20 different commercial layer flocks were completed. Flock ages ranged from 46 weeks to two years. A variety of management styles, housing, breeds, feeds, antibiotic usage, flock sizes and locations were represented. Fifty fresh fecal samples from each flock were tested by PCR for *BI*, *BP*, and, in some cases, other *Brachyspira* spp. Preliminary data on *BI* and *BP* show that 13 flocks were positive for *BI* only (in 10 to 100% of samples), one flock was positive for *BP* only (64% of samples), four flocks were positive for both *BI* (58 – 100% of samples) and *BP* (8- 82% of samples), and two flocks were negative for both. Of the 11 flocks for which the managers noted increased dirty eggs on the survey, nine had *BI* (in 70% or more of samples) and no *BP*, one had *BP* (in 64% of samples) and no *BI*, and one had neither. Of the seven flocks for which the managers reported antibiotic use within three months prior to the sampling date, six were positive for *BI* and negative for *BP*, and one was positive for both. The results show high rates of infection by two potentially pathogenic intestinal spirochetes in older layer flocks in our state. More studies are needed in North America to further define epidemiology and possible clinical, subclinical and economic significance in poultry.

Layer Breeder: A flock of 7,000 57-week-old leghorn breeders was visited in response to a complaint of a sudden onset of depression, respiratory signs, swollen eyes and face, decreased feed and water consumption and decreased egg production. Inflamed nasal passages and infraorbital sinuses (unilateral and bilateral) containing copious turbid mucoid to caseous exudates were present in most birds examined, and caseous peritonitis was also present in several hens. *Avibacterium paragallinarum* (serovar C, Modesto strain) was isolated from sinus cultures, and *E. coli* was isolated from most peritoneal/air sac swabs. Infectious bronchitis virus (IBV) (Mass serotype) was isolated from pooled cecal tonsils, but no IBV was recovered from pooled tracheas. Avian influenza, avian paramyxovirus infection, infectious laryngotracheitis, and mycoplasmosis were ruled out.

Additional history revealed that the problem began two days after many of the males, that had been moved off site for about one week, were reintroduced into the flock. Over the ~ 10 day clinical course, morbidity was nearly 100%, mortality was ~10%, and egg production decreased by ~75%. The flock was buried on the farm at 58 weeks. No other flocks in the vicinity were affected. Infectious coryza had not been diagnosed in a commercial flock in PA for many years, and the specific source of the infection could not be identified in this case.

Turkey: A series of cases with common findings have been submitted by one commercial turkey company over the past several years. Early onset of

"leg problems" and stunting were noted in the field. Increased first week mortality, decreased overall flock livability, decreased slaughter weights, and increased whole bird condemnations were also reported in affected flocks. Various medications and vitamin supplementations had been ineffective. Nutritional problems were not found, and correlation with better versus worse flock management could not be made. This company received poults from a breeder/hatchery operation in another state, and poult placement information suggested that those houses with a high percentage of progeny of certain breeder flocks were more likely to be affected than others.

Most submitted birds ranged in age from three to 26 days, and both toms and hens were represented. In general, younger birds (two to 10 days) had slightly increased clear or mildly cloudy joint fluid in hock joints (most bilateral) and mild splenomegaly and older poults (two to four weeks) were small for age and had shortened long bones and widened hocks and other joints of legs and feet. Microscopic lesions included synovitis, perichondritis and chondritis in legs, and diffuse lymphoid depletion and reticuloendothelial cell hyperplasia in spleens. Bacteria were not isolated from most swabs taken of joint and bone lesions and spleens by routine aerobic culture, nor detected in gram-stained impression smears of synovial fluid. *Mycoplasma* spp. culture attempts were also negative in our laboratory. By PCR, some joint swabs were positive for *Mycoplasma* spp., but negative for *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. Swab pools from most cases were sent to University of Georgia Poultry Diagnostic and Research Center for PCR for *Mycoplasma iowae* (*MI*), culture for *MI* or both. Tests for *Mycoplasma meleagridis* (*MM*) were also requested in some cases, but all results were negative. In 17 of these cases to date, one or more pools were positive for *MI*. Twelve other cases were considered suspect for *MI* because of findings consistent with the pattern, although *MI* was not detected. In 2008, one submission of legs from 16-day-old toms from an out of state company that receives poults from the same hatchery source was also positive for *MI* by PCR. In our laboratory, *MI* infection was diagnosed in very young poults with high mortality from an independent turkey farm in the early 1990s. No cases had been confirmed in PA before that or in the ~14 year interval between then and the onset of the currently described cases.

Game Bird: A single case consisting of three two-year-old chukar partridge breeders (one male and two females) from a breeder/hatchery operation was submitted in the winter of 2008 with a flock history of lethargy, weight loss, and increased mortality. The flock was kept on raised wire floors in an enclosed house. The house was described as "used for many years" and ventilation was reported as "not the best." The owner reported some problems with mice in the house, but no problems with wild birds. The birds were fed a commercial game bird breeder ration. Production during last year's breeding season was considered normal by the owner.

Gross lesions included unkempt plumage (two birds), overgrown beaks (three birds), decreased body weight (two birds), multiple tan or gray nodular masses in lungs (all birds) and gizzard and intestines (two birds), firm and mottled liver (two birds), mild splenomegaly (one bird), and exudate in conjunctiva and sinuses (one bird). Tests for mycoplasmosis and fungal infections were negative. Microscopic examination of lung, proventriculus, ventriculus, intestine, liver, spleen, nose, and sinus showed focal or multifocal granulomas. Acid fast-stained sections of these tissues were positive for acid fast rod-shaped bacteria which were more numerous in lung, intestine and gizzard than in liver and spleen. *Mycobacterium avium* subspecies *avium* was isolated and confirmed by DNA probe at the National Veterinary Services Laboratory.

The remaining breeders were depopulated, and the owner's intentions were to thoroughly clean and disinfect the house, and to keep breeders through one breeding season only. Although avian mycobacteriosis has been diagnosed sporadically in aviary and hobby collections of several species in our laboratory, it had not been diagnosed in any game bird farm submissions in memory.

All Species – Avian Influenza (AI): The most interesting and appreciated aspect of AI in our state is the absence of "significant" outbreaks during the past 7+ years. ("Significant" in this context is defined as H5 or H7 AI virus (AIV) in multiple flocks of commercial-size flocks of gallinaceous birds.) The only notifiable AI detected in our laboratory this year was in an out of state submission of young pheasants in a mixed species game bird operation from which H5N8 low pathogenicity (LP) AIV, waterfowl strain, was isolated. The four significant AI outbreaks in PA in the past 26 years have all involved H5 or H7 viruses that matched the predominant strains circulating in the northeast urban live bird markets (lbm) concurrently, and that had likely adapted from waterfowl strains to gallinaceous strains under multispecies selection pressure over time. During the past eight to nine years, a more rigorous, multifactorial approach has been taken to decrease the likelihood of AIV introduction into and AIV persistence within the lbm system. The most recent, persistently resident strain in the Northeast lbm (H7N2 LPAIV) appears to be eliminated from the system at this point. From the perspectives of supply states such as ours, the major market states of New York and New Jersey, and the commercial poultry

industry at large, it is hoped that these efforts continue to be successful.

(The full length article on *Brachyspira* spp. in Pennsylvania will be submitted for publication in *Avian Diseases*.)

INTERESTING DIAGNOSTIC CASES IN WESTERN CANADA

C. B. Annett

Alberta Agriculture and Rural Development, 97 East Lake Ramp N.E., Airdrie, AB T4A 0C3

SUMMARY

The following cases were included in this paper because they were interesting presentations of existing diseases in poultry or were cases that were diagnostically or epidemiologically challenging when determining the cause of the mortality or how the pathogen arrived to affect the flock. Poultry veterinarians and pathologists from the four Western Provinces were invited to contribute and submit cases they felt would fit into this category.

CASES

Case 1. Sustained low mortality in a backyard flock of adult laying hens.

Dr. Ann Britton. Abbotsford, B.C.

History: The owner of an eight month old commercial flock of Brown Hyline laying hens that were organically raised, submitted two dead hens for post mortem examination. The producer stated that the birds were vaccinated and every morning for the past four or five weeks, he would find one or two dead birds in the chicken house, otherwise the birds appeared healthy. The producer was concerned that a predator was gaining access to his flock.

Gross Pathology: Both birds were in good body condition (1.3 and 1.4 kg). One bird had fibrinous exudate around and within the pericardial sac and the spleen was moderately enlarged. In both birds, there was widely disseminated fibrinous to cooked yolk-like material over serous surfaces in the abdominal cavity with friable adhesions to the abdominal wall. Many large ova were present in the ovary containing inspissated yolk.

Gross Diagnosis: Yolk peritonitis and fibrinous pericarditis

Histopathology: Numerous tissues with scattered colonies of intravascular coccobacilli. The liver, spleen and ovary had multifocal areas of necrosis.

Diagnosis:

Comments:

Case 2. Tumors in turkeys.

Dr. Catherine Graham, Lethbridge, AB

History: A meat inspector from a nationally-regulated poultry processing plant was monitoring the slaughter of a 74 day old turkey flock. One bird was pulled from the line and condemned because it had multiple firm white masses in the liver and the spleen. Subsequently, tissues from this condemned turkey carcass were submitted to the Canadian Food Inspection Agency pathologist in Lethbridge, Alberta.

Gross Diagnosis: Lymphoid leukosis

Histopathology: Cells look to have originated from lymphoid or histiocytic lines. There is a lack of uniformity in the cell population. Numerous larger masses have a necrotic centre with macrophages and giant cells surrounding them.

Diagnosis:

Comments:

Case 3. Increased mortality broiler chicken flocks in Saskatchewan.

Dr. Bob Goodhope, Saskatoon, SK

History: Five farms in the past year had had outbreaks. All farms had multibarn production and mortality was reported at 3 to 10%. The birds were typically affected at five to six weeks of age. There was no apparent seasonal effect with respect to the affected flocks. Producers reported that the birds were found dead and rotted quickly.

Gross Pathology: Most birds were very depressed but were in good body condition. The skin over the abdominal area and the shoulders was red to purple and would tear easily and was undermined by blood tinged fluid with tiny bubbles. Gas bubbles were also present in breast muscle, spleen and liver. The breast and leg muscles were pale and had a 'cooked' appearance.

Gross Diagnosis: Acute myositis with sub-cutaneous emphysema.

Histopathology: There is massive necrosis in the skin overlying the affected muscles. The pectoral and leg muscles had acute necrosis and were heavily

infiltrated with rod bacteria. There as focal necrosis in the myocardium.

Diagnosis:

Comments:

Case 4. Spike in mortality in a flock of meat turkeys.

Dr. Darko Mitevski, Airdrie, AB

History: Ten birds from a 63 day old flock of 11,000 turkeys were submitted. The producer reported that there had been six birds found dead the day before, but 50 were found dead the day of submission.

Gross Pathology: All 10 birds had swollen kidneys and were dehydrated. There were petechial hemorrhages on the mucosal surface of the proventriculus and the cecal tonsils. 5/10 had white chalky material on the pericardium.

Gross Diagnosis: Visceral urate deposits and mild, multifocal proventricular hemorrhage.

Diagnosis:

Comments:

Case 5. Sudden increase in mortality in spiker roosters.

Dr. Sandra Stephens, Saskatoon, SK

History: One barn of 24 week old roosters, which were used for spiking broiler breeder barns, experienced a sudden severe, increase in mortality of 36% on day one and 40% the following day. The Canadian Food Inspection Agency (CFIA) visited the farm and collected oropharyngeal and cloacal swabs from birds on the farm and submitted these for influenza A testing by polymerase chain reaction (PCR). Additional swabs and tissue samples for virus isolation and blood for serological examination were sent to the National Centre for Foreign Animal Diseases (NCFAD) in Winnipeg. A quarantine was placed on the premises pending laboratory results.

Diagnosis: Influenza A H7N3

Comments:

Case 6. Suspected Newcastle disease in a pheasant breeding flock.

Dr. Tom Inglis, Airdrie, AB

History: A mixed animal practitioner who monitors the export of pheasants from this breeding flock of 75,000 birds, contacted Dr. Inglis when he was alerted to the fact that there was a sudden rise in mortality. Over a 10 day period the mortality was recorded to be overall, only 1%, but with 75% of these dead coming from one pen (300/2500). The mixed animal veterinarian was asked to submit birds representative of the problem so that a post mortem examination could be performed. The CFIA was notified of this sudden increase in mortality and tissues were submitted for influenza A and Newcastle disease testing. A quarantine was placed on the premises pending testing results.

Gross Pathology: Three adult males were submitted. Two of the three birds had petechial to ecchymotic hemorrhages on the mucosal surfaces of the proventriculus, cecal tonsils, cloaca, leg muscles and trachea. As well, hemorrhages were also present in the cerebellum. One bird had swollen kidneys and the ureters were dilated and contained white material.

Gross Diagnosis: Multifocal hemorrhages of numerous tissues. Dehydration.

Diagnosis:

Comments:

Case 7. Emaciation in a Leghorn hen.

Dr. Colleen B. Annett, Airdrie, AB

History: A producer submitted one Lohman light hen from a flock of 6,000. The producer described the bird as having a growth 'in the front of the neck' which prevented the bird from accessing feed.

Gross Pathology: The bird was moderately depressed and had a large pouch-like extension from the thoracic inlet region. Post mortem examination revealed an extremely emaciated bird (Body Condition Score 1.0/5.0). Upon incision, the pouch exuded a foul-smelling liquid grain material. There was very little food in the proventriculus, ventriculus or the remainder of the intestinal tract. This bird was not in production.

Gross Diagnosis: Emaciation with severe dilation of the crop.

Diagnosis:

Comments:

Case 8. Mortality attributed to respiratory disease in layers and pullets.

Dr. Tom Hutchison, Winnipeg, MB

a) *History*: Producer reports that in the past four weeks there have been five to 10 birds dying everyday (up to 10% final number) in his flock of 42 week old laying hens. The birds would gasp several times and fall over dead. Farm has tried electrolytes and stress aid with no luck. Birds are only found dead on the young bird side of the barn. Production is excellent.

Gross Pathology: Four birds were submitted. All were in excellent body condition. There was a light grey exudate that entirely covered, and was slightly adhered to, the laryngeal mucosa and a small portion of the proximal trachea. The underlying mucosa was hyperemic.

Gross Diagnosis: Necrotizing tracheitis

Histopathology: Laryngeal and tracheal sections have inflammatory exudate with bacteria overlying a necrotic mucosa. Laryngeal sections also have focal, epithelial proliferation and hyperplasia with vacuolar degeneration and several intracytoplasmic, eosinophilic inclusion bodies.

b) *History*: Two separate flocks (11,000 birds and 5,000 birds) of 20 week old pullets (that were raised on the same farm as the affected hens described above) were described as having increasing mortality for approximately one week.

Gross Pathology: Two birds were submitted from one flock and 13 birds were submitted from another. There was a granular, thickened appearance to the mucosa of the larynx and adjacent trachea in some of the birds.

Comments:

Histopathology: Laryngeal and tracheal sections have a thickened proliferative and hyperplastic epithelium with many eosinophilic, intracytoplasmic inclusions.

Diagnosis:

ACKNOWLEDGEMENTS

This manuscript was a joint effort of numerous veterinarians from Western Canada, including Tom Hutchison, Rosemary Postey, Mark Swendrowski, Tomy Joseph, Bob Goodhope, Susantha Gomis, Sandra Stephens, Catherine Graham, Bob Holowaychuk, Gord Kopp, Tom Inglis, Darko Mitevski, Bill Cox, Ann Britton, and John Robinson.

ENTEROCOCCUS CECORUM OSTEOMYELITIS

B. R. Charlton, G. L. Cooper, S. Stoute, A. A. Bickford, and C.G. Senties-Cue

California Animal Health and Food Safety Laboratory System - Turlock Branch, University of California, Davis
P.O. Box 1522, Turlock, CA 95381

SUMMARY

Diseases caused by *Enterococcus* spp. in the California poultry industry are not too frequent or significant. They tend to be individual bird problems or secondary to some other flock problem. *Enterococcus* spp. are normal inhabitants of the intestinal flora of poultry and were commonly referred to as the "fecal streps." *E. faecalis* and *E. faecium* are the most prevalent of intestinal enterococci but *E. gallinarum*, *E. durans*, *E. hirae*, and *E. cecorum* are also present. Identification of the *Enterococcus* spp. to the species level is not frequently performed. Few disease conditions warrant such identification although exceptions may be made on a case by case basis or when pure growth is obtained from a specimen.

During the summer of 2008, a commercial broiler company began to notice several houses of a multi-house complex were experiencing leg weakness. The mortality was elevated and a number of birds were down and/or had difficulty in walking. The addition of vitamin D had no effect. An additional ranch from the same company also experienced a similar problem during this same time period. Several submissions to CAHFS-Turlock Branch showed predominately an osteomyelitis of the proximal tibiotarsus frequently with synovitis. An *Enterococcus cecorum* was isolated in pure culture from a number of these accessions. In addition to the proximal tibiotarsus, a few birds had similar lesions in the vertebra. These birds showed the classic clinical sign of kinky-back. Histopathology of the bones showed typical osteomyelitis with large areas of heterophilic inflammation and necrosis in the bone marrow.

The identification of *Enterococci* spp. is primarily based on gram-positive cocci which are catalase-negative, grow in 6.5% NaCl, and hydrolyze esculin in the presence of bile salts. Species identification can be accomplished primarily by sugar fermentation and other biochemical reactions (1,2). The CAHFS system also utilizes the API 20 STREP (bio-Mérieux) as an additional resource. In these particular cases in which pure cultures of *Enterococcus* spp. were obtained from bone, the identification of the cultures was somewhat problematic. Over a period of six months, seven isolations of *E. cecorum* were made. The only consistent reactions obtained were the gram stain and catalase tests. Following the CAHFS identification criteria for poultry *Enterococcus* spp. strains frequently narrowed the identification down to *E. hirae* or *E. cecorum.* Neither of these two species is in the API database, so this identification kit was not too beneficial. Partial sequencing of the 16S rRNA gene was performed on three separate isolates of *E. cecorum*

and one isolate of *E. hirae* to confirm the identities of these organisms.

A ten year retrospective examination of poultry accessions in which *E. hirae* or *E. cecorum* were identified showed 80 accessions of *E. hirae* and only six accessions of *E. cecorum*. For *E. hirae*, birds less than a week of age were the most frequently involved (32% of cases), and the liver or yolk sac were the site of most isolations. The bone was a site of isolation in two cases. For the six accessions in which *E. cecorum* was identified, bone (including vertebrae and toe) was the most frequent site but isolations were also made from the blood, liver, and synovial fluid. A system wide search of accessions over the last 10 years showed two accessions from CAHFS – Fresno in 2007, both from the same producer.

E. cecorum osteomyelitis is not a new disease (3). The sudden appearance in multiple submissions over a short period of time suggests a common origin. Further investigation into the epidemiology of this condition will require cooperation among several segments of the poultry industry. Diagnosticians should consider complete bacterial identification when osteomyelitis is encountered.

REFERENCES

1. Thayer, S.G. and W.D. Waltman. Streptococcosis and enterococcoccus. In L. Dufour-Zavala, *et al*. (eds.). A laboratory manual for the isolation, identification and characterization of avian pathogens. 5th ed. Avian Association of Avian Pathologists, Jacksonville, FL. 44-46. 2008.

2. Devriese, L.A., J. Hommez, R. Wijfels, and F. Haesebrouck. Composition of the enterococcal and streptococcal intestinal flora of poultry. J Appl Bacteriol. 71:46-50. 1991.

3. Wood, A.M., *et al*. Isolation of *Enterococcus cecorum* from bone lesions in broiler chickens. Vet Rec. 150:27. 2002.

FIBRINONECROTIC TYPHLITIS IN TURKEY POULTS IN CALIFORNIA

M. Franca[A], R. Crespo[A], R. Chin[A], P. Woolcock[A], G. Senties-Cue[AB], and H.L. Shivaprasad[A]

[A]California Animal Health and Food Safety Laboratory System – Fresno
University of California, Davis, 2789 S. Orange Avenue, Fresno, CA 93725
[B]Present address: California Animal Health and Food Safety Laboratory System, Turlock
1550 N. Soderquist, Turlock, CA 95381

Fibrinonecrotic typhlitis of unknown etiology in turkey poults have been seen in California as well as in other parts of USA. Turkey poults from 32 cases were submitted to the California Animal Health and Food Safety Laboratory System, Fresno Branch, between 1991 and 2008. The age of the affected birds with typhlitis ranged from four days to one week in 30 cases. In one case, the birds were nine days old and in another case the birds were 25 days old. Both toms and hens were affected. Clinical history reported included increased mortality in 26 cases and decreased feed consumption in 13 cases. Grossly, isolated birds had mild to severely distended ceca with fibrinonecrotic cores in the lumen. Occasionally serosal and mucosal hemorrhages were observed in three cases. Microscopically, various degrees of necrosis of the mucosa with fibrin exudation and inflammation were seen. There were large numbers of bacteria within the fibrinonecrotic debris in 19 cases. *Salmonella arizonae* was isolated from the intestine in three cases. Other *Salmonella* species were isolated from the intestine in 12 cases. Culture for anaerobic bacteria was performed in 11 cases and for *Campylobacter* spp. in three cases and yielded isolation of *Clostridium perfringens* in four cases and *Campylobacter jejuni* in one case. Direct electron microscopy in portions of the small and large intestines and its contents was performed in 24 cases which revealed the presence of Rotavirus-like particles in five cases and 25 to 30 nm viral particles in four cases. *Blastocysts* spp. was seen in association with the lesion in six cases and coccidia of *Eimeria* spp. in two cases.

The exact cause of the fibrinonecrotic typhlitis in turkey poults is still unknown but it appears to be due to multifactorial etiologies including bacteria, enteric viruses, parasites, and perhaps others.

(A full-length article will be published in *Avian Diseases*.)

MYCOPLASMA IOWAE ASSOCIATED WITH SKELETAL LESIONS IN COMMERCIAL TURKEYS

David H. Ley, Rosemary A. Marusak, Eduardo J. Vivas, Oscar J. Fletcher, and H. John Barnes

Department of Population Health and Pathobiology
College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606
E-mail to: david_ley@ncsu.edu

Husbandry practices, metabolic and infectious diseases, and rapid growth may all play a role in the development of skeletal diseases in poultry (3). Skeletal lesions and diseases in poultry include: tibial dyschondroplasia, spondylolisthesis, chondrodystrophy, osteomyelitis, synovitis, mycoplasmosis, and others (2,4). *Mycoplasma iowae* (MI) infection in turkey breeders causes reduced hatchability (2 to 5%), embryo mortality (in later stages of incubation), and leg deformities (1,5). Trampel (5) described 17-day-old turkey poults with leg weakness, dehydration, chondrodystrophy of the hock joints, clear fluid in hock joint spaces, valgus deformities and shortening of the tarsometatarsal bones, and curled toes associated with MI. Experimentally in chickens and turkeys, MI has also induced airsacculitis, tenosynovitis, and arthritis, rupture of digital flexor tendons, rotated tibia and cartilage erosion (1,5).

Swollen hock joints (arthritis) and skeletal lesions occurred at very low incidences in commercial turkey flocks from two primary breeders. MI was identified by culture/immunofluorescence and MI-specific polymerase chain reaction (PCR) from some of the vertebral (back and neck) lesions, but not from swollen joints. Mycoplasma culture of vertebral lesion samples was a more sensitive diagnostic method than MI PCR. Gross skeletal lesions were consistent with chondrodystrophy. Histologic lesions were consistent with osteochondrosis, with dyschondroplasia and osteomyelitis also present. This is the first report of MI associated with vertebral chondrodystrophy and osteochondrosis in turkeys, and should now be considered in the differential diagnosis of turkeys with these lesions.

(The full-length article will be published in *Avian Pathology*.)

REFERENCES

1. Bradbury, J.M., and S.H. Kleven. Mycoplasma iowae Infection. p. 856-862. *In* Y. M. Saif (ed.), Diseases of Poultry, 12th ed. Blackwell Publishing, Ames, Iowa. 2008.

2. Crespo, R., and H.L. Shivaprasad. Diseases of the Skeleton, p.1154-1162. *In* Y.M. Saif (ed.), Diseases of Poultry, 12th ed. Blackwell Publishing, Ames, Iowa. 2008.

3. Morrow, C.J., J.M. Bradbury, M.J. Gentle, and B. H. Thorp. The development of lameness and bone deformity in the broiler following experimental infection with *Mycoplasma gallisepticum* or *Mycoplasma synoviae*. Avian Path. 26:169-187. 1997.

4. Sullivan, T.W. Skeletal problems in poultry: estimated annual cost and descriptions. Poult. Sci. 73:879-882. 1994.

5. Trampel, D.W. and F. Goll Jr. Outbreak of *Mycoplasma iowae* infection in commercial turkey poults. Avian Dis. 38:905-909. 1994.

TRICHOMONIASIS PRIMARILY INVOLVING THE RESPIRATORY TRACT OF PIGEONS IN NORTHERN CALIFORNIA

S. T. Stoute, B. R. Charlton, A. A. Bickford, and M. C. Bland

California Animal Health and Food Safety Laboratory System- Turlock Branch
University of California, Davis
1550 N. Soderquist Road, Turlock, CA 95381

INTRODUCTION

Trichomonas gallinae is the etiological agent involved in pigeon trichomoniasis or pigeon canker. It is a flagellated protozoan parasite that primarily affects the upper digestive tract in avian species. The organism typically infects squabs during feeding and infected birds usually remain carriers for life. Almost all Columbidae are carriers of *T. gallinae* but manifestations of disease do not always occur. The typical form of the disease usually affects the upper digestive tract and organisms invade the mucosa of the oral cavity, sinuses, pharynx, esophagus, crop and occasionally the conjunctiva and proventriculus. Lesions can also extend into tissues of the head, neck, nasopharynx, orbits, and cervical soft tissue. Systemic trichomoniasis involving the liver, lung, mesentery, and heart has been documented. Degenerative lesions due to trichomoniasis have also been reported in the kidney and genitalia in pigeons infected with the virulent *T. gallinae*, Eiberg strain. The following report documents a novel manifestation of trichomoniasis with the primary tissue of involvement being the respiratory tract.

MATERIALS AND METHODS

Case history. The trichomoniasis outbreak occurred in a newly constructed squab facility in Northern California. The facility consisted of one building with 20 in-line lofts separated by wire fencing. The facility housed 1000 cross-bred white feathered breeder squab candidates with 25 breeding pairs in each loft. The affected breeder candidates were the first to be housed on the new facility and were purchased at four to five weeks of age from a single source. Increased morbidity and mortality was observed in these pigeons within two weeks of the birds being transferred to the new facility. Birds six weeks of age and older were primarily affected and exhibited signs of coughing and dyspnea. Subcutaneous emphysema in the region of the thoracic inlet was also noted in some birds and may have resulted from ruptured interclavicular air sacs, secondary to dyspnea. There was a slight increase in mortality from 5% to 7% within one month of the initial outbreak in January 2008. Mortality related to respiratory trichomoniasis continued for the next three months before gradually tapering off.

Necropsy findings. A total of 11 pigeons, four live and seven dead were submitted to the CAHFS-Turlock Branch. The predominant necropsy lesions were in the respiratory tract of dead pigeons with all seven dead birds exhibiting moderate to severe tracheal lesions. Hemorrhage was present throughout the tracheal mucosa (5/7), and a solid reddish tracheal plug was present in the lumen of the other two dead birds. Two dead birds also had a yellow, caseous exudate on the abdominal air sacs, with one of these birds also exhibiting areas of consolidation in the left lung. Only one of the four live submissions exhibited a moderate reddening of the tracheal mucosa but no tracheal plug was observed. Microscopic examination of wet mount preparations of crop fluid from the four live bird submissions revealed rare numbers of trichomonad organisms with no corresponding gross lesions in the oral cavity or upper digestive tract. There were no significant diagnostic findings based on serology, virology, parasitology, and bacteriology.

Histopathology. Hematoxylin and eosin (H&E). The most significant lesion was a severe necrotizing and hemorrhagic tracheitis (10/11) characterized by an infiltration of the tracheal epithelium with a mixed population of lymphoplasmacytic cells, heterophils, fibrin, denuded epithelium, and infiltration of large numbers of protozoan-like structures throughout the lamina propria of multiple tracheal sections. Lung sections had focal areas of fibrinopurulent pneumonia characterized by caseous necrotic debris, infiltration of coccoid bacterial organisms, heterophils, lymphoplasmacytic cells, and protozoan-like parasites especially around blood vessels (5/8). Most crop and esophageal sections were devoid of trichomonads or had only a few of the organisms. One esophageal section had a focal area of ulceration with trichomonads and bacterial colonies associated with it.

Immunohistochemistry. This stain detected trichomonads in multiple tracheal and lung tissue

sections. Multiple tracheal sections exhibited brownish red staining indicative of an extensive infiltration of the lamina propria and tracheal lumen with trichomonad organisms. The staining also correlated to regions where trichomonad-like organisms were observed on H&E. Extensive brownish red staining of trichomonads were also observed in the necrotic debris of lung parenchyma and around pulmonary blood vessels of lung sections.

DISCUSSION

This case, to the best of the authors' knowledge, is the first report of a natural infection of trichomoniasis that primarily involves the trachea and lung without significant involvement of other organs. The unique presentation and the severity of tracheal and lung lesions may be associated with the pathogenicity of the infecting strain of *T. gallinae*. It may also be possible that trichomonad organisms were aspirated from the oral and upper digestive tract and were able to invade and subsequently adapt to the tissues of the respiratory tract. Clinical disease may have also been triggered by the stress of relocation from one facility to another.

The primary pathological lesions in these submissions were a hemorrhagic, caseous tracheal exudate due to an infiltration of the tracheal mucosa and lung parenchyma with trichomonads, the associated tissue inflammatory response, and build up of necrotic debris. An interesting finding in this case was a lack of significant gross and microscopic trichomonad lesions in the oral mucosal cavity and upper digestive tract where the organism is usually identified. While the H&E staining of trachea and lung microscopic sections was highly suggestive of trichomoniasis, immunohistochemistry was a useful diagnostic tool in confirming this etiological agent. Immunohistochemistry testing was specific for the *Trichomonas* genus but not specific for the species *T. gallinae*. No morphological identification or molecular sequencing was undertaken to confirm that the species was *T. gallinae*; in spite of this, other species of *Trichomonas* have never been unequivocally demonstrated to be pathogenic for the avian host (4). Antibiotic therapy is not a standard regimen in the treatment of trichomoniasis as administration may exacerbate other subclinical conditions such as candidiasis. In the United States drugs active against avian trichomoniasis, such as dimetronidazole and ipronidazole are no longer available for use in food producing birds (1,2,5). Management of this condition depends on reducing stress in birds and removing severely affected birds from the flock. The establishment of a carrier state and production of humoral immunity have been observed to have a protective effect post exposure in adults (3). Pigeons appear to be immune to disease from virulent strains after recovery from sublethal trichomoniasis (4).

REFERENCES

1. Anonymous. Animal drugs, feeds, and related products; ipronidazole. (21 CFR parts 520, 556, and 558). Code of Federal Regulations No. 10. U.S. Government Printing Office, Washington, D.C. pp. 1685-1686. 1989.
2. Anonymous. Dimetronidazole; withdrawal of new animal drug applications. Code of Federal Regulations No. 128. U.S. Government Printing Office, Washington, D.C. p. 25312. 1987.
3. Kocan, R.M. A method for producing healthy carriers of the Jones' Barn strain of Trichomonas gallinae. J. Parasitology. 55:397. 1969.
4. McDougald, L.R. Other protozoan diseases of the intestinal tract- Histomoniasis (blackhead). In: Diseases of poultry, 11th ed. Y.M. Saif, H.J. Barnes, J.R. Glisson, A.M. Fadly, L.R. McDougald, and D.E. Swayne, eds. Iowa State University Press, Ames, IA. pp. 1001-1010. 2003.
5. McLoughlin, D.K. Observations on the treatment of Trichomonas gallinae in pigeons. Avian Dis. 10:288-290. 1966.

ISOLATION AND IDENTIFICATION OF AN ADENOVIRUS FROM DUCKLINGS WITH A PROLIFERATIVE TRACHEITIS IN ONTARIO, CANADA

Marina L. Brash[A], Janet N. Swinton[A], Alexandru Weisz[B], and Davor Ojkić[A]

[A]Animal Health Laboratory, University of Guelph, Box 3612, Guelph, ON, Canada, N1H 6R8
[B]Smith and Weisz Poultry Veterinary Services, Guelph, ON, Canada, N1L 1G3

SUMMARY

Increased mortality was reported in two flocks of Muscovy ducklings from two consecutive hatches originating from the same breeder flock. Coughing, dyspnea and gasping were observed in some ducklings between six and 11 days of age. Opaque white plugs of exudate were seen in the tracheas with some ducklings having multiple tracheal plugs. Tracheal and bronchial epithelium was hyperplastic and superficial epithelial cells contained eosinophilic intranuclear viral inclusions. Virus particles compatible with adenovirus morphology were observed in tracheal epithelial cells by electron microscopy and in the supernatant from cell cultures inoculated with filtered tracheal homogenates. The isolated virus was genetically indistinguishable from duck adenovirus (DAdV-1). Our report confirms for the first time the presence of DAdV-1 in Canada and also reports for the first time adenovirus associated respiratory disease in ducklings and supports previous findings that some DAdV-1 can be pathogenic even in waterfowl.

(A full length manuscript will be submitted for publication to *Avian Diseases*.)

CHALLENGE STUDIES TO ASSESS PROTECTION OF INFECTIOUS BURSAL DISEASE VIRUS VACCINE AGAINST FIELD CHALLENGE: THE ROLE OF SPF CONTROLS

G. Avellaneda and T. Girshick

Charles River Laboratories - Avian Products and Services (SPAFAS), 67 Baxter Road, Storrs, CT 06268

Historically, chick challenge studies have been performed to assess the protection afforded for infectious bursal disease virus (IBDV) vaccines in broilers against the challenge with field isolates using the bursa: body weight (B:BW) ratios as a measurement parameter. This vaccine evaluation system gives good indication of whether or not a vaccination program is appropriate for a particular challenge strain. However, in these studies the uniformity of the challenge dose from experiment to experiment is difficult to establish creating the need of standardizing the challenge model.

The main objective of this experiment was to establish the base line of information on bursa: body weight ratios of normal specific pathogen free (SPF) birds and SPF birds challenged with different strains of IBDV. For this purpose groups of 20 SPF birds were challenged with 10^3 EID_{50} /bird of Standard, Variant E, and Al- strains of IBDV. Three replicates of each experiment were performed at different times. The B:BW ratios obtained (Figure 1) served as background to determine whether challenge experiments performed in broilers with the goal of evaluating vaccine efficacy based on B:BW ratios had the appropriate challenge dose level. This challenge model was evaluated in subsequent chick challenge where IBDV vaccinated one day old broilers were brooded and challenged at two weeks of age with Variant E or AL-2 IBDV strains. The B:BW ratios obtained for the SPF groups in the chick challenge experiments (Table1) were 4.77 for the non-infected control, 1.22 for the group infected with Variant E, and 1.09 for the group infected with Al-2. When these values where compared with the base line data (Figure 1) they showed to be within the range with the exception of the AL-2 which was slightly lower than the range of 1.3 to 1.5 obtained in the SPF studies indicating that the challenge dose for the chick challenge in broilers had been higher for this particular strain.

Interestingly the B:BW values of the different groups of broilers showed a large variation after challenge with either strain due possibly to different levels of maternal antibodies, immune response to the vaccine, or genetic make up of the different sources among other reasons. But because the B:BW ratios of the SPF birds were within the expected values, confident conclusion can be made about the protection afforded for the vaccines in the face of these two challenge strains.

Figure 1. Average of the B:BW ratios in SPF birds after infection with Standard, Variant E or Al-2 strains of IBDV in three different experiments.

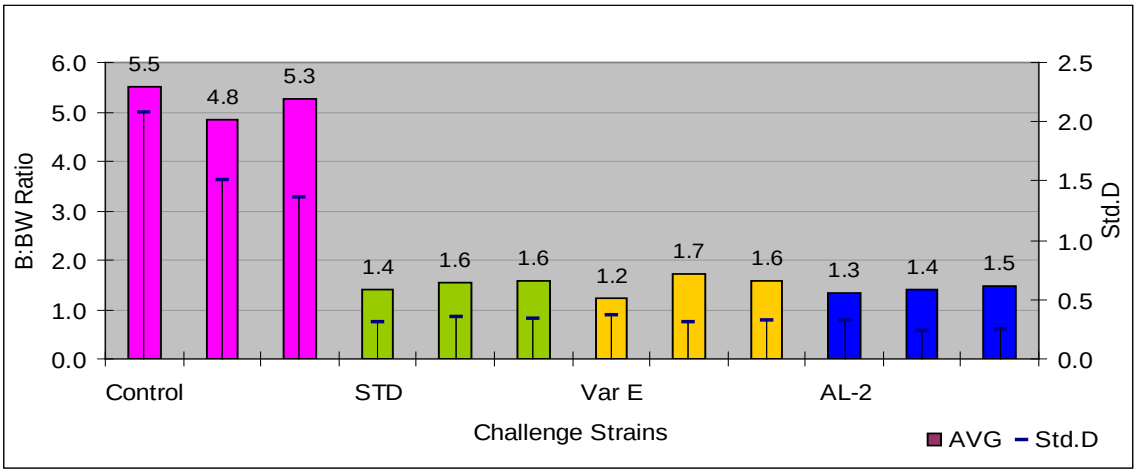


Table 1. Bursa:Body Weight Ratio in chickens of different sources challenged with different strains of IBDV

Birds Source	Non-infected Controls	Var-E	Al-2
	AVG B:BW (SD)	AVG B:BW (SD)	AVG B:BW (SD)
SPF***	4.77* (0.67)**	1.22 (0.21)	1.09 (0.23)
1	2.04 (0.51)	1.10 (0.42)	0.60 (0.11)
2	1.86 (0.43)	1.38 (0.55)	0.65 (0.12)
3	2.00 (0.53)	1.11 (0.48)	0.59 (0.11)
4	2.23 (0.57)	1.41 (0.56)	0.67 (0.14)
5	2.51 (0.47)	1.88 (0.75)	0.93 (0.32)
6	2.19 (0.44)	1.57 (0.52)	0.81 (0.16)
7	2.20 (0.27)	1.18 (0.54)	0.67 (0.18)
8	2.07 (0.52)	1.21 (0.56)	0.66 (0.17)
9	1.95 (0.37)	1.00 (0.41)	0.66 (0.13)

* Average bursa:body weight ratios
** Standard Deviation (%)
***Non-vaccinated SPF birds used as challenged control group

PREVALENCE OF *NETB* GENE IN *CLOSTRIDIUM PERFRINGENS* FIELD STRAINS ISOLATED FROM CHICKENS

Luca Bano, Ilenia Drigo, Cosetta Bacchin, Monia Cocchi, Angela Guolo, Barbara Marcon, Michela Bonci, and Fabrizio Agnoletti

Istituto Zooprofilattico Sperimentale delle Venezie, Laboratorio di Treviso, viale Brigata Treviso 13/A, 31100 Treviso, Italy

ABSTRACT

Clostridium perfringens (CP) is well known as the etiological agent of necrotic enteritis (NE) in chicken. For over 30 years α toxin was considered the key virulence factors in this type of pathology. Recently a new toxin related to the appearance of NE, called NetB, has been described. The aim of this work was to evaluate the presence of genes coding for α (*cpa*), β (*cpb*), ε (*cpetx*), ι (*cpi*), β2 (*cpb2*), enterotoxin (*cpe*) and NetB (*netB*) toxin in CP field strains collected from chickens affected or not by enteric diseases. Seventy-two CP field strains were toxin typed: 22 isolated from chickens affected by NE, 38 from chickens with intestinal lesions not ascribable to NE and 12 from healthy chickens. 91,6% strains were positive for *cpa* gene (toxintype A) and 8,3% for *cpa* and *cpb2* genes (toxintype A+β2). 33.3% CP resulted *netB* positive and 91.6% of these were isolated from chickens affected by intestinal diseases: 14 with NE and 8 with macroscopic lesions other than NE. The number of *netB* positive strains was significantly higher (p = 0,002) in chickens affected by NE (61%) than in birds with different intestinal disorders (23%). Our preliminary results seem to confirm the presence of *netB* gene in CP involved in NE outbreaks, even if, its role should be verified by means of the evaluation of the toxin expression.

INTRODUCTION

Clostridium perfringens (CP) is an important enteropathogenic agent in humans and animals. It is often found in the intestinal tract of healthy animals but it can cause outbreaks of serious enteric diseases through the production of a variety of toxins. The differential production of the four major toxins (α, β1, ε, and ι) is used to classify strains into five toxin-types (8). Some CP strains, in addition to α toxin, produce β2 and enterotoxin: two toxins that have been proposed as being important in the pathogenesis of intestinal disorders in animals and humans respectively.

In poultry, CP is well known as the causative agent of both acute and sub clinical necrotic enteritis (NE). Acute NE is characterized by high mortality rates without premonitory signs and at necropsy the mucosa of the small intestine appears strewn of large necrotic foci while, in severe cases, necrotic material covers large tracts of the intestinal mucosa surface. In sub-clinical NE the intestinal mucosa damage is limited and this condition is characterized by malabsorption with consequent reduced weight gain and increased feed-conversion ratio (6).

Historically, the α toxin has been recognized as the key virulence factor in this type of pathology but Keybourn and co-workers (5), using α-toxin knock-out mutant of CP, brought evidences that it is not an essential virulence factor in NE. The same Authors in 2008 described a novel toxin, NetB, that displays a moderate amino acid sequence similarity with CP β toxin and that seems to be expressed in most strains isolated in NE outbreaks (4). On the contrary, a recent prevalence study on *netB* gene in clinical isolates of CP from animals in US, arrived at different conclusions (7).

The aim of our study was to evaluate the presence of genes coding for α (*cpa*), β (*cpb*), ε (*etx*), ι (*cpi*), β2 (*cpb2*), enterotoxin (*cpe*) and NetB toxins in CP field strains collected from healthy chickens and from subjects affected by enteric diseases.

MATERIALS AND METHODS

Source of isolates. 72 CP field strains were analysed. The strains were isolated from the intestine (5 cm back and 2 cm after Mekel's diverticolum) of 22 chickens affected by NE, 38 chickens with intestinal disease not ascribable to NE and 12 healthy chickens.

Strains and growth conditions. All strains were obtained streaking on Perfringens Agar Base (Oxoid) 0.1 mL of 24 h broth (Cooked Meat Medium, Difco) previously inoculated with intestinal samples. CP ATCC 27324 (toxin-type E + enterotoxin), CCUG 2036 (toxin-type C), CCUG 2037 (toxin-type D), ATCC 10543 (toxin-type A+ β2) were used as reference strains. All strains were incubated in anaerobic conditions at 37°C for 48 hours.

DNA extraction. Five colonies of each CP strain included in the study were recovered from the agar plate and the DNA was extracted with DNeasy Blood & Tissue Kit (Qiagen) according to manufacturer's instructions.

Toxin coding gene detection. One multiplex PCR for *cpa*, *cpbl*, *cpetx*, and *cpi* genes and three single PCR for *cpb2*, *cpe* and *NetB* genes detection were used (1,8,3,4). The sequencing of the amplified product confirmed that the targeted *netB* gene was indeed amplified with the PCR assay.

Parasitological examination. Intestinal mucosa of all chickens was scraped in different districts and observed by optic microscope searching for protozoa and helminths (eggs and worms).

Statistical analysis. For statistical analysis Fisher exact test has been used.

RESULTS

All strains resulted positive for α toxin gene (toxin-type A) and only six (8.3%) of these were positive also for β2 toxin (toxin-type A + β2). No CP cpe-positive strains were detected.

24/72 (33.3%) CP were *netB* positive and 91.6% of these was isolated from chickens affected by intestinal disorders. *NetB* positivity percentage in each group is summarized in Table 1.

22/72 (30.5%) chickens resulted positive for coccidia. The number of *netB* gene positive strains was significantly higher with a p-value of 0.002 in chickens affected by NE (14/22, 63.6%) than in birds with different intestinal disorders (8/38, 21%). This result is more significant ($p = 0.0001$) if we consider only chickens tested negative at parasitological examination but with necrotic enteritis.

DISCUSSION

Our preliminary results seem to support the involvement of NetB toxin in the pathogenesis of NE, even if, its role should be verified by means of the evaluation of the real toxin expression and in a larger number of chicken isolates.

It is interesting observe that NE, in coccidia negative subjects, is significantly associated with the presence of CP *netB* positive strains. This result sustains the theory that CP *netB* positive strains cause NE without the support of other pathogens (such as coccidia). Otherwise, when coccidia parasite the intestine, not only CP *netB* positive but also CP with pathogenic mechanisms different from NetB toxin, could be able to produce NE.

On the contrary, the observation that strains isolated from two healthy animals were positive for *netB* gene, confirm that other virulence factors such as proteolytic enzymes or predisposing factors could have an important role on NE appearance (9).

Anyway, in this study NE lesions were defined by macroscopic observations and for this reason the number of sub-clinical necrotic enteritis could be underestimated. In future investigations histological examination must be take into account to detect necrotic intestinal lesions.

Hence, caution is required when interpreting surveys of isolates from disease outbreaks because isolates might change during the culturing process or there might even be a mixture of pathogenic and non-pathogenic strains present in the same diseased birds.

The absence of the *cpe* gene in the CP strains included in this study, lead to suppose that chicken products do not represent an important risk for transmission of enteropatogenic CP from this species to humans. However, this last sentence must be supported by further investigations in a larger number of CP strains of chicken origin.

REFERENCES

1. Yoo, H.S., S.U. Lee, K.Y. Park, and Y.H. Park. Molecular typing and epidemiological survey of prevalence of *Clostridium perfringens* types by multiplex PCR. J. Clin. Microbiol. 35:228-232. 1997.
2. Meer, R.R. and G. Songer. Multiplex polymerase chain reaction assay for genotyping *Clostridium perfringens*. Am. J. Vet. Res. 58:702-705. 1997.
3. Baums, C.G., U. Schotte, G. Amtsberg, and R. Goethe. Diagnostic multiplex PCR for toxin genotyping of Clostridium perfringens isolates. Vet. Microbiol. 100:11-16. 2004.
4. Keyburn, L., J.D. Boyce, P. Vaz, T.L. Bannam., M.E. Ford, D. Parker, A. Di Rubbo, J.I. Rood, and R.J. Moore. NetB, a new toxin that is associated with avian necrotic enteritis caused by *Clostridium perfringens.* PLoS Pathog. 4(2): e26.doi: 10.1371/ journal.ppat.0040026. 2008.
5. Keyburn, A., L., S.A. Sheedy, M.E. Ford, M.M. Williamson, M.M. Award, J.I. Rood, and R.J. Moore. Alpha Toxin of *Clostridium perfringens* is not an essential virulence factor in necrotic enteritis in chickens. Infect. Immun. 74:6496-6500. 2006.
6. Kaldhusdal, M., C. Schneitz, M. Hofshanger, and E. Skjerve. Reduced incidence of *Clostridium perfringens*-associated lesion and improved per-formance in broiler chickens treated with normal intestinal bacteria from adult fowl; Avian. Dis. 2001.
7. Martin, T., G. and J.A. Smyth. Prevalence of netB among some clinical isolates of *Clostridium perfringens* from animals in the Unated States. Vet. Microbiol. doi:10.1016/j.vetmic.2008.10.026. 2008.
8. Meer, R., R. and J. Songer. Multiplex polymerase chain reaction assay for genotyping *Clostridium perfringens*. Am. J. Vet. Res. 58:702-705. 1997.

9. Van Immerseel, F., J.I. Rood., R.J. Moore and R.W. Titball. Rethinking our understanding of the pathogenesis of necrotic enteritis in chickens; Trends in Microbiology. 17(1): 32-36. 2008.

Table 1. Number of *netB* positive strains isolated from the gut of diseased and healthy chickens: correlation between presence and absence of intestinal lesions ascribable to NE and *netB* gene positivity.

		TOTAL	NetB +	%	NetB -	%
Healthy chickens		12	2	16.6	10	83.4
Enteric diseases	NE	22	14	63.6	8	36.4
	no NE	38	8	21	30	79.0

EPIDEMIOLOGY OF AVIAN METAPNEUMOVIRUS INFECTION IN ITALY

E. Catelli[A], M. Cecchinato[B], G. Ortali[C], E. Morandini[C], L. Sperati Ruffoni[C], P. Pesente[C], C. Franciosi[A], and C. J. Naylor[D]

[A]Dipartimento di Sanità Pubblica Veterinaria e Patologia Animale, University of Bologna, Ozzano Emilia (BO), Italy
[B]Dipartimento di Sanità Pubblica, Patologia Comparata e Igiene Veterinaria, Università di Padova, Legnaro (PD), Italy
[C]Agricola Tre Valli, Gruppo Veronesi, S. Martino B.A. (Verona), Italy;
[D] Department of Veterinary Pathology, University of Liverpool, Neston, Cheshire, United Kingdom

INTRODUCTION

Avian *Metapneumovirus* (AMPV) is a negative sense RNA virus, envelope virus which is the type species in the genus *Metapneumovirus* in the *Paramyxoviridae* family. It is the casual agent of turkey rhinotracheitis (TRT), and also causes a respiratory infection in chickens, which can result in swollen head syndrome. At present four AMPV subtypes have been characterised: the A and B European subtypes (5), the United States C subtype (6) and a subtype, referred to as D, which includes two old French isolates (1). At the beginning of 2000 little information was available on circulation and molecular epidemiology of AMPV Italian poultry except for the data regarding the first Italian isolates made in the late 80s, which were shown to be B subtypes (5; Sperati Ruffoni L., personal communication). Moreover even though the vaccination was widely applied, disease still occurs occasionally in young as well older turkeys. This paper reports some studies performed in Italy since 2001 in order to understand better the epidemiology of AMPV and prophylactic problems encountered in the field.

STUDY-1

Virological, molecular and serological AMPV field survey on turkey, chicken and layer Italian farms. Since 2001 a survey of AMPV infection in Italian turkey and broiler farms, in a highly populated area of Northern Italy has been performed. Nine turkey farms and six broiler farms were sampled. Sixteen birds from each group were doubly swabbed from the choanal cleft for virus isolation on tracheal organ cultures and RT nested PCR (A and B subtype specific) using extracted RNA. At the same time blood samples were collected for a blocking ELISA serological assay. AMPV was isolated and detected by RT-PCR in 19 day-old turkeys, and in 34, 42, and 48 day-old broilers. All AMPV strains were found to be subtype B. All turkeys of more than four weeks old were AMPV

positive by ELISA. AMPV infection was found to be widely spread and the B subtype was found to be prevalent in the area sampled (2). In order to extend the epidemiological survey to a wider area, swabs from field TRT outbreaks in central regions of Italy were tested and found to contain subtype A virus. This was the first evidence of AMPV subtype A in Italy (4).

The current information on prevalence of AMPV in layers is fragmentary and the impact on egg production remains unknown. In order to draw an epidemiological picture of AMPV spreading in the layers flocks a survey was performed in 2007 in 20 flocks, on five pullets and eight layer farms. AMPV was detected by RT-PCR and blood samples were collected for ELISA serological assay. Where possible, egg production data and respiratory disease were recorded. Results confirmed the high prevalence of AMPV. All viruses were of B subtype. In two occasions AMPV infection was correlated with drop in egg production. Surveys of pullets confirmed that most groups prior to coming into lay become infected without showing clear respiratory signs. At the point of lay these groups are serologically positive to AMPV. It will be interesting to know if these birds will be protected from drop in egg production in case of further contact with the virus.

STUDY-2

Sequence analysis of fusion (F) and attachment (G) protein genes of Italian B subtype AMPVs. To establish the identity and heterogeneity in Italian AMPV strains, the nucleotide sequences of selected genes were determined and compared with previously published AMPV field strains and commonly available vaccine strains. B subtype and A subtype AMPV strains isolated in Italy from 1987 to 2007, were considered. After RNA extraction, independent overlapping RT-PCRs, covering the entire the Fusion (F) and Attachment (G) genes, were performed and amplicons sequenced. Phylograms for both gene sequences were constructed with the MEGA package, version 3.1. The B subtype sequences were clustered with the previously published AMPV B sequences. Within the European strains, significant sub clustering was apparent with the more recent isolates forming a cluster separated from viruses isolated in the previous decade. Comparison of sequences of viruses isolated prior to the introduction of B subtype vaccines shows there were very few differences in either predicted protein sequence. Viruses isolated after 2001 had similar F protein sequences but numerous G protein mutations. These mutations are likely to have produced antigenic differences between the two virus groups because they all altered highly charged, potentially charged amino acids or potential O-linked glycosylation sites. Moreover three strains isolated over a period of a few months from chickens and turkeys, showed high sequence identities. Genetic differences in these genes did not correlate to the species of the host.

STUDY-3

AMPV field evolution avoiding vaccine induced immunity. Longitudinal studies in Italian turkey farms demonstrated that subtype B viruses were frequently detected some period after subtype B vaccination. Sequencing showed that these later viruses were not derived from the previously applied vaccine. More detailed sequence analysis of fusion and attachment protein genes showed that these later subtype B detections formed a cluster (see Study-2). The attachment protein genes in this cluster were dissimilar to those found in early B subtype viruses, including the established vaccines, and these themselves formed another cluster. One day old poults were vaccinated with subtype B vaccine in experimental conditions and later challenged with either early (240 TRT-VR87) or later (205-16/04) subtype B field isolates. Protection was very poor as assessed by both clinical disease and shedding of virus after 205-16/04 challenge. The limited immunity observed may explain the dominance of the later subtype subtype B field viruses over a six year period in this Italian region. This may have resulted from immune pressure induced by mass subtype B vaccination.

STUDY-4

Field and experimental evidences of AMPV vaccine reversion to virulence. Subtype A and B AMPV vaccines are widely used in Italian commercial growing turkeys and turkey and chicken breeders. When tested under experimental conditions, these empirically derived vaccines were shown to be fully protective whilst not causing detectable disease themselves. However, they do not perform as well when used in the field and unstable attenuation has been considered to be a possible factor. Since AMPV are single-stranded RNA viruses their relatively high mutation rates have been thought to be the underlying reason for instances of reversion to virulence observed in experimental conditions. This study reports the evidence of reversion of an AMPV subtype A vaccine in the field. We isolated an AMPV a vaccine derivative from an outbreak of TRT in an Italian flock of 18 day old turkeys previously vaccinated at day old with A subtype, The vaccine derivative virus was shown to be able to cause clinical disease when applied to one day old poults in secure isolation conditions (3).

Afterwards another subtype A AMPV was isolated in association with respiratory disease typical of TRT from turkeys which had been vaccinated with a B subtype licensed AMPV vaccine. Sequencing of the virus showed that the virus had originated from a licensed live A subtype vaccine. In this instance the disease was much later, at 50 days of age, and there had been no recent history of use of the vaccine. This may indicate that AMPV vaccines are able to circulate in the environment for longer than was previously envisaged.

CONCLUSION

Since 1987 mainly B subtype AMPV has been found in Italy, in turkeys, broilers, and layers. Since 2003 subtype A has also been found, to limited degree. Two of these strains have so far been considered and genome sequence analysis and assessment of virulence by experimental infection of naïve turkeys showed these to be vaccine revertants.

The nucleotide sequences of F and G genes of AMPV subtype B strains isolated from 1987 to 2007 were determined and compared with previously published AMPV field and vaccine strains. Comparison of sequences of subtype B viruses isolated prior to the introduction of mass B vaccination shows there were very few differences. Viruses isolated after had numerous attachment protein mutations. These are likely to have produced antigenic differences that may have resulted from vaccine immune pressure.

Outbreaks of TRT still occur in the field in spite of vaccination. Use of inappropriate vaccine subtypes, evolution of field viruses able to avoiding existing vaccines and reversion to virulence are all likely to be playing a role.

REFERENCES

1. Bayon-Auboyer, M-H, C. Arnauld, D. Toquin, and N. Eterradossi. Nucleotide sequence of the F, L and G protein genes of two non-A/non-B avian pneumoviruses (APV) reveal a novel APV subgroup. J Gen Virol 81:2723-2733. 2000.
2. Catelli, E., M. Cecchinato, M. Delogu, P. De Matteo, G. Ortali, C. Franciosi, M.A. De Marco, and C.J. Naylor. Avian Pneumovirus infection in turkey and broiler farms in Italy: a virological, molecular and serological field survey. Ital J of Anim Sci 3(3):286-292. 2004.
3. Catelli, E., M. Cecchinato, C.E. Savage, R.C. Jones, and C.J. Naylor. Demonstration of loss of attenuation and extended field persistence of a live avian Metapneumovirus vaccine. Vaccine 24:6476-6482. 2006.
4. Cecchinato, M., E. Catelli, C.E. Savage, P. De Matteo, M. Faenzi, and C.J. Naylor. Evidenza di pneumovirus aviare sottotipo A in corso di un focolaio di TRT in tacchini da carne in italia. XLII Convegno Società Italiana Patologia Aviare, Forlì 2-3 ottobre 2003. Large Animal Review 9 (6): 121-122. 2003.
5. Juhasz, K. and A.J. Easton. Extensive sequence variation in the attachment (G) protein gene of avian pneumovirus: evidence for two distinct subgroups. J of Gen Virol 75:2873-2880. 1994.
6. Seal, B. Matrix Protein gene nucleotide and predicted amino acid sequence demonstrate that the first US avian pneumovirus isolate is distinct from European strains. Virus Research 58, 45-52. 1998.

ENTERIC VIRUS STATUS OF TURKEY FLOCKS OVER TIME: MOLECULAR DIAGNOSTIC STUDIES BEGINNING ON THE DAY OF PLACEMENT

J. Michael Day[A], Laszlo Zsak[A], and H. John Barnes[B]

[A]USDA/ARS Southeast Poultry Research Laboratory, 934 College Station Road, Athens, GA 30605
[B]College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606

Despite considerable evidence of the presence of certain enteric viruses in the poultry population in the United States (4,8), little is known concerning the etiology of the recurring enteric disease syndromes such as poult enteritis complex (PEC) in young turkeys and runting-stunting syndrome in broiler chickens (RSS). There is evidence that the age at virus exposure may play a part in enteric disease progression and severity in turkey poults (2,12,13). Analysis of intestinal contents from flocks showing signs of enteric disease—and from otherwise healthy flocks—often reveals infections with numerous suspect viruses. The complex nature of these enteric syndromes makes laboratory diagnosis and field management of affected

flocks difficult. Recent regional and national enteric virus surveys of commercial turkey and chicken flocks has revealed the ongoing presence of avian reoviruses, rotaviruses, astroviruses and parvoviruses in flocks, with the viruses often detected in combination (4,8). The turkey astroviruses, particularly turkey astrovirus type 2, appear to be ubiquitous in U.S. flocks, where they may affect the performance of commercial turkeys (5,9). Several turkey-origin reoviruses have recently been isolated and described at the molecular level and in pathogenesis studies, but they do not appear to be the sole causative agent in syndromes such as PEC and PEMS (1-3,7,10,11). A recent RT-PCR test targeting the NSP4 gene of turkey-origin rotavirus has revealed the presence of rotavirus in numerous field samples, including in poults prior to placement on farms (8). Avian parvovirus has also recently been described from turkeys and chickens, and its role in poultry enteric disease is currently being investigated (14). For the present study, we utilized molecular tests for several enteric viruses to assess the colonization of six turkey flocks over time. One of these flocks was placed at a Teaching Animal Unit (TAU) farm at North Carolina State University (NCSU), which was monitored for enteric viruses from the day of placement and periodically thereafter. The remaining five flocks were commercial turkey flocks placed with a turkey integrator in North Carolina, USA, one of which was a "sister flock" to the TAU flock, with poults from the same hatching being placed at the TAU farm and the commercial farm housing the sister flock.

During the early fall of 2007, in cooperation with poultry industry representatives and university researchers, we received intestinal and composite fecal samples collected from commercial and university "teaching" turkey flocks in North Carolina, USA. The barn housing the poults at the NCSU TAU had undergone a complete barn cleaning and three months of down time prior to placing the poults on fresh litter. Over the course of five weeks, beginning the week of placement, entire intestinal tracts were collected weekly from five poults on each of five commercial farms and shipped overnight on ice packs to Southeast Poultry Research Laboratory (SEPRL) in Athens, GA. Further, composite fecal samples were collected from the day of placement through day 63 post-placement from the TAU flock at NCSU. The composite fecal samples were collected every three days except for days 12 through 21, when daily composite samples were collected. The fecal samples were shipped in batches on wet ice to SEPRL. Upon receipt at SEPRL, all samples were held at 4°C and processed within 24 hours. Intestinal and fecal samples were processed into 10% homogenates in sterile phosphate buffered saline, clarified via centrifugation, and total RNA was extracted using TRIZOL reagent (Invitrogen) and the MagMax RNA extraction kit (Ambion), and total DNA was extracted using the DNeasy blood and tissue kit (Qiagen). Samples were tested via RT-PCR or PCR for the presence of the avian astrovirus polymerase (Pol) gene, avian rotavirus NSP4 gene (viral enterotoxin) the avian reovirus σNS gene, and avian parvovirus NS gene (4,8,14).

All five flocks placed on the commercial farms were positive via RT-PCR for avian astrovirus during the first week after placement, and three of the five flocks remained positive for avian astrovirus through the fifth week after placement. Avian astrovirus was not detected in the TAU flock until day 31 following placement, and was detected intermittently through day 52 following placement. Sequencing of selected amplicons revealed the presence of turkey astrovirus type 2 in all cases (9). Avian rotavirus was detected in all flocks during the sampling period, with three of the five commercial flocks testing positive for rotavirus during the first week following placement, including the sister flock. The TAU flock tested positive for rotavirus on day 17 following placement and intermittently through day 63 following placement. Parvovirus was detected in all of the commercial flocks by the fourth week following placement, with one commercial flock testing positive during the second week. Turkey-origin avian reovirus was detected intermittently in four of the five commercial flocks and only once, on day 14 following placement, in the TAU flock. Observations of flock performance taken in the field noted non-specific poult enteric disease in three of the five commercial flocks by day ten following placement; one of the commercial flocks with observed enteric disease was the sister flock to the TAU flock. The observation of enteric disease in the commercial flocks correlated with the detection of rotavirus in those flocks in the first week following placement. No enteric disease was noted in the TAU flock during the sampling period. The poults that were placed at the TAU facility and in the commercial sister flock were also colonized with *Escherichia coli* prior to placement.

The results of this study are generally consistent with other, earlier longitudinal studies which have followed the enteric virus status of poultry, in that the simple presence of enteric viruses alone or in combination is not necessarily correlated with the onset or eventual occurrence of enteric disease. Interestingly, the detection of avian rotavirus during the first week following placement in the commercially-reared poults did correlate with a field diagnosis of enteric disease in those flocks at ten days. Poults from the same hatching did not develop enteric disease during grow out on the TAU farm, despite the appearance of rotavirus at day 17 following placement. The early appearance of astrovirus in all of the commercial flocks and the

eventual appearance of parvovirus in all flocks did not correlate with the diagnosis of enteric disease. Turkey astrovirus can cause enteric signs in young poults and can have an effect on poult body weights (6). Little is known about the pathogenesis of parvovirus in turkeys. The intermittent detection of turkey-origin avian reovirus did not correlate with enteric disease. The turkey-origin avian reoviruses probably do not play a major role in enteric disease signs, although they can result in immune dysfunction in young poults (2,7,11). Further research should concentrate on the role of avian rotavirus in turkeys, particularly in birds concomitantly infected with bacteria. This study also suggests that management techniques and farm location may play significant roles in the development of enteric disease in turkeys infected with combinations of enteric viruses.

REFERENCES

1. Day, J., M. Pantin-Jackwood, and E. Spackman. Sequence and phylogenetic analysis of the S1 genome segment of turkey-origin reoviruses. Virus Genes. 2007.
2. Day, J.M., E. Spackman, and M. Pantin-Jackwood. Turkey origin reovirus induced immune dysfunction in specific pathogen free and commercial turkey poults. Avian Dis 52:387-391. 2008.
3. Kapczynski, D.R., H.S. Sellers, V. Simmons, and S. Schultz-Cherry. Sequence analysis of the S3 gene from a turkey reovirus. Virus Genes 25:95-100. 2002.
4. Pantin-Jackwood, M., J.M. Day, M.W. Jackwood, and E. Spackman. Enteric viruses detected by molecular methods in commercial chicken and turkey flocks in the United States between 2005 and 2006. Avian Dis 52:235-244. 2008.
5. Pantin-Jackwood, M., E. Spackman, and P. Woolcock. Phylogenetic Analysis of Turkey Astroviruses Reveals Evidence of Recombination. Virus Genes 32:187-192. 2006.
6. Pantin-Jackwood, M.J., E. Spackman, and J.M. Day. Pathogenesis of type 2 turkey astroviruses with variant capsid genes in 2-day-old specific pathogen free poults. Avian Pathol 37:193-201. 2008.
7. Pantin-Jackwood, M.J., E. Spackman, and J.M. Day. Pathology and virus tissue distribution of Turkey origin reoviruses in experimentally infected Turkey poults. Vet Pathol 44:185-195. 2007.
8. Pantin-Jackwood, M.J., E. Spackman, J.M. Day, and D. Rives. Periodic monitoring of commercial turkeys for enteric viruses indicates continuous presence of astrovirus and rotavirus on the farms. Avian Dis 51:674-680. 2007.
9. Pantin-Jackwood, M.J., E. Spackman, and P.R. Woolcock. Molecular characterization and typing of chicken and turkey astroviruses circulating in the United States: implications for diagnostics. Avian Dis 50:397-404. 2006.
10. Sellers, H.S., E.G. Linnemann, L. Pereira, and D.R. Kapczynski. Phylogenetic analysis of the sigma 2 protein gene of turkey reoviruses. Avian Dis 48:651-657. 2004.
11. Spackman, E., M. Pantin-Jackwood, J. Day, and H. Sellers. The pathogenesis of turkey origin reoviruses in turkeys and chickens. Avian Pathology 34:291-296. 2005.
12. Yason, C.V., and K.A. Schat. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: clinical signs and virology. Am J Vet Res 48:977-983. 1987.
13. Yason, C.V., B.A. Summers, and K.A. Schat. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: pathology. Am J Vet Res 48:927-938. 1987.
14. Zsak, L., K.O. Strother, and J. Kisary. Partial genome sequence analysis of parvoviruses associated with enteric disease in poultry. Avian Pathol 37. 2008.

EVALUATION OF PRODUCTIVE PARAMETERS AFTER USING AN O78 *E. COLI* VACCINE IN BROILERS

Veronica Flamenco

Fort Dodge Animal Health, San Salvador, El Salvador

INTRODUCTION

Escherichia coli is recognized as an inhabitant of the intestines of healthy birds. Sometimes, due to stressing factors, some *E. coli* strains can become opportunistic causing cellulitis and airsacculitis as secondary infections. Other strains, known as APEC (avian pathogenic *E. coli*) are considered highly pathogenic and affect birds by elevating mortality, increasing the percentage of condemned broilers at the slaughter-house, and generally, by affecting productive parameters.

The objective of this study is to evaluate productive parameters in broilers after using an O78 *E. coli* vaccine, as well as the percentage of condemned carcasses due to *E. coli* at the slaughter-house.

MATERIALS AND METHODS

E. coli isolates were sent to the Wiley Laboratory at The Pennsylvania State University for serotyping. These came from a poultry farm located in El Salvador that has problems related to *E. coli*. The serotypes isolated were O5, O79, and O143. Three houses containing 26,000, 25,000 and 13,000 broilers respectively, were vaccinated with one dose of an O78 modified live *E. coli* vaccine at the third day of age via spray. Another house of 13,000 broilers was left as a control group. All of these were housed on the same farm. At the hatchery, they received their normal vaccination program which included a live and inactivated ND vaccine, plus BI and AI. In the field, their vaccination program consisted of one dose of IBD and ND at day eight and at day 18. The flocks were monitored throughout the growing period and at the slaughter-house. Parameters were recorded as well were necropsies done on daily mortality. Since other variables influenced the productive parameters, it was decided to focus the trial on the percentage of clinical signs related to mortality, plus the percentage of condemned birds at the slaughter-house due to *E. coli*. The results shown here are from the second round of flocks using the O78 *E. coli* vaccine, from the same houses and farm.

RESULTS

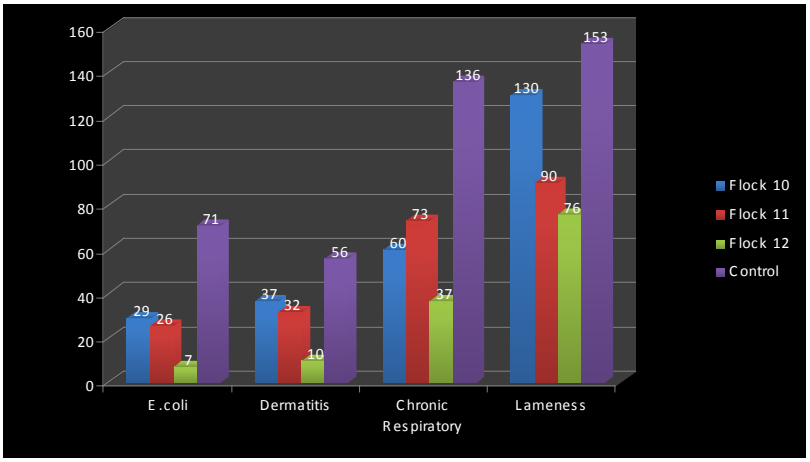
Chart 1 shows that the vaccinated flocks presented less *E. coli*-related mortality due to chronic respiratory disease and dermatitis versus the control group. The vaccinated flocks also had a lower incidence of lameness than the control group.

Chart 2 shows the number of birds condemned at the slaughter house due to dermatitis and airsacculitis, resulting in a lower number of condemned broilers from the vaccinated groups versus the control group.

CONCLUSIONS

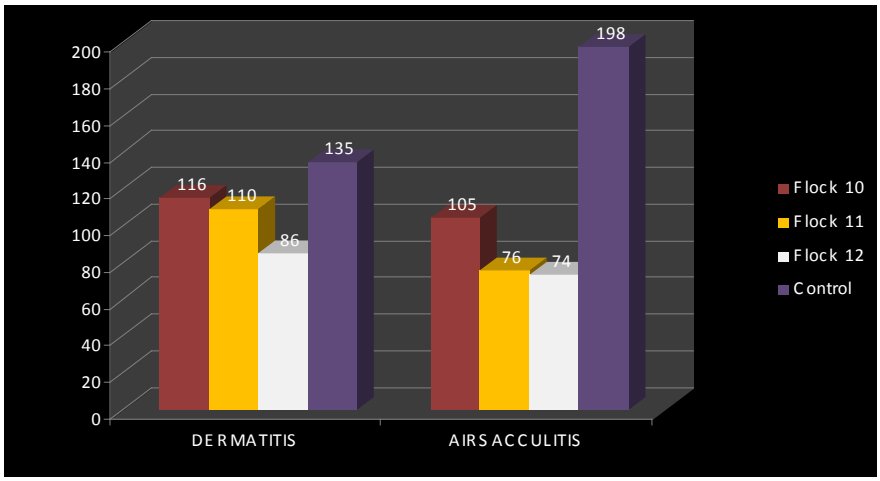
- Productive parameters such as body weight increased a 14% in the vaccinated flocks.
- The vaccine worked as an aid by reducing the number of condemned birds, and therefore, increasing profits. The vaccinated group had 32% less airsacculitis than the control group and 30% less dermatitis than the control group.
- The O78 vaccine demonstrated cross-protection against the O5, O79, and O143 serotypes from this farm.

Chart 1. *E. coli*-related mortality in vaccinated vs. control flocks.



	# of Birds
Flock 10	26000
Flock 11	25000
Flock 12	13000
Control	13000

Chart 2. Condemned birds at slaughter – vaccinated vs. control groups.



UNUSUAL AVIAN MYCOBACTERIOSIS IN COMMERCIAL BROWN LAYERS

D. Gallazzi, G. Manarolla, T. Rampin, and G. Sironi

Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria – Sezione di Anatomia Patologica Veterinaria e Patologia Aviare – Università degli Studi di Milano –via Celoria 10 - 20133 Milano – Italy

ABSTRACT

An unusual outbreak of avian mycobacteriosis in 46-week-old commercial brown layers is described. Reduced feed intake, gradual drop in egg production and increased mortality rate were observed in the flock housed in Northern Italy. Eight birds were humanely euthanatized and necropsied. The birds were in poor body condition, with pale, shrivelled combs, and principally presented prominent orbital lesions. Grossly, multiple, variable-sized nodular lesions were found in the intestinal wall, liver, spleen, lungs, bone marrow, and conjunctiva. Histologically, both visceral and orbital lesions displayed granulomatous nature with variable numbers of acid fast bacilli. Polymerase chain reaction confirmed *Mycobacterium avium* as the causative agent. The peculiarity of this outbreak of mycobacteriosis regards type and age of the affected birds and the uncommon involvement of the soft orbital tissues.

INTRODUCTION

Avian mycobacteriosis is a well known disease with a world-wide distribution. In poultry, the disease is usually termed tuberculosis and is principally caused by *Mycobacterium avium*. This disease is chronic and affected flocks are characterized by unthriftiness, decreased egg production, and finally death. Although tuberculosis in commercial poultry is now rare, it still occurs sporadically in backyard poultry and game birds. In poultry, the onset of mycobacterial infections is usually confined to the gastro-intestinal tract (2,6). Here we describe an unusual avian mycobacteriosis in commercial brown layers.

MATERIALS AND METHODS

Animals. Eight 46-week-old commercial brown layers out of a flock with egg drop and increased mortality were submitted for diagnosis. The animals were humanly euthanized and immediately necropsied.

Histopathology. Samples of periocular tissues, eyes, heart, lungs, spleen, liver and intestine were fixed in 10% buffered formalin and routinely included for histopathology. Four-micron sections were stained with hematoxylin and eosin and with Zeehl-Neelsen (ZN).
Additional samples of conjunctiva were store at -20°C until use.

PCR. DNA was extracted from frozen tissues using a DNA minikit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The identification of the mycobacterial species was based on the amplification and sequence analysis of a

hypervariable region of the 16S rRNA gene as previously described (1,3,4). A 5 µL sample of the PCR products was electrophoresed in 2% agarose (Promega, Milan, Italy) and stained with ethidium bromide (Euroclone, Milan, Italy). PCR products of the expected length (555 bp) were purified from agarose gel slices using a QIAquick PCR purification kit (Qiagen) and sequenced using an ABI Prism 310 genetic analyser (Applied Biosystems, Foster City, California, USA). The sequence was submitted to the GenBank database (FJ639163). The percentage of similarity with reference sequences was evaluated by BLAST search in the NCBI website.

RESULTS

Animals. All the birds were in poor body condition with atresic ovary and oviduct. Three layers showed monolateral, moderate to extremely severe enlargement and thickening of the soft periocular tissues with chemotic conjunctivitis and subconjunctival nodular lesions. Disseminated nodular lesions of varying dimensions were also observed in intestine, spleen, liver, lungs bone marrow of all the eight birds.

Histopathology. The sections of both orbital and visceral nodular lesions revealed granulomatous nature characterized by numerous hystiocytes and multinucleated giant cells surrounded by variable numbers of lymphocytes and lesser numbers of plasma cells and viable heterophils. Larger granulomas were centred on necrotic areas. The sections of orbital tissues showed the granulomas were immediately beneath the conjunctiva which was diffusely hyperplastic and infiltrated by large numbers of lymphocytes and plasma cells. Erosion of conjunctival epithelium was associated with larger granulomas which merged inwardly with massive necrosis. Mild uveitis was observed in the eye sections. ZN stain revealed scarce numbers of acid fast bacilli (AFB) within the visceral granulomas whereas AFB were extremely rare in the orbital granulomas.

PCR and sequencing. Molecular analysis revealed *Mycobacterium avium* as the causative agent with a percentage of similarity of 99%.

DISCUSSION

As in other countries around the world, *Mycobacterium avium* infections are occasionally reported in commercial Italian poultry whereas they are still diagnosed in backyard and game birds. Typically, clinical signs appear in the second year of age and are characterized by disseminated granulomas associated with numerous AFB. Here we describe an outbreak of tuberculosis caused by *Mycobacterium avium* in 46-week old commercial layers with peculiar involvement of the orbital tissues and scarce AFB within the lesions. It was not possible to ascertain the source of infection and no follow-up data were available. It is possible to hypothesize that the precocious onset of the disease with exuberant paucibacillary granulomas was due to a hyperergic response as well-known in mammals (5). As mycobacteria are usually shed via faeces from intestinal lesions, the uncommon orbital involvement can be explained with superinfection via aerosolized bacteria in the environmental dust. Extreme paucibacillarity of the orbital lesions and the low numbers of birds with these signs seems to sustain a secondary involvement of the periocular tissues.

REFERENCES

1. Dumonceau, J.M., P.A. Fonteyne, L. Realini, A. van Gossum, J.P. van vVoren, and F. Portaels. Species-specific Mycobacterium genavense DNA in intestinal tissues of individuals not infected with Human Immunodeficiency Virus. J. Clin 33, 2514-2515. 1995.

2. Fulton, R.M and C.O. Thoen. Other bacterial diseases. Tuberculosis. In: Diseases of poultry, 11th ed. Y.M. Saif, H.J. Barnes, A.M. Fadly, J.R. Glisson, L.R. McDougald, and D.E. Swayne, eds. Iowa State University Press, Ames, Iowa, USA. pp 836-844. 2003.

3. Hughes, M.M., G. James, N. Ball, M. Scally, R. Malik, D.I. Wigney, P. Martin, S. Chen, D. Mitchell, and D.N. Love. Identification by 16S rRNA gene analyses of a potential novel Mycobacterial species as an etiological agent of canine leproid granuloma syndrome. J. Clin. Microbiol. 38, 953-959. 2000.

4. Manarolla G., E. Liandris, G. Pisoni, D. Sassera, G. Grilli, D. Gallazzi, G. Sironi, P. Moroni, R. Piccinini, and T. Rampin. Avian mycobacteriosis in companion birds: 20-year survey. Vet. Microbiol. 133: 323-327. 2009.

5. Snyder, P.W. Diseases of Immunity. In: Pathologic basis of veterinary disease, M.D. Mc Gavin and J.F. Zachary, eds. Mosby Elsevier, St. Luis, Missouri, USA. pp 193-251. 2003.

6. Tell, L.A., L. Woods, and R.L. Cromie. Mycobacteriosis in birds. Rev. Sci. Tech. Ser. Sci. Hum. (International Office of Epizootics). 20, 180-203. 2001.

PATHOGENICITY MARKERS OF *CLOSTRIDIUM* SPP. IN COMMERCIAL TURKEYS

M. Saita[A], L. Bano[B], G. Grilli[A], and D.Gallazzi[A]

[A]Department of Veterinary Pathology, Hygiene and Public Health, University of Milan, Via Celoria 10, 20133 Milano, Italy, E-mail: daniele.gallazzi@unimi.it
[B]Istituto Zooprofilattico Sperimentale delle Venezie, Laboratorio di Treviso, Italy

SUMMARY

Since the growth promoter ban in Europe, enteritis of different etiologies (virus, bacteria, and protozoa) are increasingly becoming the main cause of economic loss in commercial turkeys production. This study is focused on typing of *Clostridium* spp. isolated from samples of jejunum and ileum of 82 birds out of 17 turkey flocks. The birds were six-day to 104-day old, both male and female, with enteric disorders. The presence of toxin NetB was investigated. Multiplex PCR to detect cpa, cpb1, cpetx, cp1, cpb2 and cpe toxin genes were used for *Clostridium* typing. No lesions of necrotic enteritis were observed. *Clostridium perfringens* type A was isolated from 25 enteric samples, *Clostridium difficile* was found in four cases and *Clostridium sordelli* in one case. *Clostridium perfringens* was present from six to 104 days of age indicating its possible role in the enteric disorders of commercial turkeys. NetB toxin was found in no sample. Three out of four isolates of *Clostridium difficile* were characterized by the presence of toxin genes.

INTRODUCTION

The main clostridia responsible for a wide range of diseases in avian species are: *Clostridium colinum*, *C. botulinum*, *C. septicum*, and *C. perfringens*, *C. fallax*, *C. novyi*, *C. sporogenes*, and *C. difficile* (1).

Pathological signs are caused by the different toxins but in many cases cofactors such as dietary ingredients or changes, severe stress, coccidiosis, and other protozoal diseases of the intestinal tract or immunosuppressive infections can enhance the disease (1). *Clostridium perfringens* (CP) is often isolated from the intestinal tract of healthy birds but can also cause outbreaks of disease in poultry, and especially in broiler and turkey flocks. CP is a gram-positive, spore forming, and anaerobic bacterium responsible for a wide range of diseases in humans and animals. Its pathogenicity is associated with the production of 17 toxins, of which α, β, ε, and ι are the major lethal ones (2). A commonly used classification scheme divides CP isolates into five types (A-E) on the basis of their capability to produce the major lethal toxins (2). Some CP strains, in addition to α toxin, produce β2 and enterotoxin: two toxins that have been proposed as being important in the pathogenesis of intestinal disorders in animals and humans respectively (3,4,5).

Clostridiosis occurs as acute or subclinical disease. The acute clinical disease is characterized by necrotic enteritis (NE). Intestinal focal necrosis and hepatitis are typical signs frequently associated with subclinical clostridiosis (6). The role of CP toxin types in the pathogenesis of NE in poultry is still not clear. Studies conducted in Finland, Sweden, Belgium, and Denmark demonstrated that CP isolated from chickens affected by NE belong to toxin type A (6,7,8,9,10), and demonstrated that α toxin is not essential in causing NE in broilers. Very few studies are focused on turkeys, although since the growth promoters ban in Europe in 2006, it has become a pathology of major concern. Recently, NetB, a novel toxin that is associated with broiler NE, has been described (10). The toxin was identified using screens for proteins from the supernatant of *C. perfringens* cultures that were cytotoxic for chicken hepatocellular carcinoma cells (LMH) *in vitro*.

The aim of this study was to perform toxin genotyping of CP field strains collected from the intestines of diseased turkeys by multiplex PCR for detection of α, β, ε, ι, β2, NetB, and enterotoxin genes.

MATERIALS AND METHODS

Birds. Eighty-two birds from 17 commercial turkey flocks showing enteric disorders were humanly euthanized and necropsied. The turkeys were six-day to 104-day old, both male and female.

Strains and growth conditions. All strains were obtained streaking on Perfringens Agar Base (Oxoid) 0.1 mL of 24 h broth (Cooked Meat Medium, Difco) culture of jejunum and ileum fragments (5 cm back and 3 cm after the Merkel's diverticulum) collected from sick commercial turkeys. CP ATCC 27324 (toxin-type E+enterotoxin), CCUG 2036 (toxin-type C), CCUG 2037 (toxin-type D), ATCC 10543 (toxin-type A + β2) were used as reference strains. All strains were incubated in anaerobic conditions at 37°C for 48 hours.

DNA extraction. Colonies of each CP strain were recovered from the agar plate and the DNA was

extracted with DNeasy Blood & Tissue Kit (Qiagen) according to manufacturer's instructions.

Toxin coding gene detection. One multiplex PCR for cpa, cpb1, cpetx , and cpi genes and three single PCR for cpb2 (2), cpe (11) and NetB (10) genes detection were used. PCR primers and fragment length are listed in table 1. The sequencing of the amplified product confirmed that the targeted netB gene was indeed amplified with the PCR assay.

Parasitological examination. Intestinal mucosa of all chickens was scraped in different districts and observed by optic microscope searching for protozoa and helminths (eggs and worms).

RESULTS

At necropsy, all 82 turkeys showed enteric lesions. In younger subjects (one to three weeks) intestinal lesions were consistent with viral enteritis, a common finding in Italian flocks. In older turkeys (three to six weeks of age) coccidiosis was diagnosed. Twenty-five (30.48%) out of 82, aged from six to 104 days old, were positive for *C. perfringens* type A. All strains resulted positive for α toxin gene (toxin-type A) and only one (1.2%) of these was also positive for β2 toxin (toxin-type A + β2). No CP cpe-positive or NetB positive strains were detected. Four (4.8%) turkeys were positive for *C. difficile*. Among these, one was negative for both toxin genes while two were positive for TcdA and TcdB, and one was positive only for TcdB. One (1.2%) was positive for *C. sordelli*.

DISCUSSION

The data highlight that the CP isolates included in the study were of toxin type A and a relatively low percentage of isolates carried the β2 toxin gene, irrespective of enteric lesions. No CP toxin type C was found also in birds affected by NE. Our findings confirm the most recent results reported from different countries, and the data suggest that the role of CP type C should be revaluated in the pathogenesis of NE. The presence of *Clostridium* spp. was often associated with other pathogens, such as viral enteritis in the first three weeks, coccidiosis between three and five weeks and hemorrhagic enteritis between six and 12 weeks of age.

These observations underline the importance of predisposing factors (nutrition, drug treatments, concomitant diseases) in poultry clostridiosis. It must be kept in mind that the presence of CP type A already exists in six-day-old turkeys. The role of this pathogen at such a young age must be clearly understood, but surely it could play an important role in developing enteric disorders. After the growth promoter ban in 2006, enteric imbalances are a main concern. The lack of NetB positive findings, which seems to play a major role in NE of chickens, is an important result as there is no data available for this toxin in turkeys. Moreover, the presence of *C. difficile* in four samples – three of them toxin genes positive – is quite interesting because of its potential zoonotic role.

REFERENCES

1. Barnes E.M., C.S. Impey, and D.M. Cooper. Manipulation of the crop and intestinal flora of newly hatched chick, Am. J. Clin. Nutr. 33: 2426-2433. 1980.
2. Meer, R.R. and J. Songer. Multiplex polymerase chain reaction assay for genotyping *Clostridium perfringens*. Am. J. Vet. Res. 58:702-705. 1997.
3. Sarker, M.R., R.J. Carman, and B.A. McClane. Inactivation of the gene (*cpe*) encoding *Clostridium perfringens* enterotoxin eliminates the ability of two cpe-positive *C. perfringens* type A human gastrointestinal disease isolates to affect rabbit ileal loops. Mol. Microbiol. 33:946-958. 1999.
4. Thiede, S., R. Goethe, and G. Amtsberg. Prevalence of β2 toxin gene of *C. perfringens* type A from diarrhoeic dogs. Vet. Rec. 149:276-274. 2001.
5. Manteca, C., G. Daube, T. Jauniaux, A. Linden, V. Prison, J. Detileux, A. Ginter, P. Coppe, A. Kaeckenbeeck, and J.G. Mainil. A role for the *Clostridium perfringens* β2 toxin in bovine enterotoxaemia? Vet. Microbiol. 86: 191-202. 2002.
6. Engström, B.E., C. Fermér, A. Lindberg, E. Saarinen, V. Båverud, and A. Gunnarsson. Molecular typing of isolates of *Clostridium perfringens* from healthy and disease poultry. Vet. Microbiol. 94: 225-235. 2003.
7. Nauerby, B., K. Pedersen, and M. Madsen. Analysis by pulsed-field gel electrophoresis of the genetic diversity among *C. perfringens* isolates from chickens. Vet. Microbiol. 94:257-266. 2003.
8. Heikinheimo, A. and H. Korkeala. Multiplex PCR assay for toxinotyping *Clostridium perfringens* isolates obtained from Finnish broiler chickens. Lett. Appl. Microbiol. 40:407-411. 2005.
9. Gholamiandekhordi, A.R., R. Ducatelle, M. Heyndrickx, F. Haesebrouck, and F. Van Immerseel. Molecular and phenotypical characterization of *Clostridium perfringens* isolates from poultry flocks with different disease status. Vet. Microbiol. 113:146-152. 2006.
10. Keyburn, A.L., S.A. Sheedy, M.E. Ford, M.M. Williamson, M.M. Awad, J.I. Rood, and R.J. Moore. Alpha-toxin of *Clostridium perfringens* is not an essential virulence factor in necrotic enteritis in chickens. Infect. Immun. 74:6496-6500. 2006.

11. Baums, C.G., U. Schotte, G. Amtsberg, and R. Goethe. Diagnostic multiplex PCR for toxin genotyping of *Clostridium perfringens* isolates. Vet. Microbiol. 100:11-16. 2004.

Table 1. Primers used to detect *C. perfringens* toxin coding genes.

GENE	Primers	Sequence (5'-3')	Fragment length
cpa	cpa_F	GTT GAT AGC GCA GGA CAT GTT AAG	402
	cpa_R	CAT GTA GTC ATC TGT TCC AGC ATC	
cpb	cpb_F	ACT ATA CAG ACA GAT CAT TCA ACC	236
	cpb_R	TTA GGA GCA GTT AGA ACT ACA GAC	
cpetx	etx_F	ACT GCA ACT ACT ACT CAT ACT GTG	541
	etx_R	CTG GTG CCT TAA TAG AAA GAC TCC	
cpi	cpi_F	GCG ATG AAA AGC CTA CAC CAC TAC	317
	cpi_R	GCG ATG AAA AGC CTA CAC CAC TAC	
cpe	cpe_F	GGG GAA CCC TCA GTA GTT TCA	506
	cpe_R	ACC AGC TGG ATT TGA GTT TAA TG	
cpb2	cpb2_F	AGA TTT TAA ATA TGA TCC TAA CC	567
	cpb2_R	CAA TAC CCT TCA CCA AAT ACT C	
NetB	AKP78_F	GCT GGT GCT GGA ATA AAT GC	384
	AKP79_R	TCG CCA TTG AGT AGT TTC CC	

EFFICACY STUDY OF A LIVE *E. COLI* VACCINE IN COMMERCIAL TURKEYS RAISED IN FLOOR PENS

Cheryl Gustafson[A], Stephen W. Davis[B], and Kalen Cookson[A]

[A]Fort Dodge Animal Health, Overland Park, KS 66210
[B]Colorado Quality Research, Inc., Wellington, CO 80549

ABSTRACT

A recently licensed live *Escherichia coli* vaccine has proved to reduce losses due to *E. coli* infections and provide added protection for chickens in the field. It is the purpose of this report to present the results of a floor pen study demonstrating the efficacy of this live *E. coli* vaccine in commercial turkeys. Two vaccination programs were tested, one at three days of age and the second treatment group at three days of age and three weeks of age. Birds were challenged at six weeks of age with a virulent strain of *E. coli.* Protection was evaluated by necropsy of the birds one week following challenge. These results show that both vaccination programs provide protection in turkeys against *E. coli* infection.

INTRODUCTION

E. coli infections cause severe losses both in chickens and turkeys. *E. coli* was first isolated in chickens in the late 1800s and over a century later is still causing mortality and disease in not only chickens but turkeys as well. It is recognized as one of the more damaging and economically important disease causing bacteria in the poultry industry. Colibacillosis can involve either localized or systemic infections of *E. coli*. It is the disease causing *E. coli*, known as avian pathogenic *E. coli* (APEC) that appear to be increasing in virulence and resistance (1,2), keeping this age-old bacterium an ever increasing concern to the poultry industry.

MATERIALS AND METHODS

The poults (Nicholas toms) were raised on wood shavings and housed at 36 birds per floor pen. Feed and water was provided *ad libitum.* Each treatment group consisted of 4 repetitions (pens) for a total of 144 birds per group. Treatment group B received the live *E. coli* test vaccine (*E. coli* aroA - attenuated strain) at three days and three weeks of age. Treatment group C received the test vaccine at three weeks of age. The test vaccine was administered by coarse spray using the ShurFlo Backpack sprayer with the XR TeeJet nozzle at 40psi. Groups A and D did not receive the test

vaccine. All birds received live Newcastle disease B1, B1 vaccine on days 14 and 35 via drinking water and HE (hemorrhagic enteritis) vaccine on day 28. No other treatments or medications were given during the study.

Groups A, B, and C were challenged and group D was the negative control. At 42 days of age the designated birds were challenged according to the established model for intratracheal (IT) inoculation with a virulent (APEC) *E. coli* serotype O78 isolate. Titer determined at time of challenge was 1.1 x 10^9 CFU/bird. All birds were observed daily for clinical signs and mortality. Dead or morbid birds were removed and a post-mortem examination was done to determine the cause of morbidity/mortality. At 49 days of age, seven days post-challenge, all remaining birds were posted and lesions recorded.

RESULTS

The mortality for this study before challenge was 0.7 to 3.3%. The two control groups had mortality greater than 1% and the two vaccinated groups fell below 1% mortality prior to challenge. The three-day vaccinated group was the only group without mortality in the first two weeks. The mortality for the challenged treatment groups ranged from 24 to 51%. The unvaccinated challenged group suffered 51% mortality, while the vaccinated groups were at 24% and 33% mortality. Percent severe airsacculitis was also less in the vaccinated group compared to the unvaccinated challenged group which was 94%. Negative controls suffered no severe airsacculitis or mortality.

DISCUSSION

The APEC challenge in this study was very severe with over 50% mortality and over 90% severe airsacculitis in the unvaccinated control group. Despite this strong challenge the live *E. coli* vaccine did provide protection and decreased mortality and incidence of severe airsacculitis. Prior to challenge, it was noted that the vaccinated groups had better livability. This may suggest added protection from low level infection. Both treatment groups provided protection against the six-week challenge. With *E. coli* being a ubiquitous bacterium, early vaccination may be beneficial, especially when early challenge is imminent.

REFERENCES

1. Nolan, L.K. Emergence of avian pathogenic *Escherichia coli* with enhanced resistance and disease causing capacity. In: Iowa Egg Symposium Proceedings. Iowa State University, Ames, IA. 2007.

2. Rodríguez-Siek, K.E., *et al.* Characterizing the APEC pathotype. Vet. Res. 36:241-256. 2005.

IDENTIFICATION OF *CAMPYLOBACTER JEJUNI* IN A LOCAL POULTRY FARM WITH STUDY OF TRANSMISSION ROUTE

Ning Jia, Yip Chi Wai, and Frederick Chi-Ching Leung

School of Biological Sciences, The University of Hong Kong, Hong Kong SAR, China

ABSTRACT

Campylobacter jejuni is the leading cause of food-borne human gastroenteritis in developed countries but its occurrence in Hong Kong still remains unclear. In this study, 24 chickens and certain amount of environmental samples were collected from a local poultry farm in Hong Kong for the detection of *Campylobacter jejuni*. Chicken samples including skins, toes, intestines, and feces, and environmental samples including feed and water were tested respectively. We cultured for Campylobacter isolates from each sample and examined them by polymerase chain reaction assay and DNA sequencing analysis of flaA gene. Seven out of the 24 chickens were confirmed as *Campylobacter jejuni* positive, indicating the existence of *Campylobacter jejuni* in Hong Kong. A phylogenetic tree was eventually constructed according to the sequence of flaA. This study revealed the potential threat of our isolated *Campylobacter jejuni* to the public because the phylogenetic tree shows its close relationship with some human-infecting strains discovered in USA.

THE EFFICACY OF BIOSEALED IN PREVENTING *SALMONELLA* SPP. FROM COLONIZING CONCRETE SURFACES

K.S. Macklin, D.M. Paiva, and M. Singh

Dept. of Poultry Science, Auburn University, Auburn, AL 36849-5416

SUMMARY

One of the challenges facing the poultry industry is controlling *Salmonella*. Biosealed™ is a cement sealer that claims to seal pores inside cement and to have antimicrobial activity. Using this product in the hatchery should help minimize bacterial contamination. A series of experiments were performed to determine the efficacy of this product against *Salmonella.* Cement blocks were divided into one of four treatments: Bacterial inoculation (CON), Biosealed applied before inoculation (BA), Biosealed applied after inoculation (BI) Biosealed applied before and after inoculation (BAI). Sampling was performed on the interior and exterior of the cement blocks. The exterior swabbing results showed that BI and BAI had significantly ($P < 0.05$) lower *Salmonella* levels compared to CON and BA. *Salmonella* was not isolated from the interior of blocks in BA, BI and BAI; however it was isolated from CON. It is concluded that Biosealed is an effective cement sealer that appears to have anti-microbial activity.

(The full-length article will be published in *Poultry Science*.)

SEROLOGIC RESPONSE AGAINST H5N2 OIL EMULSION AI VACCINE IN DOMESTIC DUCKS

Andres Morales Garzon, Donaji Garcia Lopez, Raul Montalvo Ramirez, Ruth Larios, and Eduardo Lucio Decanini

Investigación Aplicada S. A. DE C.V., 7 Norte 416 Tehuacan Puebla, Mexico, 75700

SUMMARY

Thirty-five two-week-old commercial ducks were divided into five groups and maintained on wire cages. Groups 1 and 3 received 1.0 and 2.0 mL respectively of a water killed LAI vaccine (H5N2, strain Chicken/Mexico/232/94/CPA) identified as Optimune AIV by subcutaneous route (SC). Groups 2 and 4 received 0.4 and 0.8 mL, respectively, a concentrated water killed LAI vaccine (H5N2) identified as Optimune C-AIV by SC. Group 5 was the control group without treatment. All birds were bled before vaccination and at 1, 2, 3, 4, and 5 wks post vaccination to quantify antibodies level against AI by Inhibition Hemagglutination (IH) test. All groups were negative to AI by IH before vaccination. Groups 2 and 4 (concentrated vaccine) showed higher titer than Groups 1 and 3 (normal vaccine) after five wks with a geometrical mean (GM) of 296 (8.2 log 2) and 485 (8.9 log 2) respectively, Group 5 showed a GM of 5 (2.3 log 2) during all experiment period. We conclude that oil emulsion vaccine containing subtype H5H2 could be an alternative to protect commercial ducks and avoid transmission to other species, mainly poultry.

INTRODUCTION

There was a theory that wild aquatic birds are believed to be the primordial reservoir of type A influenza viruses. Avian influenza causes asymptomatic infection in natural host; however, in aberrant hosts including poultry, swine, and humans, clinical disease could be observed. Avian influenza virus is classified in 15 hemagglutinin subtypes (HA) but high pathogenicity has been associated with some strains of the H5 or H7 HA subtypes. Since 1994 H5N2 subtype AI has been present in Mexico, and an extensive vaccination program has been established to avoid high pathogenicity virus in farms. However, commercial and backyard ducks could be affected by H5N1 subtype and infect poultry or humans. The objective of this work was to investigate if two commercial vaccines to H5N2 could be an alternative to protect against H5N1.

MATERIALS AND METHODS

Birds. Thirty-five two-week-old commercial ducks were maintained in wire cages.

Vaccines. *Normal vaccine*: Killed oil emulsion Avian Influenza Vaccine Optimune AIV®; *concentrated vaccine*: Optimune C-AIV® (Investigacion Aplicada S.A. de C.V. Tehuacan, Puebla, Mexico). The schedule of vaccination was according to Table 1.

Serology. A sample of serum was collected from each group at 0, 1, 2, 3, 4, and 5 wks post vaccination. IH test was run and geometric mean was calculated.

RESULTS AND DISCUSION

The aim of this paper was to show seroprevalence in commercial ducks using two commercial vaccines against H5N2 LPAI killed vaccine. All birds had not antibodies against avian influenza virus before vaccination, and negative control remained with very low titers until they were slaughtered. The first one is a normal vaccine containing AIV strain Chicken/Mexico/ 232/94/CPA and the other is a concentrated vaccine containing same strain. Both met minimal requirements established by Mexican authorities. In the case of poultry, normal vaccine is recommended to a dose of 0.5 mL per bird and concentrated vaccine is recommended to be administered at 0.2 mL. So, in ducks manufacturer recommended use double doses for each vaccine. Serologic results are showed in Table 2. As we can see, the best serologic response was obtained with concentrated vaccine reached a GM of 296 (8.2 log2) and 485 (8.9 log2) with a single or double doses at end of trial. Nevertheless, normal vaccine could be used.

In 2006, Swayne *et al*. (1) showing that H5N2 vaccines protect chickens from Asian H5N1 high pathogenicity avian influenza virus. So, we believe that H5N2 vaccines could be used to protect commercial ducks not only in Mexico but in other countries to reduce environmental contamination by H5N1 HPAI. Also, we recommend that challenge trials be carried out with H5N1 AI strains.

REFERENCES

1. Swayne, D.E., Chang-Won Lee, and E. Spackman. Inactivated North American and European H5N2 avian influenza virus vaccines protect chickens from Asian H5N1 high pathogenicity avian influenza virus. Avian Pathol. 35(2): 141-146. 2006.

Table 1. Vaccination schedule.

Group	Treatment	Doses (mL)	Administration	Number of birds
1	Normal	1.0	Subcutaneous	10
2	Concentrated	0.4	Subcutaneous	10
3	Normal	2.0	Subcutaneous	5
4	Concentrated	0.8	Subcutaneous	5
5	Control	-	None	5

Table 2. Serologic results with (H2N2) AI vaccines in commercial ducks.

W.P.	Group 1		Group 2		Group 3		Group 4		Group 5	
	GM	Log 2	GM	Log 2	GM	Log 2	GM	Log 2	GM	Log 2
1	5	2.3	5	2.3	5	2.3	5	2.3	5	2.3
2	20	4.3	83	6.3	27	4.7	98	6.6	5	2.3
3	74	6.7	109	6.7	80	6.3	320	8.3	5	2.3
4	109	5.5	148	7.2	121	6.9	422	8.7	5	2.3
5	127	7.0	296	8.2	160	7.3	485	8.9	5	2.3

W.P. Wks post vaccination

GM. Geometric mean

FIELD TRIALS TO TEST SAFETY AND EFFICACY OF A LIVE ATTENUATED VACCINE OF AVIAN PATHOGENIC *ESCHERICHIA COLI* SEROVAR O78 IN BROILER CHICKENS

Tetsuji Nagano, Rie Kitahara, and Shinya Nagai

Nippon Institute for Biological Science, 9-2221-1, Shin-machi, Ome, Tokyo 198-0024, JAPAN

INTRODUCTION

In the 56th Western Poultry Disease Conference, we showed that a rational attenuated *crp* mutant of avian pathogenic *E. coli* (APEC) serovar O78 can be a candidate as a safe and effective live vaccine against avian colibacillosis. Administration of the mutant strain via various routes (fine spray, coarse spray, eye drop, and *in ovo*) evoked an effective immune response that could protect chickens from challenge with the virulent wild-type *E. coli* O78 strain under laboratory conditionS. In this presentation, we will describe the results of the field trials using the mutant strain and will further evaluate safety and efficacy of the mutant as a vaccine candidate.

MATERIALS AND METHODS

In four broiler chicken farms where colibacillosis had frequently occurred, a total of 63,208 birds were vaccinated with the mutant strain and 61,508 chickens were served as non-vaccinated control birds. In each farm, approximately 7,400 to 25,500 birds were housed separately. Both sexes of Ross308 chickens were used in Farms A and B, male Cobb500 chickens were used in Farm C and female Ross308 chickens were used in Farm D. The lyophilized trial vaccine (Lot.19, 1,000 doses per vial) was dissolved in 300 mL of sterilized physiological saline. Chickens of Farms A and B were first vaccinated via fine spray at one day of age and were secondarily vaccinated via coarse spray three weeks later. In Farms C and D, chickens were vaccinated twice via fine spray at one day and four-weeks of age. The efficacy and safety of the vaccine were assessed by comparatively monitoring the status of individually housed birds: general clinical signs, existence or nonexistence of colibacillosis, weight gain, and productivity until slaughter and experimental challenge exposure. When clinical colibacillosis was not observed in both the vaccinated and the non-vaccinated birds, ten randomly selected birds from each household were introduced to the authors' laboratory and experimentally challenge-exposed to the virulent wild-type *E. coli* O78 strain via intravenously injection. After the challenge exposure, all birds were monitored daily for signs of illness and deaths. One week later, the surviving chickens were euthanized, and gross lesions representing colibacillosis (pericarditis and perihepatitis) were recorded.

RESULTS

After each vaccination, no adverse symptoms were observed in any of the treated birds. In Farm A, where clinical colibacillosis was observed, mortality and condemnation rates of the vaccinated birds were significantly lowered compared to those of the control. In the other three farms (Farms B, C, and D), where clinical colibacillosis was not observed, no significant differences were detected between the vaccinated and control birds except that the mean body weight was higher in the vaccinated birds in Farm C. In the experimentally challenge-exposed birds, improved results were observed in vaccinated birds; in survival rate and clinical score in Farm B, in survival rate, clinical score and weight gain in Farm C and in weight gain and pericarditis score in Farm D.

DISCUSSION

In field trials using a total of 63,208 birds, the trial vaccine was shown safe for commercial broiler chickens. The efficacy of the vaccine was indicated by reduction of deleterious effects by the colibacillosis, *i.e.* reduced mortality rate, clinical scores and other increased productivity. Additionally the trial vaccine protected against the experimental challenge-exposure of APEC strain.

The results presented here demonstrate that this vaccine can confer protection against acute and chronic manifestation of colibacillosis caused by APEC infection.

INACTIVATION AND DEGRADATION OF INFLUENZA VIRUS AND NEWCASTLE DISEASE VIRUS DURING COMPOSTING

J. Guan, M. Chan, C. Grenier, D.C. Wilkie, B.W. Brooks, and J.L. Spencer

Canadian Food Inspection Agency, Ottawa Laboratory (Fallowfield), 3851 Fallowfield Road, P.O. Box 11300, Station H, Ottawa, Ontario, Canada, K2H 8P9

The fate of avian influenza (AI) and Newcastle disease (ND) viruses during composting was investigated by virus isolation and real-time RT PCR (1,2). Experiments included a comparison of survival of these viruses in cage layer manure and in litter taken from a floor pen of chickens. The latter was decomposed and resembled compost. These materials were contained within nylon mesh bags and were buried in compost or were held outside of compost at ambient temperatures. For control purposes, bacteria-free allantoic fluid that contained viruses was dispensed in vials that were sealed and held with other specimens. There were at least 5 $\log_{10}$ of virus per g of each specimen when the study began on day 0, but by day seven, the temperatures in compost had reached at least 50°C and the viruses had been inactivated. At ambient temperatures that ranged from 13° to 28°C, the viruses were inactivated by day 21. In sealed vials buried in compost the viruses survived to day 10, but at ambient temperatures they were viable to the termination of the experiment on day 21. At both the compost and ambient temperatures, the degradation of viral RNA was more rapid in used litter than in cage layer manure, suggesting that differences in microbial activity may have been a factor. In the absence of microbial activity, the viral RNA in sealed vials remained stable at ambient temperatures to day 21.

A role for microbial activity in the degradation of viral RNA was supported by other experiments, similar to the above, where the RNA of ND virus in embryonated eggs with intact shells, persisted during 21 days in compost. In comparison, the RNA of AI and ND viruses in muscle and liver specimens were fully degraded during this period. Likewise, the RNA of AI virus in eggs whose shells were crushed during composting was fully degraded. This suggested that the intact shells had prevented composting activity within the eggs that could have degraded the viral RNA.

In vitro studies compared the survival of viruses in water extracts containing microbes from compost with similar extracts from manure. At temperatures ranging from 35° to 55°C, survival of viruses was similar in the two extracts. However, at 25°C the killing of viruses was significantly more rapid in the suspension that contained microbes from compost than in the one that contained microbes from cage layer manure ($P < 0.05$). The findings suggest that microbes in manure, compost and water on poultry farms can influence the killing and degradation of AI and ND viruses and that this should be considered in formulating cleaning and disinfection programs following outbreaks of these diseases.

REFERENCES

1. Guan, J., M. Chan, B-L Ma, C. Grenier, D.C. Wilkie, J. Pasick, B.W. Brooks, and J.L. Spencer. Development of methods for detection and quantification of avian influenza virus and Newcastle disease viruses in compost by real time RT-PCR and virus isolation. Poultry Science 87: 838-843.

2. Guan, J., M. Chan, C. Grenier, D.C. Wilkie, B.W. Brooks, and J.L. Spencer. Survival of avian influenza and Newcastle disease viruses in compost and at ambient temperatures based on virus isolation real-time reverse transcriptase PCR. Avian Diseases, in press.

MAREK'S DISEASE VIRUS DETECTION IN THE FIELD BY PCR METHOD

Tamayo Maritza[A], Gallardo Jaime[A], Mireia Toldra[B], and Amanda Cook[C]

[A]Fort Dodge Animal Health, Mexico
[B]Fort Dodge Animal Health Olot, Spain
[C]Fort Dodge Animal Health Charles City, Iowa

SUMMARY

Marek's disease (MD) is a common lympho-proliferative disease of chickens, usually characterized by mononuclear cellular infiltrates in peripheral nerves and other organs and tissues including iris and skin. The disease is caused by a herpesvirus and has a major economic impact on the poultry industry because of cost of vaccination, mortality, condemnations, and loss of egg production.

There is no treatment is available, the main tool for prevention and control of the MD is the use of attenuated strains. Vaccine development for the control of MD was a highlight in avian medicine and basic cancer research, as this was the first example of a neoplasic disease preventable by immunization. For diagnostic purposes, we relied on histopathology and clinical signs, being also difficult to differentiate vaccinated from unvaccinated birds.

A PCR analysis has been specially designed to detect serotype 1 Marek's disease virus (MDV), and it consists in two different techniques:

-Real-Time PCR (quantitative PCR): permits the calculation of the number of MDV serotype 1 genomes present in 10.000 chicken cells.

-132 bp PCR (qualitative PCR): permits the ability to distinguish between the two different serotype 1 MDV strains (i.e. field and vaccine).

The qualitative PCR test detects the 132 bp repeat region of the MDV genome. This region is specific to serotype 1 and shows differences between Rispens vaccine strain and field strains. Since feather tips can be sampled in a non-invasive manner, and since they have higher virus levels than other tissues, they are selected as the tissue of choice for sampling. Non-invasive sampling also provides the opportunity to sample the same chick at several different time-points and to sample many birds in the field in order to get a statistic significance. Using FTA cards facilitates sampling and transportation.

CONCLUSION

This test allows the detection of serotype 1 MDV and to differentiate Rispens vaccine strain from field viruses. The use of this technique will be another tool that will help reduce losses due to vaccine failures and to detect susceptible birds prior to challenge.

REFERENCES

1. Baigent, S.J., L.J. Petherbridge, K. Howes, L.P. Smith, R.J.W. Currie,and V.K. Nair. Absolute quantitation of Marek's disease virus genome copy number in chicken feather and lymphocyte samples using real-time PCR. Journal of Virological Methods. 123, 53-64. 2005.

2. Baigent, S.J., K., L.P. Smith, R.J. W. Currie, and V.K. Nair. Replication Kinetics of Marek´s disease vaccine virus in feathers and lymphoid tissues using PCR and virus isolation. Journal of General Virology, 86,2989-2998. 2005.

3. Baigent, S.J., K., L.P. Smith, V.K. Nair, and R.J.W. Currie. Vaccinal Control of Marek´s disease: Current challenge and future strategies to maximize protection. Veterinaty Immunology and Immunopathology. 112,78-86. 2006.

4. Landman, W.J.M. and S.B.E. Verschuren. Titration of Marek's disease cell-associated vaccine virus (CVI988) of reconstituted vaccine and vaccine ampoles from dutch hatcheries. Avian Diseases. 47, 1458-1465. 2003.

5. Nair, V. Evolution of Marek's disease – a paradigma for incessant race between the pathogen and the host. The Veterinary Journal. 170, 175-183. 2005.

6. Venugopal, K. Marek's disease: an update on oncogenic mechanisms and control. Research in Veterinary Science. 69, 17-23. 2000.

7. Witter, R.L. and K.A. Schat. Neoplasic diseases: Marek's disease. In: Saif, Y.M., H.J. Barnes, A.M. Fadly, J.R. Clisson, L.R. Mcdougald, and D.E. Swayne. (2003). Diseases of poultry. 11th ed. (pp.407-465). Ames: Iowa State University. 2003.

AVIAN H5N1 - AN ANIMAL VIRUS!

Frederick C. Leung

University of Hong Kong
fcleung@hkucc.hku.hk

As of November 12, 2007, the cumulative number of confirmed human cases of avian influenza A/(H5N1) reported to World Health Organization (WHO) was 335, with 206 deaths. Avian influenza including H5N1 refers to a large group of different influenza viruses for which the primary host is birds and only on rare occasions will these cross over and infect other species including pigs and humans. Pandemic influenza disease occurs when a new subtype emerges that has not previously circulated in humans. Since H5N1 is a strain which posses high potential for causing serious disease in humans, WHO and other health experts have been priming the world to prepare for this threat along with OIE and FAO calling for culling million of poultry world-wide.

During my presentation, I will present a model based on the most recent event of an animal virus "crossing over" to become a human virus. I will argue that the scale of the warnings appears to outstrip the magnitude of the real threat. Further, that the culling of millions of chicken may not have actually lowered the actual risk for human health. Rather that efforts and resources should be directed to re search in understanding the molecular and genetic mechanisms which are underlying the virus crossing between species. Only then can effective barriers can then be put in place: specifically to limit the direct contact of susceptible species; to lower the transmission rate; and to avoid establishing/adaptation to a new host. In addition, I shall review recent scientific findings that avian H5N1 has remained an animal virus for the past ten years since the first scientific evidence was obtained that avian H5N1 can infect human without an intermediate host.

The probability and feasibility that the avian H5N1 will successfully adapt to human as a new host is therefore assessable in my opinion as remaining low at this particular moment.

(This paper was presented at the Fifty-Seventh Western Poultry Disease Conference, April 9-12, 2008.)

Return to main menu

SIGNIFICANCE OF ANTIBODY DETECTION IN THE DIAGNOSTIC OF CHICKEN CRYPTOSPORIDIOSIS

Hayet Abbassi[A] and Muriel Naciri[B]

[A]College of Food, Agricultural, and Natural Resource Sciences, Animal Science Department, University of Minnesota, 1364 Eckles Avenue, Saint Paul MN 55108.
[B]INRA, Station de Pathologie Aviaire et de Parasitologie, 37380, Nouzilly, France

ABSTRACT

The kinetics of anti-*Cryptosporidium baileyi* antibodies were compared with the oocyst shedding in four groups of SPF chickens. Group I received orally *C. baileyi* oocysts at 11 days of age (Day11) and a challenge with the parasite at Day 60. The second group II, did receive in addition a very virulent strain of infectious bursal disease virus (vvIBDV) at Day 7. Two control groups, one (group III), had the vvIBDV at Day 7 and the parasite at day 60, and the other (group IV), had only the parasite at Day 60. A fifth group of chicken had only placebos and served as uninfected control group. The first group shed oocysts during 24 days, showed a seroconversion for IgM and IgA 10 dpi that last 24 days and over 52 days respectively. The IgG seroconversion was evident 17 dpi reached a peak 38dpi and stayed relatively high until the end of the experiment, Day 83. In the group IV, seroconversions were evident for all immunoglobulin isotypes (IgG, IgM and IgA) 10 dpi, and reach a peak 17dpi. Both IgG and IgA were positive until the end of the experiment 23dpi (Day 83), while IgM was not anymore detectable at that time. The oocyst shedding, last only five days in this group. Compared to group I the birds in group II shed about the double amount of oocysts during a longer period of time (45 *versus* 24 days); however, they did not show any detectable antibodies for all isotypes at all time points. More surprisingly, the birds in group III, having the virus at one week of age and a primo-infection with the parasite 53 days later shed lower quantity of *C. baileyi* oocysts during five days when compared to the group IV, but no antibodies were detected in these birds. These results were not expected, knowing that the follicular regeneration in the bursa of Fabricius takes place few weeks after an infection with vvIBDV.

The results in this study allow as having new considerations for our interpretations of the serology. Taken together with our previous findings (1,2), we start to build a clearer idea about the significance of each antibody isotype in the diagnostic of a cryptosporidial infection. The current question is: What will be the effect of an IBDV strain that is less virulent? Also, what will be the effect of variant IBDV strains that are very different from the vvIBDV strains in many aspects?

(The full-length article will be published in *Avian Diseases*.)

REFERENCES

1. Abbassi, H., F. Coudert, Y. Chérel, G. Dambrine, J. Brugère-Picoux, and M. Naciri. Renal Cryptosporidiosis (Cryptosporidium baileyi) in specific-pathogen free chickens experimentally coinfected with Marek's disease virus. Avian Dis. 43:738-744.1999.

2. Abbassi, H., F. Coudert, G. Dambrine, Y. Chérel, and M. Naciri. Effect of Cryptosporidium baileyi in specific pathogen free chickens vaccinated (CIV988/Rispens) and challenged with HPRS-16 strain of Marek's disease virus. Avian Pathol. 29:623-634. 2000.

INTERESTING DIAGNOSTIC CASES IN WESTERN CANADA

C. B. Annett

Alberta Agriculture and Rural Development, 97 East Lake Ramp N.E., Airdrie, AB T4A 0C3

SUMMARY

The following cases were included in this paper because they were interesting presentations of existing diseases in poultry or were cases that were diagnostically or epidemiologically challenging when determining the cause of the mortality or how the pathogen arrived to affect the flock. Poultry veterinarians and pathologists from the four Western Provinces were invited to contribute and submit cases they felt would fit into this category.

CASES

Case 1. Sustained low mortality in a backyard flock of adult laying hens.

Dr. Ann Britton. Abbotsford, B.C.

History: The owner of an eight month old commercial flock of Brown Hyline laying hens that were organically raised, submitted two dead hens for post mortem examination. The producer stated that the birds were vaccinated and every morning for the past four or five weeks, he would find one or two dead birds in the chicken house, otherwise the birds appeared healthy. The producer was concerned that a predator was gaining access to his flock.

Gross Pathology: Both birds were in good body condition (1.3 and 1.4 kg). One bird had fibrinous exudate around and within the pericardial sac and the spleen was moderately enlarged. In both birds, there was widely disseminated fibrinous to cooked yolk-like material over serous surfaces in the abdominal cavity with friable adhesions to the abdominal wall. Many large ova were present in the ovary containing inspissated yolk.

Gross Diagnosis: Yolk peritonitis and fibrinous pericarditis

Histopathology: Numerous tissues with scattered colonies of intravascular coccobacilli. The liver, spleen and ovary had multifocal areas of necrosis.

Diagnosis:

Comments:

Case 2. Tumors in turkeys.

Dr. Catherine Graham, Lethbridge, AB

History: A meat inspector from a nationally-regulated poultry processing plant was monitoring the slaughter of a 74 day old turkey flock. One bird was pulled from the line and condemned because it had multiple firm white masses in the liver and the spleen. Subsequently, tissues from this condemned turkey carcass were submitted to the Canadian Food Inspection Agency pathologist in Lethbridge, Alberta.

Gross Diagnosis: Lymphoid leukosis

Histopathology: Cells look to have originated from lymphoid or histiocytic lines. There is a lack of uniformity in the cell population. Numerous larger masses have a necrotic centre with macrophages and giant cells surrounding them.

Diagnosis:

Comments:

Case 3. Increased mortality broiler chicken flocks in Saskatchewan.

Dr. Bob Goodhope, Saskatoon, SK

History: Five farms in the past year had had outbreaks. All farms had multibarn production and mortality was reported at 3 to 10%. The birds were typically affected at five to six weeks of age. There was no apparent seasonal effect with respect to the affected flocks. Producers reported that the birds were found dead and rotted quickly.

Gross Pathology: Most birds were very depressed but were in good body condition. The skin over the abdominal area and the shoulders was red to purple and would tear easily and was undermined by blood tinged fluid with tiny bubbles. Gas bubbles were also present in breast muscle, spleen and liver. The breast and leg muscles were pale and had a 'cooked' appearance.

Gross Diagnosis: Acute myositis with sub-cutaneous emphysema.

Histopathology: There is massive necrosis in the skin overlying the affected muscles. The pectoral and leg muscles had acute necrosis and were heavily infiltrated with rod bacteria. There as focal necrosis in the myocardium.

Diagnosis:

Comments:

Case 4. Spike in mortality in a flock of meat turkeys.

Dr. Darko Mitevski, Airdrie, AB

History: Ten birds from a 63 day old flock of 11,000 turkeys were submitted. The producer reported

that there had been six birds found dead the day before, but 50 were found dead the day of submission.

Gross Pathology: All 10 birds had swollen kidneys and were dehydrated. There were petechial hemorrhages on the mucosal surface of the proventriculus and the cecal tonsils. 5/10 had white chalky material on the pericardium.

Gross Diagnosis: Visceral urate deposits and mild, multifocal proventricular hemorrhage.

Diagnosis:

Comments:

Case 5. Sudden increase in mortality in spiker roosters.

Dr. Sandra Stephens, Saskatoon, SK

History: One barn of 24 week old roosters, which were used for spiking broiler breeder barns, experienced a sudden severe, increase in mortality of 36% on day one and 40% the following day. The Canadian Food Inspection Agency (CFIA) visited the farm and collected oropharyngeal and cloacal swabs from birds on the farm and submitted these for influenza A testing by polymerase chain reaction (PCR). Additional swabs and tissue samples for virus isolation and blood for serological examination were sent to the National Centre for Foreign Animal Diseases (NCFAD) in Winnipeg. A quarantine was placed on the premises pending laboratory results.

Diagnosis: Influenza A H7N3

Comments:

Case 6. Suspected Newcastle disease in a pheasant breeding flock.

Dr. Tom Inglis, Airdrie, AB

History: A mixed animal practitioner who monitors the export of pheasants from this breeding flock of 75,000 birds, contacted Dr. Inglis when he was alerted to the fact that there was a sudden rise in mortality. Over a 10 day period the mortality was recorded to be overall, only 1%, but with 75% of these dead coming from one pen (300/2500). The mixed animal veterinarian was asked to submit birds representative of the problem so that a post mortem examination could be performed. The CFIA was notified of this sudden increase in mortality and tissues were submitted for influenza A and Newcastle disease testing. A quarantine was placed on the premises pending testing results.

Gross Pathology: Three adult males were submitted. Two of the three birds had petechial to ecchymotic hemorrhages on the mucosal surfaces of the proventriculus, cecal tonsils, cloaca, leg muscles and trachea. As well, hemorrhages were also present in the cerebellum. One bird had swollen kidneys and the ureters were dilated and contained white material.

Gross Diagnosis: Multifocal hemorrhages of numerous tissues. Dehydration.

Diagnosis:

Comments:

Case 7. Emaciation in a Leghorn hen.

Dr. Colleen B. Annett, Airdrie, AB

History: A producer submitted one Lohman light hen from a flock of 6,000. The producer described the bird as having a growth 'in the front of the neck' which prevented the bird from accessing feed.

Gross Pathology: The bird was moderately depressed and had a large pouch-like extension from the thoracic inlet region. Post mortem examination revealed an extremely emaciated bird (Body Condition Score 1.0/5.0). Upon incision, the pouch exuded a foul-smelling liquid grain material. There was very little food in the proventriculus, ventriculus or the remainder of the intestinal tract. This bird was not in production.

Gross Diagnosis: Emaciation with severe dilation of the crop.

Diagnosis:

Comments:

Case 8. Mortality attributed to respiratory disease in layers and pullets.

Dr. Tom Hutchison, Winnipeg, MB

a) *History*: Producer reports that in the past four weeks there have been five to 10 birds dying everyday (up to 10% final number) in his flock of 42 week old laying hens. The birds would gasp several times and fall over dead. Farm has tried electrolytes and stress aid with no luck. Birds are only found dead on the young bird side of the barn. Production is excellent.

Gross Pathology: Four birds were submitted. All were in excellent body condition. There was a light grey exudate that entirely covered, and was slightly adhered to, the laryngeal mucosa and a small portion of the proximal trachea. The underlying mucosa was hyperemic.

Gross Diagnosis: Necrotizing tracheitis

Histopathology: Laryngeal and tracheal sections have inflammatory exudate with bacteria overlying a necrotic mucosa. Laryngeal sections also have focal, epithelial proliferation and hyperplasia with vacuolar degeneration and several intracytoplasmic, eosinophilic inclusion bodies.

b) *History*: Two separate flocks (11,000 birds and 5,000 birds) of 20 week old pullets (that were raised on the same farm as the affected hens described above) were described as having increasing mortality for approximately one week.

Gross Pathology: Two birds were submitted from one flock and 13 birds were submitted from another. There was a granular, thickened appearance to the mucosa of the larynx and adjacent trachea in some of the birds.

Histopathology: Laryngeal and tracheal sections have a thickened proliferative and hyperplastic epithelium with many eosinophilic, intracytoplasmic inclusions.

Diagnosis:

Comments:

ACKNOWLEDGEMENTS

This manuscript was a joint effort of numerous veterinarians from Western Canada, including Tom Hutchison, Rosemary Postey, Mark Swendrowski, Tomy Joseph, Bob Goodhope, Susantha Gomis, Sandra Stephens, Catherine Graham, Bob Holowaychuk, Gord Kopp, Tom Inglis, Darko Mitevski, Bill Cox, Ann Britton, and John Robinson.

CHALLENGE STUDIES TO ASSESS PROTECTION OF INFECTIOUS BURSAL DISEASE VIRUS VACCINE AGAINST FIELD CHALLENGE: THE ROLE OF SPF CONTROLS

G. Avellaneda and T. Girshick

Charles River Laboratories - Avian Products and Services (SPAFAS), 67 Baxter Road, Storrs, CT 06268

Historically, chick challenge studies have been performed to assess the protection afforded for infectious bursal disease virus (IBDV) vaccines in broilers against the challenge with field isolates using the bursa: body weight (B:BW) ratios as a measurement parameter. This vaccine evaluation system gives good indication of whether or not a vaccination program is appropriate for a particular challenge strain. However, in these studies the uniformity of the challenge dose from experiment to experiment is difficult to establish creating the need of standardizing the challenge model.

The main objective of this experiment was to establish the base line of information on bursa: body weight ratios of normal specific pathogen free (SPF) birds and SPF birds challenged with different strains of IBDV. For this purpose groups of 20 SPF birds were challenged with 10^3 EID_{50} /bird of Standard, Variant E, and Al- strains of IBDV. Three replicates of each experiment were performed at different times. The B:BW ratios obtained (Figure 1) served as background to determine whether challenge experiments performed in broilers with the goal of evaluating vaccine efficacy based on B:BW ratios had the appropriate challenge dose level. This challenge model was evaluated in subsequent chick challenge where IBDV vaccinated one day old broilers were brooded and challenged at two weeks of age with Variant E or AL-2 IBDV strains. The B:BW ratios obtained for the SPF groups in the chick challenge experiments (Table1) were 4.77 for the non-infected control, 1.22 for the group infected with Variant E, and 1.09 for the group infected with Al-2. When these values where compared with the base line data (Figure 1) they showed to be within the range with the exception of the AL-2 which was slightly lower than the range of 1.3 to 1.5 obtained in the SPF studies indicating that the challenge dose for the chick challenge in broilers had been higher for this particular strain.

Interestingly the B:BW values of the different groups of broilers showed a large variation after challenge with either strain due possibly to different levels of maternal antibodies, immune response to the vaccine, or genetic make up of the different sources among other reasons. But because the B:BW ratios of the SPF birds were within the expected values, confident conclusion can be made about the protection afforded for the vaccines in the face of these two challenge strains.

Figure 1. Average of the B:BW ratios in SPF birds after infection with Standard, Variant E or Al-2 strains of IBDV in three different experiments.

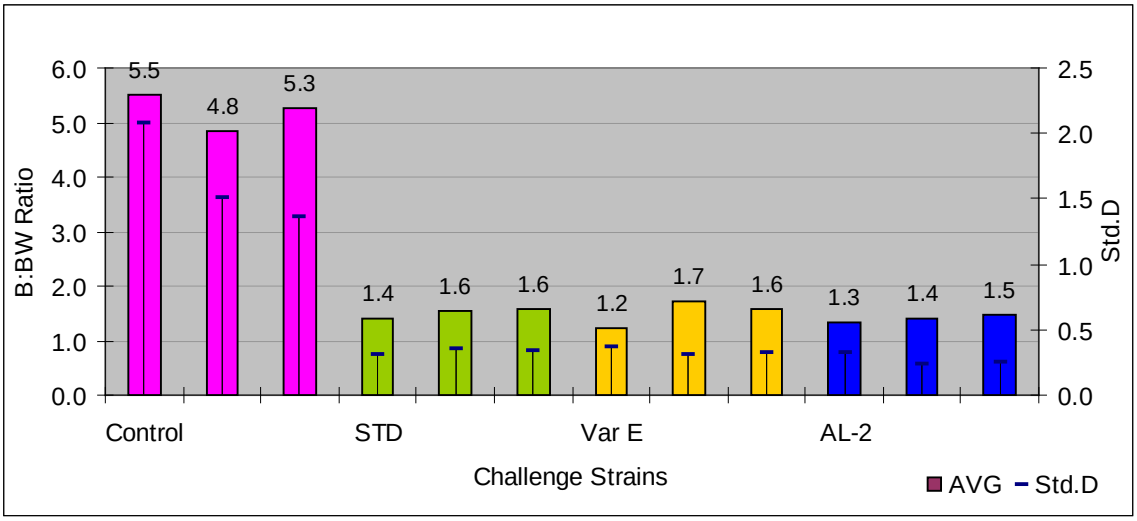


Table 1. Bursa:Body Weight Ratio in chickens of different sources challenged with different strains of IBDV

Birds Source	Non-infected Controls	Var-E	Al-2
	AVG B:BW (SD)	AVG B:BW (SD)	AVG B:BW (SD)
SPF***	4.77* (0.67)**	1.22 (0.21)	1.09 (0.23)
1	2.04 (0.51)	1.10 (0.42)	0.60 (0.11)
2	1.86 (0.43)	1.38 (0.55)	0.65 (0.12)
3	2.00 (0.53)	1.11 (0.48)	0.59 (0.11)
4	2.23 (0.57)	1.41 (0.56)	0.67 (0.14)
5	2.51 (0.47)	1.88 (0.75)	0.93 (0.32)
6	2.19 (0.44)	1.57 (0.52)	0.81 (0.16)
7	2.20 (0.27)	1.18 (0.54)	0.67 (0.18)
8	2.07 (0.52)	1.21 (0.56)	0.66 (0.17)
9	1.95 (0.37)	1.00 (0.41)	0.66 (0.13)

* Average bursa:body weight ratios
** Standard Deviation (%)
***Non-vaccinated SPF birds used as challenged control group

A STUDY ON PRODUCTION STATUS OF *MYCOPLASMA GALLISEPTICUM*-FREE AND MG-INFECTED COMMERCIAL LAYER FLOCKS IN TABRIZ REGION OF IRAN

A. Azizpour[A], M.H. Bozorgmehri Fard[B], S. Charkhkar[C], N. Shikhi[C], A. Feizi[D], A .Zakeri[E], and S. Farashi Bonab[F]

[A]Post Graduate Student of Poultry Diseases, Faculty of Specialized Veterinary Sciences, Islamic Azad University, Science and Research Campus, Tehran and Member of Young Researchers Club (Ardabil branch), Islamic Azad University, Ardabil branch, Ardabil, Iran
[B]Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[C]Department of Clinical Sciences, Faculty of Specialized Veterinary Sciences, Islamic Azad University, Science and Research Campus, Tehran, Iran
[D]Department of Clinical Sciences, Faculty of Veterinary Medicine, Islamic Azad University, Tabriz Branch, Tabriz, Iran
[E]Department of Animal Sciences, Agriculture Faculty, Islamic Azad University, Tabriz branch, Tabriz, Iran
[F]Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
Corresponding author's email: Aidin_azizpour@yahoo.com

ABSTRACT

Mycoplasma gallisepticum (MG) is the most pathogenic and economically significant mycoplasma of poultry and has a worldwide distribution. The aim of this study was to determine the effects of MG on performance of commercial layer flocks in Tabriz, one-day old, Hyline W-36-strain eight female flocks of commercial layer chickens from a breeding company. Four MG-free flocks and four MG-infected flocks were compared for production factors during rearing and production periods. The production factors data were analyzed by using analysis of variance (one-way ANOVA models) and in cases of significant difference, Tukey' test was used. In rearing period, livability and body weight at the age of 17 weeks in MG-infected flocks were significantly lower than MG-free flocks ($P < 0.05$). The most total feed intake in rearing period was in MG-infected group; however, difference between this group and MG-Free group wasn't significant. At production period (18-80 weeks), livability, hen housed egg production to 80 weeks, and peak production in MG-infected group were less than MG-Free group; whereas total feed intake was higher in MG-infected group; however, these factors weren't significantly different between MG-infected and MG-Free groups. According to the results of this research peak production of MG-infected group was 4.73% less than MG-Free group, and MG-infected group produced 8.5 eggs per hen fewer than MG-free group in the laying cycle. This investigation indicated that overall, MG-infected group had lower economic performance.

INTRODUCTION

Avian mycoplasmosis is an important disease in poultry industry of many countries and causing economic loss (1,6). The disease can be caused by several species of genus *Mycoplasma* (class Mollicutes, order Mycoplasmatales, family Mycoplasmataceae), such as *Mycoplasma gallisepticum*, *Mycoplasma iowae*, *Mycoplasma meleagridis*, and *Mycoplasma synoviae* (1,7,14). It mainly occurs in chickens and turkeys but many other domestic and wild birds may be infected (10,15). Mycoplasmas are transmitted both horizontally, through infectious aerosols, contaminated feed and water, close contact between birds, and vertically in laid eggs (transovarian transmission), and can be remain in the flock constantly as subclinical form (5,10,14). *M. gallisepticum* (MG) is the most important mycoplasmal pathogen in poultry and is responsible for significant economic losses in the poultry industry worldwide (3,5). This organism is the etiologic agent of chronic respiratory disease (CRD) in chickens and infectious sinusitis in turkeys. Clinical symptoms of MG infections in these avian species include rales, coughing, nasal discharge, sinusitis, and severe air sac lesions (6,10,14). The mortality rate is low unless a secondary microorganism infection is present (6,11). The aim of this study was to determine the effects of MG on performance of commercial layer flocks during the rearing and production periods in Tabriz.

MATERIALS AND METHODS

Flocks. For this study, one-day old Hyline W-36-strain eight female flocks of commercial layer chickens from a breeding company in Tabriz-Iran were chosen.

Four flocks were MG-free (capacity overall 40000) and four flocks were infected with MG (capacity overall 40000). The flocks were reared in nearly the same conditions and were compared for production factors during rearing and production periods from February 2006 to November 2007.

Preparation of sera samples. On day two, 20 chicks were randomly chosen per flock and blood samples were obtained aseptically from the selected birds. The blood was allowed to clot and kept for 1-2 h at room temperature. After clotting, sera were separated, centrifuged at 1500 g for 6 min, poured into sterile vials, individually labeled, and stored at 4ºC.

Serum plate agglutination (SPA) test. The SPA test was carried out with crystal violet stained MG antigen (Nobilis®, Intervet International, Holland) for detection of MG antibodies in collected sera to determine the infection. Briefly, 20 µL of antigen and 20 µL of chicken sera were mixed and followed by gentle rocking. Results were read within two min. In positive cases granules formed slowly which was seen during rocking, but in negative case no such granules formed within two min. All serum samples were first tested at a dilution of 1:2. Subsequently, positive samples were serially diluted from 1:4 to 1:32 in saline and re-tested. Serum samples that showed agglutination at a dilution of 1:16 or higher were considered positive. The flock was considered MG-infected if 10% or more serum samples were positive in SPA test (4,7,14).

Measurements. Performance factors that were investigated in the MG-infected and MG-free flocks during growing period (1-17 weeks) included livability (%), body weight at 17 weeks (g), and total feed intake (kg). During the production period (18-80weeks), livability (%), total feed intake (kg), hen housed egg production to 80 weeks, and peak production (%) were evaluated.

Statistical analysis. The data of production factors were analyzed by using analysis of variance (one-way ANOVA models) and in cases of significant difference, Tukey' test was used. Results are expressed as mean±SD and differences were considered significant at $P < 0.05$.

RESULTS

The results of production factors analysis of MG-free and MG-infected flocks are shown in Table 1. At the rearing period, livability and body weight at the age of 17 weeks in MG-infected flocks were significantly lower than MG-free flocks ($P < 0.05$). The most total feed intake in rearing period was in MG-infected group; however, difference between this group and MG-free group wasn't significant. At production period (18-80 weeks), livability, hen housed egg production to 80 weeks, and peak production in MG-infected group were less than MG-free group; whereas total feed intake was higher in MG-infected group; however, these factors weren't significantly different between MG-infected and MG-free groups.

DISCUSSION

Due to the substantial performance and production losses, MG has been described as the most economically important pathogenic *Mycoplasma* species affecting poultry (3,6). Production losses between 10 and 20% have been reported in layers and broiler breeder chicken flocks infected with MG (1). The annual economic impact of MG infections in the United States was estimated at between $118 and $150 million for the layer industry alone in 1994 (12). The economic losses is due to prevention and treatment costs, mortality, decreases egg production and hatchability, and reduced feed conversion efficiency and weight gain (2,3,6,10,13,15). In a study, layers maintained free from infection with MG laid on the average 15.7 more eggs/hen housed than the layers infected with MG (2). In another study using data collected from 366 commercial layer flocks in California, an MG-infected flock produced 12 and five fewer eggs per hen than an uninfected flock during first and second cycles, respectively (9). Similar to previous studies, this investigation indicated that MG-infected group in general had lower economic performance. Livability and body weight at the age of 17 weeks in MG-infected flocks were significantly lower than MG-free flocks ($P < 0.05$). Peak production of MG-infected group was 4.73% less than MG-free group, and MG-infected group produced 8.5 eggs per hen fewer than MG-free group in a laying cycle, which was about 60 weeks in duration. Based on this information we can assume that MG infection in 1000 hens might causes 8500 fewer eggs production costing about $625. It is necessary to mention that this calculation of egg production is without the livability of birds on laying point.

REFERENCES

1. Bradbury, J.M. Avian Mycoplasmosis. In: Frank Jordan *et al*. (eds) Poultry Diseases. 5th edn. W.B. Sanders Company, Lowa. pp: 178-193. 2001.

2. Carpenter, T.R., E.T. Mallinson, K.F. Miller, R.F. Gentry, and L.D. Schwartz. Vaccination with F stirian *Mycoplasma gallisepticum* to reduce production losses in layer chickens. Avian Dis 25: 404-409. 1981.

3. Evans, J.D., S.A. Leigh, S.L. Branton, S.D. Collier, G.T. Pharr, and S.M.D. Bearson. *Mycoplasma gallisepticum*: Current and Developing Means to Control the Avian Pathogen. J. Appl. Poult. Res 14, 757–763. 2005.

4. Feberwee, A., D.R. Mekkes, J.J. De Wit, E.G. Hartman, and A. Pijpers. Comparison of culture, PCR and different serologic tests for detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infections. Avian Dis 49, 260-268. 2005.

5. Feberwee, A., D.R. Mekkes, D. Klinkenberg, J.C.M. Vernooij, A.L.J. Gielkens, and J.A. Stegeman. An experimental model to quantify horizontal transmission of *Mycoplasma gallisepticum*. Avian Pathology 34 (4), 355-361. 2005.

6. Levisohn, S. and S.H. Kleven. Avian mycoplasmosis. Rev Sci Tech 19 (2): 425-442. 2000.

7. Lierz, M., N. Hagen, D. Lueschow, and H.M. Hafez. Use of polymerase chain reactions to detect *Mycoplasma gallisepticum*, *Mycoplasma imitans*, *Mycoplasma iowae*, *Mycoplasma meleagridis* and *Mycoplasma synoviae* in birds of prey. Avian Pathology 37(5), 471-476. 2008.

8. Mushi, E.Z., M.G. Binta, R.G. Chabao, M. Mathaio, and R.T. Ndebele. Detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* antibodies in the sera of indigenous chicken by Rapid Plate agglutination test at Mnopane, Gabornone, Botswana, Onderstepeort. J. Vet. Res 66: 333-334. 1999.

9. Mohammad, H.O., T.E. Carpenter, and R. Yamamoto. Economic impact of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in commercial layer flocks. Avian Dis 31 (3): 478-482. 1987.

10. Nascimento, E.R., V.L.A. Pereira, M.G.F. Nascimento, and M.L. Barreto. Avian Mycoplasmosis Update. Brazilian Journal of Poultry Science 7 (1), 1-9. 2005.

11. Pakpinyo, S., P. Pitayachamrat, S. Saccavadit, T. Santaswang, A. Tawatsin, and J. Sasipreeyajan. Laboratory Diagnosis of *Mycoplasma gallisepticum* (MG) Infection in Experimental Layer Chicken Receiving MG Vaccines and MG Organisms. TJVM 36(2): 29-37.

12. Patterson, P.H. Coping with *Mycoplasma gallisepticum*. Internews 7:1–3. 1994.

13. Roussan, D.A., E.A. Abu-Basha, and R.R. Haddad. Control of *Mycoplasma gallisepticum* Infection in Commercial Broiler Breeder Chicken Flocks Using Tilmicosin (Provitil Powder®) Oral Formulation. International Journal of Poultry Science 5 (10): 949-954. 2006.

14. Ley, D.H. *Mycoplasma Gallisepticum* Infection. In:Saif, Y.M. *et al*. (eds). Disease of poultry 12th edn. Blackwel Publishing Company. Iowa State Press University, Chapter 21, pp: 807-833. 2008.

15. Sarkar, S.K., M.B. Rahman, M. Rahman, K.M.R. Amin, M.F.R. Khan, and M.M. Rahman. Sero-Prevalence of *Mycoplasma gallisepticum* Infection of Chickens in Model Breeder Poultry Farms of Bangladesh. International Journal of Poultry Science 4 (1): 32-35. 2005.

Table 1. Performance factors of MG-free and MG-infected groups during rearing period (1-17 weeks) and production period (18-80 weeks).

Rearing period

Groups	Livability (%)	Body weight at the age of 17 weeks (g)	Total feed intake (kg)
MG-Free	97.18 ± 0.17a*	1190 ± 0.002 a	5.40 ± 0.12
MG-infected	96.30 ± 0.18b	1180 ± 0.006 b	5.54 ± 0.18

Production period

Groups	Livability (%)	Total feed intake (kg)	Hen housed egg to 80 weeks	Peak production (%)
MG-Free	92.12 ± 1.54	42.78 ± 0.80	313.75 ± 6.74	89.25 ± 2.20
MG-infected	90.30 ± 1.50	44.80 ± 1.86	305.25 ± 4.10	84.52 ± 3.92

*a.b: Means having different superscript in a column differ significantly ($P < 0.05$).

PREVALENCE OF *NETB* GENE IN *CLOSTRIDIUM PERFRINGENS* FIELD STRAINS ISOLATED FROM CHICKENS

Luca Bano, Ilenia Drigo, Cosetta Bacchin, Monia Cocchi, Angela Guolo, Barbara Marcon, Michela Bonci, and Fabrizio Agnoletti

Istituto Zooprofilattico Sperimentale delle Venezie, Laboratorio di Treviso, viale Brigata Treviso 13/A, 31100 Treviso, Italy

ABSTRACT

Clostridium perfringens (CP) is well known as the etiological agent of necrotic enteritis (NE) in chicken. For over 30 years α toxin was considered the key virulence factors in this type of pathology. Recently a new toxin related to the appearance of NE, called NetB, has been described. The aim of this work was to evaluate the presence of genes coding for α (*cpa*), β (*cpb*), ε (*cpetx*), ι (*cpi*), β2 (*cpb2*), enterotoxin (*cpe*) and NetB (*netB*) toxin in CP field strains collected from chickens affected or not by enteric diseases. Seventy-two CP field strains were toxin typed: 22 isolated from chickens affected by NE, 38 from chickens with intestinal lesions not ascribable to NE and 12 from healthy chickens. 91,6% strains were positive for *cpa* gene (toxintype A) and 8,3% for *cpa* and *cpb2* genes (toxintype A+β2). 33.3% CP resulted *netB* positive and 91.6% of these were isolated from chickens affected by intestinal diseases: 14 with NE and 8 with macroscopic lesions other than NE. The number of *netB* positive strains was significantly higher (p = 0,002) in chickens affected by NE (61%) than in birds with different intestinal disorders (23%). Our preliminary results seem to confirm the presence of *netB* gene in CP involved in NE outbreaks, even if, its role should be verified by means of the evaluation of the toxin expression.

INTRODUCTION

Clostridium perfringens (CP) is an important enteropathogenic agent in humans and animals. It is often found in the intestinal tract of healthy animals but it can cause outbreaks of serious enteric diseases through the production of a variety of toxins. The differential production of the four major toxins (α, β1, ε, and ι) is used to classify strains into five toxin-types (8). Some CP strains, in addition to α toxin, produce β2 and enterotoxin: two toxins that have been proposed as being important in the pathogenesis of intestinal disorders in animals and humans respectively.

In poultry, CP is well known as the causative agent of both acute and sub clinical necrotic enteritis (NE). Acute NE is characterized by high mortality rates without premonitory signs and at necropsy the mucosa of the small intestine appears strewn of large necrotic foci while, in severe cases, necrotic material covers large tracts of the intestinal mucosa surface. In sub-clinical NE the intestinal mucosa damage is limited and this condition is characterized by malabsorption with consequent reduced weight gain and increased feed-conversion ratio (6).

Historically, the α toxin has been recognized as the key virulence factor in this type of pathology but Keybourn and co-workers (5), using α-toxin knock-out mutant of CP, brought evidences that it is not an essential virulence factor in NE. The same Authors in 2008 described a novel toxin, NetB, that displays a moderate amino acid sequence similarity with CP β toxin and that seems to be expressed in most strains isolated in NE outbreaks (4). On the contrary, a recent prevalence study on *netB* gene in clinical isolates of CP from animals in US, arrived at different conclusions (7).

The aim of our study was to evaluate the presence of genes coding for α (*cpa*), β (*cpb*), ε (*etx*), ι (*cpi*), β2 (*cpb2*), enterotoxin (*cpe*) and NetB toxins in CP field strains collected from healthy chickens and from subjects affected by enteric diseases.

MATERIALS AND METHODS

Source of isolates. 72 CP field strains were analysed. The strains were isolated from the intestine (5 cm back and 2 cm after Mekel's diverticolum) of 22 chickens affected by NE, 38 chickens with intestinal disease not ascribable to NE and 12 healthy chickens.

Strains and growth conditions. All strains were obtained streaking on Perfringens Agar Base (Oxoid) 0.1 mL of 24 h broth (Cooked Meat Medium, Difco) previously inoculated with intestinal samples. CP ATCC 27324 (toxin-type E + enterotoxin), CCUG 2036 (toxin-type C), CCUG 2037 (toxin-type D), ATCC 10543 (toxin-type A+ β2) were used as reference strains. All strains were incubated in anaerobic conditions at 37°C for 48 hours.

DNA extraction. Five colonies of each CP strain included in the study were recovered from the agar plate and the DNA was extracted with DNeasy Blood & Tissue Kit (Qiagen) according to manufacturer's instructions.

Toxin coding gene detection. One multiplex PCR for *cpa*, *cpbl*, *cpetx*, and *cpi* genes and three single PCR for *cpb2*, *cpe* and *NetB* genes detection were used (1,8,3,4). The sequencing of the amplified product confirmed that the targeted *netB* gene was indeed amplified with the PCR assay.

Parasitological examination. Intestinal mucosa of all chickens was scraped in different districts and observed by optic microscope searching for protozoa and helminths (eggs and worms).

Statistical analysis. For statistical analysis Fisher exact test has been used.

RESULTS

All strains resulted positive for α toxin gene (toxin-type A) and only six (8.3%) of these were positive also for β2 toxin (toxin-type A + β2). No CP cpe-positive strains were detected.

24/72 (33.3%) CP were *netB* positive and 91.6% of these was isolated from chickens affected by intestinal disorders. *NetB* positivity percentage in each group is summarized in Table 1.

22/72 (30.5%) chickens resulted positive for coccidia. The number of *netB* gene positive strains was significantly higher with a p-value of 0.002 in chickens affected by NE (14/22, 63.6%) than in birds with different intestinal disorders (8/38, 21%). This result is more significant (p = 0.0001) if we consider only chickens tested negative at parasitological examination but with necrotic enteritis.

DISCUSSION

Our preliminary results seem to support the involvement of NetB toxin in the pathogenesis of NE, even if, its role should be verified by means of the evaluation of the real toxin expression and in a larger number of chicken isolates.

It is interesting observe that NE, in coccidia negative subjects, is significantly associated with the presence of CP *netB* positive strains. This result sustains the theory that CP *netB* positive strains cause NE without the support of other pathogens (such as coccidia). Otherwise, when coccidia parasite the intestine, not only CP *netB* positive but also CP with pathogenic mechanisms different from NetB toxin, could be able to produce NE.

On the contrary, the observation that strains isolated from two healthy animals were positive for *netB* gene, confirm that other virulence factors such as proteolytic enzymes or predisposing factors could have an important role on NE appearance (9).

Anyway, in this study NE lesions were defined by macroscopic observations and for this reason the number of sub-clinical necrotic enteritis could be underestimated. In future investigations histological examination must be take into account to detect necrotic intestinal lesions.

Hence, caution is required when interpreting surveys of isolates from disease outbreaks because isolates might change during the culturing process or there might even be a mixture of pathogenic and non-pathogenic strains present in the same diseased birds.

The absence of the *cpe* gene in the CP strains included in this study, lead to suppose that chicken products do not represent an important risk for transmission of enteropatogenic CP from this species to humans. However, this last sentence must be supported by further investigations in a larger number of CP strains of chicken origin.

REFERENCES

1. Yoo, H.S., S.U. Lee, K.Y. Park, and Y.H. Park. Molecular typing and epidemiological survey of prevalence of *Clostridium perfringens* types by multiplex PCR. J. Clin. Microbiol. 35:228-232. 1997.
2. Meer, R.R. and G. Songer. Multiplex polymerase chain reaction assay for genotyping *Clostridium perfringens*. Am. J. Vet. Res. 58:702-705. 1997.
3. Baums, C.G., U. Schotte, G. Amtsberg, and R. Goethe. Diagnostic multiplex PCR for toxin genotyping of Clostridium perfringens isolates. Vet. Microbiol. 100:11-16. 2004.
4. Keyburn, L., J.D. Boyce, P. Vaz, T.L. Bannam., M.E. Ford, D. Parker, A. Di Rubbo, J.I. Rood, and R.J. Moore. NetB, a new toxin that is associated with avian necrotic enteritis caused by *Clostridium perfringens.* PLoS Pathog. 4(2): e26.doi: 10.1371/ journal.ppat.0040026. 2008.
5. Keyburn, A., L., S.A. Sheedy, M.E. Ford, M.M. Williamson, M.M. Award, J.I. Rood, and R.J. Moore. Alpha Toxin of *Clostridium perfringens* is not an essential virulence factor in necrotic enteritis in chickens. Infect. Immun. 74:6496-6500. 2006.
6. Kaldhusdal, M., C. Schneitz, M. Hofshanger, and E. Skjerve. Reduced incidence of *Clostridium perfringens*-associated lesion and improved per-formance in broiler chickens treated with normal intestinal bacteria from adult fowl; Avian. Dis. 2001.
7. Martin, T., G. and J.A. Smyth. Prevalence of netB among some clinical isolates of *Clostridium perfringens* from animals in the Unated States. Vet. Microbiol. doi:10.1016/j.vetmic.2008.10.026. 2008.
8. Meer, R., R. and J. Songer. Multiplex polymerase chain reaction assay for genotyping *Clostridium perfringens*. Am. J. Vet. Res. 58:702-705. 1997.

9. Van Immerseel, F., J.I. Rood., R.J. Moore and R.W. Titball. Rethinking our understanding of the pathogenesis of necrotic enteritis in chickens; Trends in Microbiology. 17(1): 32-36. 2008.

Table 1. Number of *netB* positive strains isolated from the gut of diseased and healthy chickens: correlation between presence and absence of intestinal lesions ascribable to NE and *netB* gene positivity.

		TOTAL	NetB +	%	NetB -	%
Healthy chickens		12	2	16.6	10	83.4
Enteric diseases	NE	22	14	63.6	8	36.4
	no NE	38	8	21	30	79.0

IMAPACT OF IN-HOUSE LITTER COMPOSTING ON COCCIDIOSIS, NECROTIC ENTERITIS, AND BROILER PERFORMANCE

Daniel A. Bautista[A], Jose Miguel Ruano[B], George W. Malone[B], Steve Fitz-Coy[C], Kathy Phillips[A], and Stephen L. Collier[A]

[A]University of Delaware-Lasher Laboratory, Carvel Research and Education Center, Georgetown, DE 19947
[B]University of Delaware, Department of Animal and Food Sciences, College of Agriculture and Natural Resources, Newark, DE
[C]Intervet/Schering-Plough Animal Health Millsboro, DE

INTRODUCTION

Coccidiosis remains one of the most expensive and common diseases in commercial broiler production. The disease can be mild or severe depending on the amount of ingested oocysts. The short direct life-cycle and high reproductive potential of coccidia in poultry often lead to severe outbreaks in the modern poultry house. Hence the need for young poultry to be on continuous medication with low-levels of anticoccidial drugs to reduce the infections to a low infection level (9). The protozoan parasites of the genus *Eimeria* multiply in the intestinal tract and cause tissue damage, with resulting interruption of feeding and digestive processes, dehydration, blood loss and increased susceptibility to other diseases. The tissue damage and changes in the intestinal tract function is a well-known predisposing factor leading to necrotic enteritis (NE). Studies have shown that coccidia species, such as *E. acervulina*, *E. maxima*, and *E. brunetti* are associated with NE (1,2,3,10).

Necrotic enteritis is an acute enterotoxemic condition of young chickens and turkeys, often associated with high sudden mortality for a short duration. The causative agent of necrotic enteritis is a gram positive, obligate, anaerobic spore-forming bacteria *Clostridium perfringens* (CP), with mortality ranging from 2 to 50%.

During the early years of NE control, in-feed antimicrobials were the preferred products for the control of NE. In recent years worldwide, there is pressure to remove or reduce the usage of in-feed antibiotics from poultry feeds. As a result, there has been a dramatic increase in the prevalence of necrotic enteritis in commercial poultry operations (10). Furthermore, the accumulation of cocci oocysts and CP in built-up old litter can place a heavier burden on anticoccidial and antimicrobial drugs, and cocci vaccines. In-house composting of litter has been shown to reduce a number of poultry pathogens and dramatically improve flock performance.

Recent studies indicated reduced incidence of necrotic enteritis (4) and gangrenous dermatitis (6). The goal of this study is to evaluate the effect of in-house litter composting conditions on coccidiosis and necrotic enteritis organisms in relation to the observed performance improvement through in-house litter composting.

MATERIALS AND METHODS

Inactivation of cocci oocysts and CP by composting temperature and gases. A mixture of broiler chicken-derived sporulated coccidia oocysts (*E. acervulina*, *E. maxima*, *E. tenella*, *E. mivati/mitis type*) suspended in potassium dichromate solution at 1 x 10^6 oocysts/mL was dispensed in six 14 mL polystyrene tubes containing 8 mL inoculum/tube. One set of tubes has the openings screw-capped and taped tightly then sealed with air-tight, heat-sealed plastic bags, and the other set's open ends covered with several layers of sterile gauze. The tubes are all taped around a wooden stick and placed in an upright position in different areas of the compost pile:

a) 1ft below the surface.
b) On the surface of the pile, covered by less than 1 inch litter.
c) Dirt floor along the sidewall of the broiler house at ambient summer conditions.

The tubes are placed on the day of the windrowing of the litter. Representative samples are taken out at three days and seven days respectively. The samples were gavaged into five-wk old SPF leghorns (n = 6). Each bird received 1 mL of the inoculum containing 1 x 10^6 oocysts. The birds were humanely euthanized by cervical dislocation and necropsied on day six dpi and evaluated by gross cocci lesion scoring by Johnson and Reid (5), oocyst counts, and histopathology. This experiment was repeated twice.

The effect of normal composting temperatures and ammonia levels on total *Clostridium perfringens* (CP) and spore counts (obtained by inactivating

vegetative CP bacteria at 65°C/149°F for 30 min) was determined using separate water baths set at 130°F, 135°F, and 140°F to simulate common composting temperatures. Overnight CP broth cultures and spores are immersed in the water bath for 1, 3, 6, and 24 h and quantified using serial dilution and plating in Shahidi-Ferguson Perfringens (SFP) Agar. Ammonia concentrations of 2000 PPM was achieved in a covered litter compost pile at ambient temp (80°F). Tube samples with gauze caps were exposed to the ammonia for three and five days, and then plated into SFP Agar as described.

Inactivation of cocci oocysts in naturally contaminated litter. To approximate natural litter conditions, clean litter from bagged pine shavings were continuously contaminated with a mixture of broiler chicken-derived cocci oocysts (*E. acervulina*, *E. maxima*, *E. tenella*, *E. mivati/mitis type*). This is done using 150 seeder birds (five-wk old SPF leghorns) inoculated with 1 x 10^5 oocysts/mL via oral gavage. The birds were kept for four weeks to allow for enough cocci shedding in the litter to occur, then replaced with a new batch of inoculated birds. Three continuous batches of seeder birds were used. The litter was broken up, collected in a mound and mixed well by shovel. One half (control) was redistributed immediately in the original room in a floor pen four inches deep at ambient temperature and humidity to avoid inadvertent composting conditions. The other half of the pile was transferred to an adjacent cocci-free room with the same physical characteristics. This litter was piled up in a 3½ ft conical mound and allowed to go through the composting process for seven days, with the pile turned/mixed once at three days. Temperature (1ft. level) inside the compost pile surface was monitored at 4 h intervals up to day seven. The pile was broken up at the end of the composting period and was redistributed in a floor pen at four in. depth.

Experimental birds. Day-old straight-run commercial broiler chicks (n = 44 per group), were placed on the composted and uncomposted cocci-contaminated litter. An equal number of birds were placed on clean pine shavings without cocci (cocci-control group) to compare against the growth performance of birds placed in the other two groups. All the birds were raised up to six weeks of age. Average weekly body weights were taken. Necropsy and cocci evaluation was performed on dead birds. Representative birds (n = 6) were euthanized and examined for intestinal cocci lesions (Johnson and Reid method), oocyst counts, and intestinal segments were collected for histopathology evaluation on at 21 days of age. Freshly voided feces from the litter was collected and pooled for fecal flotation and quantification of cocci shedding.

RESULTS AND DISCUSSION

Inactivation of cocci oocysts and CP by composting temperature and gases. Internal windrow temperatures rose to 135°F in 48 h and peak to 150°F in 60 h, but typically, compost pile temperatures consistently peak at 140°F, depending on the litter moisture content (approx. 15 to 30%). Temperatures tend to decline gradually but elevate close to peak temperatures after the windrow is turned/aerated.

Birds inoculated with the cocci mixture placed one ft. underneath the compost pile (three and seven days, gauze capped and sealed tubes) were all negative for any detectable gross coccidiosis lesions, microscopic oocyst counts and histopathological lesions. In contrast, birds inoculated with cocci exposed to the surface of the litter windrow and dirt floor at ambient temperature, regardless of whether the tubes were sealed or gauze-capped, showed acute, moderate to severe lesions compatible with *E. acervulina*, *E. maxima*, *E. tenella*, and *E. mivati/mitis* type coccidiosis. This is accompanied by high counts of oocysts in the intestinal scrapings. The severity of cocci lesions are a function of the stage of the disease at necropsy. The results were consistent when the experiment was repeated. Exposure to composting temperatures alone (135° to 150°F) for at least three days can inactivate common poultry coccidia.

Inactivation of cocci oocysts in naturally contaminated litter. There was significantly higher mortality (6/30 birds) due to severe *E. maxima* and *E. tenella* coccidiosis and necrotic enteritis at days 17-28 of age in the uncomposted, cocci-contaminated litter (control group), compared to 1/30 birds on day 30 in the composted, cocci-contaminated litter (treatment group). The cocci and necrotic enteritis lesions were supported by high *E. maxima* and *E. tenella* oocyst counts and histopathology lesions. The control birds in group 3 did not experience mortality until day 35. Six birds in this group died due to severe (+4) *E. tenella* lesions from day 35 to 42 (end of study), probably through cocci contamination by flies from the uncomposted litter group in the nearby separate room. It was speculated that flies could be responsible for the single mortality in the composted group, but putative premunity due to early low-level exposure to cocci oocysts may have prevented birds raised on composted cocci-contaminated litter group from developing severe acute cocci lesions compared to the immunologically naïve controls (see voided feces quantification data in the next paragraph below). The experiment was repeated (group 1 was composted again) with enhanced biosecurity to eliminate flies (regular pesticide fogging and fine mesh netting) in addition to the already established ban on stepping on the litter in the pens, and dedicated feed and equipment. The composted

group and the control group did not have any cocci or necrotic enteritis-associated mortality up to the end of the study. The uncomposted litter group, on the other hand, had two cocci-related mortalities on day 25 (one bird with severe necrotic enteritis and high *E. maxima* cocci oocyst counts) and day 39 (one bird with severe bloody cecal contents and thickened lining (+4 *E. tenella* cecal lesions) with high (+4) counts of *E. tenella* oocysts.

Cocci lesions and microscopic scoring in live birds. Bird samples (6/6) grown on composted litter showed no gross cocci lesions and no oocysts in intestinal scrapings of the duodenum, jejunum and cecum. This was confirmed by histopathology results. Birds from the uncomposted litter showed (+2) moderate (1/6 *E. maxima*, 2/6 *E. tenella*) and severe (+3) gross cocci lesions (2/6 *E. maxima*, 3/6 *E. tenella*). Fecal flotation of pooled samples of freshly voided feces at day 21 revealed significant different between groups. There were no oocysts found in group 3. There is a very low (+1) oocyst count of *E. maxima* in group 2 compared to markedly higher (+3) counts of *E. maxima* and *E. tenella* oocysts, consistent with the cocci lesion and microscopic oocyst count scoring. The experiment was repeated again as mentioned in the previous paragraph. Birds from the control and the composted group (n = 10/group) are negative for any gross cocci lesions and microscopic counts. The uncomposted group had 2/10 birds with mild lesions (+1) associated with *E. maxima* and *E. tenella* respectively. The lesions are supported by low counts (+1) of *E. maxima* and *E. tenella* from scrapings taken from the respective areas of the lesion scores. At the end of the study (42 days of age), another cocci check was done on 15 birds per group. The control and the composted groups were negative for cocci lesions and oocysts. The uncomposted group on the other hand showed early cocci lesions scores: 4/15 (+1) and 3/15 (+2) *E. acervulina*-type lesions; and 4/15 (+1) *E. tenella* lesions. This is supported by the corresponding cocci oocyst counts: 1/15 (+1) and 2/15 (+2) *E. acervulina*-type; 5/15 (+1), 4/15 (+2), 7/15 (+3) *E. mivati* type; *E. maxima* lesions; 4/15 (+1), 5 (+2), 4/15 (+3) and 1/15 (+4) *E. tenella* oocyst counts. Fecal flotation and quantification of cocci (day 28) in voided feces showed *E. maxima* was being shed (5.4×10^5 per g feces) in the uncomposted litter group. This is compatible with the single mortality (necrotic enteritis) in this group on day 25 (see mortality). Fecal flotation to correlate with the cocci check at the end of the study was not done.

Effect on growth performance. There are no significant differences in body weights between the three groups from day of age to three weeks. However, the mean body weights of the birds raised on the uncomposted cocci-contaminated litter were significantly lower ($\alpha = 0.05$) than the birds in the composted cocci-contaminated group from weeks four to six (end of study). The mean body weight of the birds in the composted litter and the control group are not significantly different except at six weeks, where the mean weight of the composted group is significantly heavier ($\alpha = 0.05$) than birds in the controls and uncomposted litter group. This is a result of the controls inadvertently getting infected then subsequently succumbing to severe clinical signs of *E. tenella* coccidiosis starting at five wk. The mean weights of controls and the uncomposted litter groups which both experienced severe coccidiosis are not significantly different at wk six. The divergence in mean body weights is directly linked to the onset of above-mentioned clinical coccidiosis and concomitant necrotic enteritis. The results from the repeated experiment showed the same trend, without the previous experiment's cocci-initiated decreased weight gain at week six relative to the composted litter group (data not shown).

Both repeated experiments in contaminated litter showed that in-house composting of cocci-contaminated litter significant significantly reduced the incidence of clinical coccidiosis and necrotic enteritis and the amount of cocci oocysts being shed as evidenced in the oocyst counts and voided feces. Furthermore, the first experiment also suggested that reduced levels of cocci in the litter maybe responsible for beneficial premunity that alleviates cocci clinical signs on subsequent cocci infection, relative to a completely naïve flock. In a commercial farm setting, it is realistic to expect that most, but not all litter material is effectively removed, windrowed and composted. These can be reservoirs for cocci and other poultry pathogens. Be that as it may, the significant reduction of cocci levels in the litter as a result of composting may enhance the effectiveness of cocci vaccines and anticoccidial medications by reducing the level of initial cocci field challenge. Reduction of cocci challenge will reduce the incidence of necrotic enteritis which is a common sequelae to coccidiosis.

Total *C. perfringens* counts (7.8 logs) were reduced by 2.4 logs in 1 h at 135°F and 140°F. There was a lesser degree of reduction (1.26 logs) at 130°F but showed comparable log reduction (2.35 logs) at 3 h, and similar log reduction at 6 h (2.65 logs) compared to 140°F (2.67 logs). Ammonia concentrations of 2000 PPM in a covered compost pile at ambient temp (80° to 85°F) did not decrease initial CP log counts. CP spore counts were not reduced by 130° to140°F temperatures and 2000 PPM ammonia levels indicating the durability of CP spores.

The reduced incidence of necrotic enteritis in a commercial broiler house using a tractor-attached aerator/windrowing equipment over conventional

removal of caked litter between flocks have been documented (4). It is plausible, based on our findings, that this field observation can be explained by the reduction of coccidia oocyst levels (rather than CP spore levels) due to litter composting.

REFERENCES

1. Al-Sheikhly, F. and R.B. Truscott. The pathology of necrotic enteritis of chickens following infusion of broth cultures of *clostridium perfringens* in the duodenum. Avian Diseases, 21, 230-240. 1977.

2. Al-Sheikhly, F. and A. Al-Saieg. Role of coccidia in the occurance of necrotic enteritis of chickens. Avian Diseases, 24, 324-333. 1980.

3. Baba, E., A.L. Fuller, J.M. Gilbert, S.C. Thayer, and L.R. McDougald. Effect of *Eimeria brunetti* infection and dietary zinc on experimental induction of necrotic enteritis in broiler chickens. *Avian Dz* 36: 59-62. 1992.

4. Flory, G.A., R.W. Peer, B. Barlow, D. Hughes, G.W. Malone, and A.P. McElroy. Litter Reconditioning as an Alternative Litter Management Strategy within the Commercial Poultry Industry. www.deq.state.va.us/export/sites/default/vpa/pdf/Virginia_Litter_Reconditioning_Study.pdf

5. Johnson, J. and W.M. Reid. Anticoccidial drugs: Lesions scoring techniques in battery and floor-pen experiments with chickens. *Exp Parasitol* 28:30-36. 1970.

6. Malone, G.W. Management guidelines for In-house Composting. In 2008 *Delmarva Poultry Conference*, September 10, 2008. Ocean City, MD. http://www.mdchick.umd.edu/files/malonemanagement.pdf.

7. Macklin, K. In-House Windrow Composting. *In 2007 Delmarva Breeder, Hatchery, and Growout Conference*, September 12, 2007. Ocean City, MD. 2007. www.rec.udel.edu/Poultry/proceedings2007/Macklin_In-House%20Windrowing.pdf

8. McDevitt, R.M., J.D. Booker, T. Acamovic, and N.H.C. Sparks. Necrotic enteritis: A continuing challenge for poultry industry. *World's Poultry Science Journal*. 62: 221-247. 2006.

9. McDougald, L.R. and S.H. Fitz-Coy. Coccidiosis. *In Diseases of Poultry*.12th ed. p. 1070

10. Williams, R.B., R.N. Marshall, R.M. La Ragione, and J. Catchpole. Parasitol Res 90. 19-26. 2003.

EVALUATION OF LITTER TREATMENT REGIMENS AT THREE USAGE LEVELS

J. P. Blake, J. B. Hess, and K. S. Macklin

Department of Poultry Science, Auburn University, Auburn, AL

SUMMARY

A series of experiments evaluated six litter treatment strategies in reducing ammonia volatilization during broiler production. Poultry Litter Treatment™ (PLT), granulated aluminum sulfate (Al-Clear™) (GA), Poultry Guard™ (PG), and Hydrated Lime (HL), liquid aluminum sulfate (A-7™) (LA), and concentrated sulfuric acid (SA) were applied at three levels each to evaluate the effect of application rate compared to a non-treated control. In each experiment, 70 mixed-sex broilers were placed in each of 16 pens (2.44 x 2.44 m), fed commercial diets, and weighed at 21, 42, and 49 days of age. Litter and ammonia were evaluated weekly. Results indicate that PLT, GA, LA, and SA were capable of reducing ammonia volatilization during broiler production. PG and HL treatments failed to support any reduction in ammonia volatilization.

INTRODUCTION

Interest in the use of litter treatments has increased over the last decade as growers and technical personnel alike recognize health and productivity benefits of improving the broiler house environment. It is known that high ammonia levels make birds more susceptible to respiratory diseases. Numerous laboratory and field studies have shown ammonia levels as low as 10 ppm affecting bird health and performance (1). Ammonia levels above 25 ppm in the poultry house can damage the bird's respiratory system and allow infectious agents to become established, leading to declining flock health and performance. Resistance to respiratory disease may be decreased and pathogenic bacteria can be significantly increased in the lungs, air sacs, and livers of birds exposed to ammonia because of damage that occurs to the tracheal cilia. In addition, growth and condemnation rate may be compromised in birds exposed to levels of ammonia exceeding 10 ppm. Built-up litter propagates higher in-house ammonia levels, which can adversely affect poultry health by making the birds more susceptible to respiratory diseases. Techniques to reduce ammonia levels and pathogenic microbes include changes in management practices and use of litter treatments.

Most litter treatments used in the broiler industry involve chemical reduction of litter pH so that bacteria associated with ammonia release are either inactivated, reduced in number, or both. Volatilization of ammonia has been attributed to microbial decomposition of nitrogenous compounds, principally uric acid, in poultry house litter. Once formed, free ammonia will be in one of two forms: as the uncharged form of NH_3 (ammonia) or the ammonium ion (NH_4), depending on litter pH. Ammonia volatilization remains low when litter pH is below 7.0, but can be substantial when above 8.0. Uric acid decomposition is most favored under alkaline (pH > 7) conditions. Uricase, the enzyme that catalyzes uric acid breakdown, has maximum activity at a pH of 9. As a result, uric acid breakdown decreases linearly for more acid than alkaline pH values. One principal ureolytic bacterium, *Bacillus pasteurii*, cannot grow at neutral pH, but thrives in litter above pH 8.5. Typically, litter pH in a broiler house ranges between 9-10. Gaseous emission of NH_3 can be inhibited if converted to NH_4^+ (ammonium); which can be accomplished by lowering litter pH. In general, an effective litter treatment results in the production of hydrogen ions (H^+) when it dissolves, and the hydrogen ions produced by this reaction will attach to ammonia to form ammonium, which further reacts with sulfate ions to form ammonium sulfate $(NH_4)_2SO_4$. Ammonium sulfate is a water-soluble fertilizer. As a result of these acid-based reactions, the amount of ammonia emitted from the litter will be reduced; which should increase the nitrogen (N) content of the litter.

The main goal in using a litter treatment is to effectively reduce ammonia emissions from poultry facilities, which will have a direct effect on improving litter management, nutrient enrichment, and reducing ammonia volatilization from poultry house litter. Unfortunately, most litter treatments are typically effective for only three to four weeks; whereas, broilers are housed for six or more weeks prior to slaughter. As a result, a series of experiments were conducted to evaluate six treatment regimens applied at three levels with a goal of quantifying the amount applied for its effectiveness in reducing ammonia volatilization as compared to an untreated control.

MATERIALS AND METHODS

Poultry Litter Treatment (PLT™), granulated aluminum sulfate (Al-Clear™) (GA), Poultry Guard™

(PG), and hydrated lime (HL), were applied at 24.4, 48.8, or 73.2 kg/100 m^2 (50, 100, or 150 lbs/1000 ft^2); a liquid acidified aluminum sulfate (A-7^{TM}) (LA), was applied at 81.4, 162.8, and 227.1 L/100m^2 (20, 40 or 60 gal/1000 ft^2); and concentrated sulfuric acid (98% H_2SO_4) (SA) was applied at 9.75, 19.50, and 29.26 kg/100m^2 (20, 40, or 60 lb/1000 ft^2) on new pine sawdust bedding and tested against a non-treated control (CON). In each experiment, 1120 commercial broiler chicks (Cobb X Ross) were obtained from a commercial hatchery and randomized with 70 birds placed in each of 16 pens (2.44 x 2.44 x 2.44 m; 8 x 8 x 8 ft). Birds were fed a corn-soybean meal starter (0.68 kg/bird; 22% CP, 3087 kcal/kg ME), grower (1.36 kg/bird; 20% CP, 3131 kcal/kg ME), finisher (1.81 kg/bird; 17.5% CP, 3197 kcal/kg ME) and withdrawal (c.a. 1.36 kg/bird; 16.5% CP, 3219 kcal/kg ME) to meet or exceed NRC (2) requirements. New pine shavings (54.42 kg; 120 lbs) were placed in each pen at the start of each experiment. Feed and water were provided *ad libitum* with 24 hr light. Birds and feed were weighed at 21, 42 and 49 d to determine growth and feed performance.

Litter and air quality samples were obtained initially and weekly through day 49. Ammonia measurements were conducted using a closed container of specified dimension (46 x 36 x 12 cm; 21 x 15.5 x 5 in) inverted over the litter bed and determined using a Drager CMS Analyzer equipped with a remote air sampling pump and appropriate ammonia sampling chip (0.2-5, 2-50, or 10-150 ppm). The tube from the sampling pump was located in the top center of the container. The sampling pump was evacuated (calibrated) for 60 seconds followed by a measurement period of up to 300 seconds. Most readings were usually achieved with 60 seconds following evacuation. Litter was collected weekly, starting the day prior to chick placement and continued through day 49. Collection was performed in each pen by using the grab sampling technique. Individual litter samples (3g) were mixed with 60 mL distilled water for pH measurement. Data was analyzed by analysis of variance using the General Linear Models procedure of the Statistical Analysis System (3). When significant ($P < 0.05$), means were separated by Tukey's HD multiple comparison procedure.

RESULTS AND DISCUSSION

There were no differences ($P > 0.05$) in growth performance in any experiment attributed to type or level of litter treatment. Initial litter pH was significantly lower ($P < 0.05$) for PLT, GA, PG, LA, and SA treated pens as compared to CON (ca 2.3 vs. 6.4) and was influenced by level of application. Results indicated that PLT, GA, and LA significantly ($P < 0.05$) reduced ammonia volatilization as compared to CON through day 42 at the intermediate and highest application rates. SA significantly ($P < 0.05$) reduced ammonia volatilization through day 35 at only the highest application rate as compared to CON. Although PG exhibited the ability to lower pH, it failed to elicit a significant ($P > 0.05$) reduction in ammonia. Conversely, HL elevated litter pH initially as compared to CON (12.8 vs. 6.3), but this effect disappeared after day 21. HL failed to support any reduction in ammonia volatilization. Litter analysis results did not indicate a significant ($P > 0.05$) increase in amount of nutrients retained due to treatment. Results indicate that PLT, GA, LA, and SA were capable of reducing ammonia volatilization during broiler production. Results show that higher levels of litter treatments can extend ammonia control and may contribute to improvements in bird health. In these trials, ammonia levels were often controlled at the intermediate and highest application levels for up to 42 days (starting with new pine shavings litter).

Originally, litter treatments were placed at a relatively low level (generally 50 lb/1000 ft^2) to give early ammonia control during the brooding period. More recently, higher levels have been suggested as the industry becomes more comfortable with performance benefits associated with improving air quality in the broiler house with litter treatment use. To maximize the effectiveness of any litter treatment, one must properly prepare and apply the litter treatment in addition to managing the house and litter. Prior to application of any litter treatment, the house needs to be de-caked or tilled. Afterwards, the litter treatment can be applied at the chosen level before birds are placed in the house. Spills or concentrated areas should be raked into the litter to prevent overexposure or consumption by the young birds. As with any litter treatment product, the rate selection for an individual's operation will be dependent on current management practices and needs based on such factors as ventilation control and litter moisture levels. Higher rates may be recommended when high ammonia conditions prevail.

Litter treatments have become a common means of improving the broiler house environment throughout much of the broiler industry. Although different litter treatments vary in their ability to control ammonia, each offers a unique set of characteristics that need to be considered in selecting the appropriate product to meet an individual's needs. The litter treatment that offers the best return on investment will depend on the user's ability to select the product that best meets application goals. It is likely that the use of these products will continue as growers manage reused litter to their best advantage.

REFERENCES

1. Carlile, F.S. Ammonia in Poultry Houses: A Literature Review. World's Poultry Science Journal. 40:99-113. 1984.

2. National Research Council (NRC). Nutrient Requirements of Poultry. 9th Rev. Edition. National Academy Press, Washington, DC. 1994.

3. SAS Institute. SAS/STAT User's Guide: Statistics, Version 6.12, SAS Institute, Inc., Cary, NC. 1997.

INTERACTIVE PROBLEM-SOLVING OF FIELD CASES INVOLVING COMMERCIAL POULTRY – AN AUDIENCE PARTICIPATION PRESENTATION

Mark C. Bland

Cutler Associates, Napa California

My plan is to share with you, the WPDC audience, several field cases involving small commercial poultry operations where background information will be provided regarding each case and with WPDC audience participation, we will try and formulate a plan to find the cause and resolve the issue at hand be it through proper diagnosis and treatment or through necessary management changes to mitigate the problem. This is to be an interactive presentation by both the speaker and the audience. Space is provided for you to write down your answers and comments for the presentation. I suggest for those in the audience who wish to participate (residents, interns, poultry science students, etc.) that you may find sitting in the first few rows helpful for both speaker and participants.

Case 1: Commercial brown meat pullets/ cockerels experiencing late mortality.

History: A multi-age commercial facility growing brown-feathered pullets and cockerels for the specialty markets was experiencing increased late mortality in flocks from 12 to 17 weeks of age. Daily mortality ranged from 0.3 to 0.75 %. At any one time the farm had day-old chicks to market-aged birds on site. Clinical signs included high mortality and morbidity, coughing, snicking, and with approximately 1 to 2% of the flock experiencing swollen hocks and lameness. The owner states that this and his second farm (located nearby) have had continual problems for the past year or so – more so in the late fall to early spring. The owner has been spending several thousand dollars each month on medication in the older flocks.

While you are accumulating historical information you are informed that there may be a second problem involving higher than normal mortality during the brooding period. Mortality has ranged from 5 to 15% for the first two weeks with very few if any flocks have first two-week mortality of less than 5%. In addition, the owner points out that several months ago birds were submitted to the lab and *Mycoplasma synoviae* was diagnosed. The owner is not sure what this all means to the overall problem(s) he is facing.

There are 10 production buildings on site. All are of conventional design, with curtains on the sidewalls, dirt floors, nipple drinkers and automated pan feeder system. Chick placements are approximately 20,000 and placed every other week. Current weather conditions are cold and rainy.

List or identify your possible problems/ differential diagnoses to the high mortality a) during brooding and b) prior to market?

What additional questions would you ask the producer in an attempt to get a clearer picture of the situation?

The following are your observations during the farm visit:

Brooding: First week house temperatures are in the mid-80s. Partial room brooding is in place. An adequate number of radiant stoves are present. There appears to be one feeder flat for every 200+ chicks. Feeder pans are empty. Nipple water system is in place and functioning properly. Numerous chicks are observed with pasted vents.

Grow-out: Litter conditions are very wet, curtains are open, outside weather conditions are cold and rainy, inside house temperature is in the low 50s. Nipple water system is in place and functioning properly. A good number of the older birds are coughing and depressed. You observe a few birds with swollen hocks.

What samples, procedures, tests would you submit?

Based on your farm visit, answers provided to your questions during the initial presentation and lab results what is your tentative diagnosis?

What recommendations would you suggest to the owner, which would help reduce the mortality issues currently on the farm?

What long-term recommendations would you recommend to the owner to help prevent future issues from developing?

Case # 2: Broiler farm with poor production, higher than normal condemnations and late mortality issues.

History: You meet with the live production supervisor. He wants your opinion regarding two of their worst broiler production farms (A and B). The farms are about 1/8 of a mile apart and are located about two miles from a large layer facility and two very large broiler complexes operated by another broiler company. Farm A is placed first with approximately 190,000 broilers over a seven-day period. Then farm B receives about 180,000 broilers about a week later with the same age spread of one week. For the past two years both farms A & B have been "bottom dwellers" in regards to production performance (feed conversion, body weights, mortality, and condemnations at the plant). Recent lab reports from birds grown at both farms indicate bacterial septicemia (*E. coli*) and high serological titers to infectious bronchitis virus (IBV). A Cal 99, IBV virus was isolated. The live production supervisor wants to change the IBV vaccination program to include a stronger IBV vaccine like Ark 99 or Holland strain in hopes of solving the poor performance and wants your "blessing."

So, what do you think?

What other questions or information would you like to know or discuss?

Farm A: Consists of eight buildings, six are approximately 40 x 500 feet, have a high peak roof, are partially insulated, and are curtain sided; two are 50 x 400 feet, with a high peak roof, are partially insulated, and are environmental enclosed. Partial room brooding is in place. Outside temperature is in the mid 50s. It is cold and windy.

Farm visit observations: Farm A:

Flocks range from 24-31 days of age. Brooder stoves are off. Overall flock health in all buildings is OK. Flock uniformity varies in each house. Litter conditions range from poor to barely acceptable. Daily mortality is approximately 0.1%. No other observations of note.

Farm B: Consists of 11 buildings, eight are approximately 50 x 400 feet, have a high peak roof, are partially insulated, and are curtain sided; three are approximately 20-25 x 100 feet and environmental enclosed. Partial room brooding is in place. Outside temperature is in the mid 50s. It is cold and windy.

Farm visit observations: Farm B

Curtain sided buildings, flock ages are 20-24 days of age, and stoves are off.

In two houses (24 days of age) the brooding curtain is up. House temperature is 58°F, chicks are in large groups huddled together throughout the house, and flock is uneven in size. Litter conditions are poor.

In two houses (20 to 22 days of age) the brooder curtain is partially down. House temperature is 70° to 72°F on the brood side and 58° to 60°F on the cool side. Litter conditions are poor on the brood side and good on the cool side. Eighty percent of the birds are located on the brood side and look comfortable. The 20% on the cool side are huddled against the sidewall where the sun is shining.

One house (22 days of age), the brooder curtain is partially down. House temperature is 74°F on the brood side and 60°F on the cool side. Litter conditions are good throughout both sides of the house. Birds are evenly distributed throughout both sides of the house. No huddling is seen. Birds look fairly even.

Environmental houses: Flocks range in age from 19-24 days. Brooder stoves are off.

In three houses the brooder curtain is partially down. House temperature is 74° to 76°F on the brood side and 58° to 60°F on the cool side. One fan is operating on a timer (1 min/12 min) and is located on the brood side farthest away from the brood curtain (minimum ventilation). Litter conditions are poor on the brood side and good on the cool side. Ninety-five percent of the birds are located on the brood side and look comfortable except for the fact that the birds are very crowded around the tube-hanging feeders. There is approximately one round tube-hanging feeder per 200 + birds in the house. The 5% of the birds on the cool side are huddled against the sidewalls.

Do you see any issues with the current management?

What additional questions would you ask the producer in an attempt to get a clearer picture of the situation?

What suggestions or recommendations would you provide to the ranch manager to address the current situation?

Any additional comments that you would like to make to the production supervisor?

Any take home message for the veterinarian and/or the producer?

Case # 3: Central nervous signs in two-week-old Muscovy ducklings.

History: A flock of two-week-old Muscovy ducklings placed on a multi-aged commercial production facility were experiencing signs of central nervous involvement and respiratory distress. Approximately five to seven ducklings appeared to be involved. Current mortality was within normal standards. Two additional flocks, three and four weeks of age, were in the same house showing no clinical signs of distress.

What are your possible diagnoses?

What additional questions would you ask the producer in an attempt to get a clearer picture of the situation?

What samples, procedures, tests would you submit?

What is your tentative diagnosis based on the answers provided to your questions during the presentation?

What is your recommendation to treat the flock and/or affected birds? What steps would you suggest to prevent the problem from occurring in future flocks?

Future outcome.

A NEW APPROACH TO PREDICT THE IMPACT OF COCCIDIOSIS USING THE STANDARD INDUSTRY INTESTINAL SURVEY

Charles Broussard[A], RG Teeter[B], and Linnea Newman[A]

[A]Intervet/Schering-Plough Animal Health, Summit, NJ
[B]Department of Animal Science, Oklahoma State University, Stillwater, OK
E-mail: chares.broussard@sp.intervet.com

SUMMARY

Since the introduction of in-feed anticoccidials to the poultry industry, the standard method of determining the efficacy of these products has been to perform an intestinal health survey. Typically these surveys involve the necropsy of several chickens (usually five birds) to give a representation of coccidial challenge for a house or multi-house farm. Bird samples of various ages are submitted to represent the range of potential coccidiosis challenge in the field. The focus of the intestinal health survey is to determine the efficacy of the current anticoccidial program to the coccidial challenge. The scoring method developed by Reid and Johnson (1) has been adopted as the standard method to assess severity of gross cocci lesions. In addition a standardized method for the ranking of microscopic scoring for coccidial development has been developed by Fitz-Coy (2). This has been shown to be extremely helpful with evaluating low level challenges of some coccidial species especially *E. maxima*.

A limitation of the current intestinal survey has been the interpretation of the data collected. The data is usually reported as the severity of gross and microscopic lesions for each pathogenic species of *Eimeria* affecting broiler performance. This data is summarized and expressed as a percentage of affected birds. Then this information is extrapolated to the greater population of chickens in the field operation to represent the current coccidial challenge for the anticoccidial program being evaluated.

The current methodology of evaluating only the severity of coccidial lesions ignores the impact of the timing of the coccidial challenge during the broiler growth cycle. Recent studies by Teeter *et al.* (3) demonstrated the importance of timing of coccidial challenges on broiler performance. This work showed coccidial challenges of equivalent severity had more negative impact on chickens if occurring later in the growth cycle. This correlation also held true for birds exhibiting low level coccidial challenge (+1 and +2 scores). This work substantiates the need in the field to more accurately assess coccidial challenge by evaluating both the severity and timing of coccidial challenges.

Does the current intestinal survey data collection method allow a more sensitive method to evaluate efficacy of anticoccidial programs and to predict the impact of these programs on broiler performance? The use of statistical modeling has allowed predictability in laboratory situations of coccidiosis challenge on broiler performance (3). Can this approach be taken one step further to accurately predict the impact of coccidiosis at the field level and allow the poultry industry to begin a proactive approach to coccidial disease management versus the current reactive approach?

REFERENCES

1. Reid, W.M. and J. Johnson. Pathogenicity of Eimeria acervulina in light and heavy coccidial infections. Avian Diseases 14:156-177. 1970.

2. Fitz-Coy, S. Diseases of Poultry 12th Edition. Coccidiosis. 1068-1085. 2008.

3. Teeter, R.G., A. Beker, C. Brown, C. Broussard, S. Fitz-Coy, J. Radu, and L. Newman. Calorimetry applications quantify the variable cost of subclinical at various points in the broiler growth curve. 57th Western Poultry Disease Conference Proceedings 99-101. 2008.

EPIDEMIOLOGY OF AVIAN METAPNEUMOVIRUS INFECTION IN ITALY

E. Catelli[A], M. Cecchinato[B], G. Ortali[C], E. Morandini[C], L. Sperati Ruffoni[C], P. Pesente[C], C. Franciosi[A], and C. J. Naylor[D]

[A]Dipartimento di Sanità Pubblica Veterinaria e Patologia Animale, University of Bologna, Ozzano Emilia (BO), Italy
[B]Dipartimento di Sanità Pubblica, Patologia Comparata e Igiene Veterinaria, Università di Padova, Legnaro (PD), Italy
[C]Agricola Tre Valli, Gruppo Veronesi, S. Martino B.A. (Verona), Italy;
[D] Department of Veterinary Pathology, University of Liverpool, Neston, Cheshire, United Kingdom

INTRODUCTION

Avian *Metapneumovirus* (AMPV) is a negative sense RNA virus, envelope virus which is the type species in the genus *Metapneumovirus* in the *Paramyxoviridae* family. It is the casual agent of turkey rhinotracheitis (TRT), and also causes a respiratory infection in chickens, which can result in swollen head syndrome. At present four AMPV subtypes have been characterised: the A and B European subtypes (5), the United States C subtype (6) and a subtype, referred to as D, which includes two old French isolates (1). At the beginning of 2000 little information was available on circulation and molecular epidemiology of AMPV Italian poultry except for the data regarding the first Italian isolates made in the late 80s, which were shown to be B subtypes (5; Sperati Ruffoni L., personal communication). Moreover even though the vaccination was widely applied, disease still occurs occasionally in young as well older turkeys. This paper reports some studies performed in Italy since 2001 in order to understand better the epidemiology of AMPV and prophylactic problems encountered in the field.

STUDY-1

Virological, molecular and serological AMPV field survey on turkey, chicken and layer Italian farms. Since 2001 a survey of AMPV infection in Italian turkey and broiler farms, in a highly populated area of Northern Italy has been performed. Nine turkey farms and six broiler farms were sampled. Sixteen birds from each group were doubly swabbed from the choanal cleft for virus isolation on tracheal organ cultures and RT nested PCR (A and B subtype specific) using extracted RNA. At the same time blood samples were collected for a blocking ELISA serological assay. AMPV was isolated and detected by RT-PCR in 19 day-old turkeys, and in 34, 42, and 48 day-old broilers. All AMPV strains were found to be subtype B. All turkeys of more than four weeks old were AMPV positive by ELISA. AMPV infection was found to be widely spread and the B subtype was found to be prevalent in the area sampled (2). In order to extend the epidemiological survey to a wider area, swabs from field TRT outbreaks in central regions of Italy were tested and found to contain subtype A virus. This was the first evidence of AMPV subtype A in Italy (4).

The current information on prevalence of AMPV in layers is fragmentary and the impact on egg production remains unknown. In order to draw an epidemiological picture of AMPV spreading in the layers flocks a survey was performed in 2007 in 20 flocks, on five pullets and eight layer farms. AMPV was detected by RT-PCR and blood samples were collected for ELISA serological assay. Where possible, egg production data and respiratory disease were recorded. Results confirmed the high prevalence of AMPV. All viruses were of B subtype. In two occasions AMPV infection was correlated with drop in egg production. Surveys of pullets confirmed that most groups prior to coming into lay become infected without showing clear respiratory signs. At the point of lay these groups are serologically positive to AMPV. It will be interesting to know if these birds will be protected from drop in egg production in case of further contact with the virus.

STUDY-2

Sequence analysis of fusion (F) and attachment (G) protein genes of Italian B subtype AMPVs. To establish the identity and heterogeneity in Italian AMPV strains, the nucleotide sequences of selected genes were determined and compared with previously published AMPV field strains and commonly available vaccine strains. B subtype and A subtype AMPV strains isolated in Italy from 1987 to 2007, were considered. After RNA extraction, independent overlapping RT-PCRs, covering the entire the Fusion (F) and Attachment (G) genes, were performed and

amplicons sequenced. Phylograms for both gene sequences were constructed with the MEGA package, version 3.1. The B subtype sequences were clustered with the previously published AMPV B sequences. Within the European strains, significant sub clustering was apparent with the more recent isolates forming a cluster separated from viruses isolated in the previous decade. Comparison of sequences of viruses isolated prior to the introduction of B subtype vaccines shows there were very few differences in either predicted protein sequence. Viruses isolated after 2001 had similar F protein sequences but numerous G protein mutations. These mutations are likely to have produced antigenic differences between the two virus groups because they all altered highly charged, potentially charged amino acids or potential O-linked glycosylation sites. Moreover three strains isolated over a period of a few months from chickens and turkeys, showed high sequence identities. Genetic differences in these genes did not correlate to the species of the host.

STUDY-3

AMPV field evolution avoiding vaccine induced immunity. Longitudinal studies in Italian turkey farms demonstrated that subtype B viruses were frequently detected some period after subtype B vaccination. Sequencing showed that these later viruses were not derived from the previously applied vaccine. More detailed sequence analysis of fusion and attachment protein genes showed that these later subtype B detections formed a cluster (see Study-2). The attachment protein genes in this cluster were dissimilar to those found in early B subtype viruses, including the established vaccines, and these themselves formed another cluster. One day old poults were vaccinated with subtype B vaccine in experimental conditions and later challenged with either early (240 TRT-VR87) or later (205-16/04) subtype B field isolates. Protection was very poor as assessed by both clinical disease and shedding of virus after 205-16/04 challenge. The limited immunity observed may explain the dominance of the later subtype subtype B field viruses over a six year period in this Italian region. This may have resulted from immune pressure induced by mass subtype B vaccination.

STUDY-4

Field and experimental evidences of AMPV vaccine reversion to virulence. Subtype A and B AMPV vaccines are widely used in Italian commercial growing turkeys and turkey and chicken breeders. When tested under experimental conditions, these empirically derived vaccines were shown to be fully protective whilst not causing detectable disease themselves. However, they do not perform as well when used in the field and unstable attenuation has been considered to be a possible factor. Since AMPV are single-stranded RNA viruses their relatively high mutation rates have been thought to be the underlying reason for instances of reversion to virulence observed in experimental conditions. This study reports the evidence of reversion of an AMPV subtype A vaccine in the field. We isolated an AMPV a vaccine derivative from an outbreak of TRT in an Italian flock of 18 day old turkeys previously vaccinated at day old with A subtype, The vaccine derivative virus was shown to be able to cause clinical disease when applied to one day old poults in secure isolation conditions (3). Afterwards another subtype A AMPV was isolated in association with respiratory disease typical of TRT from turkeys which had been vaccinated with a B subtype licensed AMPV vaccine. Sequencing of the virus showed that the virus had originated from a licensed live A subtype vaccine. In this instance the disease was much later, at 50 days of age, and there had been no recent history of use of the vaccine. This may indicate that AMPV vaccines are able to circulate in the environment for longer than was previously envisaged.

CONCLUSION

Since 1987 mainly B subtype AMPV has been found in Italy, in turkeys, broilers, and layers. Since 2003 subtype A has also been found, to limited degree. Two of these strains have so far been considered and genome sequence analysis and assessment of virulence by experimental infection of naïve turkeys showed these to be vaccine revertants.

The nucleotide sequences of F and G genes of AMPV subtype B strains isolated from 1987 to 2007 were determined and compared with previously published AMPV field and vaccine strains. Comparison of sequences of subtype B viruses isolated prior to the introduction of mass B vaccination shows there were very few differences. Viruses isolated after had numerous attachment protein mutations. These are likely to have produced antigenic differences that may have resulted from vaccine immune pressure.

Outbreaks of TRT still occur in the field in spite of vaccination. Use of inappropriate vaccine subtypes, evolution of field viruses able to avoiding existing vaccines and reversion to virulence are all likely to be playing a role.

REFERENCES

1. Bayon-Auboyer, M-H, C. Arnauld, D. Toquin, and N. Eterradossi. Nucleotide sequence of the F, L and G protein genes of two non-A/non-B avian pneumoviruses (APV) reveal a novel APV subgroup. J Gen Virol 81:2723-2733. 2000.

2. Catelli, E., M. Cecchinato, M. Delogu, P. De Matteo, G. Ortali, C. Franciosi, M.A. De Marco, and C.J. Naylor. Avian Pneumovirus infection in turkey and broiler farms in Italy: a virological, molecular and serological field survey. Ital J of Anim Sci 3(3):286-292. 2004.

3. Catelli, E., M. Cecchinato, C.E. Savage, R.C. Jones, and C.J. Naylor. Demonstration of loss of attenuation and extended field persistence of a live avian Metapneumovirus vaccine. Vaccine 24:6476-6482. 2006.

4. Cecchinato, M., E. Catelli, C.E. Savage, P. De Matteo, M. Faenzi, and C.J. Naylor. Evidenza di pneumovirus aviare sottotipo A in corso di un focolaio di TRT in tacchini da carne in italia. XLII Convegno Società Italiana Patologia Aviare, Forlì 2-3 ottobre 2003. Large Animal Review 9 (6): 121-122. 2003.

5. Juhasz, K. and A.J. Easton. Extensive sequence variation in the attachment (G) protein gene of avian pneumovirus: evidence for two distinct subgroups. J of Gen Virol 75:2873-2880. 1994.

6. Seal, B. Matrix Protein gene nucleotide and predicted amino acid sequence demonstrate that the first US avian pneumovirus isolate is distinct from European strains. Virus Research 58, 45-52. 1998.

EXPERIMENTAL DUAL CHALLENGE WITH *ORNITHOBACTERIUM RHINOTRACHEALE* AND *MYCOPLASMA SYNOVIAE* IN BROILERS

R. Cerdá[A,B], J. Uriarte[A], J. Origlia[A], and M. Petruccelli[A]

[A]Laboratory of Diagnosis of Avian and Piliferou Diseases, Faculty of Veterinary Medicine, National University of La Plata, CC296, 1900, La Plata, Argentina
[B]ECO Animal Health, London, UK

SUMMARY

Ornithobacterium rhinotracheale (ORT) and *Mycoplasma synoviae* (MS) have been recognized as primary respiratory pathogens in broiler chickens. However, their possible pathogenic synergism has not been demonstrated using a dual infection study in broiler chickens. In the present study, three week old broiler chickens were inoculated either with a standard ORT strain (intratracheally), a MS field strain (aerosol) or both pathogens at the same time. At seven and 14 days post challenge, chickens from each group were euthanatized and analyzed for thoracic and abdominal air sac lesions, lung and trachea lesions. Significant differences in lesion scores were observed in the ORT/MS group compared to either the uninoculated, control group or the MS group. Infections were confirmed by strain re-isolation, PCR, and serology. This is the first report of an ORT/MS dual infection study in chickens.

RESUMEN

Desafío dual experimental de *Ornithobacterium rhinotracheale* y *Mycoplasma synoviae* en pollos.

Ornithobacterium rhinotracheale (ORT) y *Mycoplasma synoviae* (MS) han sido reconocidos como patógenos respiratorios primarios en pollos. Sin embargo, su sinergismo patogénico no ha sido aun demostrado experimentalmente en este tipo de aves. En el presente estudio, pollos de tres semanas fueron desafiados ya sea con una cepa estándar de ORT (vía intratraqueal), una cepa de campo de MS (vía aerosol), o ambos agentes conjuntamente. A los 7 y 14 días postinoculación se sacrificaron pollos de cada grupo para la evaluación de lesiones en sacos aéreos torácicos y abdominales así como también de lesiones en pulmón y tráquea. Se observaron diferencias significativas en el score de lesiones entre el grupo ORT/MS y el grupo control sin inocular como así también con el grupo desafiado con MS. La confirmación de las infecciones experimentales se llevaron a cabo mediante reaislamiento de los agentes descargados, PCR y pruebas serológicas. Este es el primer reporte de una infección dual experimental de ORT y MS en pollos.

INTRODUCTION

Ornithobacterium rhinotracheale (ORT) and *Mycoplasma synoviae* (MS) have been recognized as primary respiratory pathogens in broiler chickens (6). Both bacterial species have a worldwide distribution and are associated with respiratory disease and high economic losses (3,4). Despite the known multi-factorial etiology of the respiratory complex in poultry, only a few experimental trials have been done to establish possible interactions between bacteria and viruses with ORT (1). Apart from one field report regarding dual ORT and MS infection in turkeys (7), there are no reports on natural or experimental ORT and MS interactions in broiler chickens. The present study was undertaken to evaluate the possible synergy between ORT and MS in broiler chickens.

MATERIALS AND METHODS

Experimental animals. Forty commercial Ross broiler chicks, free of mycoplasma and *Salmonella*, were hatched and later housed in isolators. Food and water was available *ad libitum*.

***Ornithobacterium rhinotracheale* inoculum.** The ORT challenge inoculum was prepared from strain ATCC51463. The bacterium was cultured in 5% sheep blood agar media supplemented with 10 ug/mL gentamicin in a 5% CO_2 atmosphere. After 48 h incubation, ten bacterial colonies were transferred into 5 mL of PBS and a challenge inoculum containing 10^9 colony forming units (CFU)/mL was prepared.

***Mycoplasma synoviae* inoculum.** The MS strain used was a recent field isolate from an outbreak of infectious synovitis in a laying hen farm in Buenos Aires province, Argentina. The strain was cloned and prepared using Frey (Gibco) broth supplemented with 12% pig serum and had an organism density of 10^8 color changing units/mL (CCU/mL).

Experimental design. Forty, one-day old, chicks were randomly allocated to four experimental groups

such that each group contained 10 chicks. At two weeks of age the birds were shown to be free of maternally- derived antibodies to ORT and MS using a commercial enzyme-linked immunosorbent assay (ELISA)(IDEXX) and a rapid serum agglutination test (RSA) (Intervet Schering Plough, The Netherlands), respectively. At this time all the chickens received a ND-IB (La Sota-Massachusetts) vaccination via eye drop. At three weeks of age the different groups received the following treatments: (ORT/MS) challenge with ORT and MS cultures, (MS) MS culture, (ORT) ORT culture and an uninoculated control group.

Lesion scores. Postmortem gross lesions were scored at seven and 14 days post-inoculation (PI) using five chickens from each group. Thoracic and abdominal air sacs, lung and trachea lesions were scored. Air sacs were examined and given a score according to the amount of cheesy exudate contained within the air sacs as follows: 0 = no visible exudate; 1 = 25% or less of the air sac contained exudate; 2 = 25 to 50% of the air sac contained exudate; 3 = more than 50% of the air sac contained exudate. The maximum air sac score per bird was 6; lungs, 0 = no abnormalities, 1 = unilateral pneumonia, 2 = bilateral pneumonia. The maximum lung score per bird was 2; trachea, 0 = no abnormalities, 1 = some exudate in the tracheal lumen, 2 = lumen of the trachea filled with exudate. The maximum trachea score per bird was 2.

Confirmation of infection. A pool of tracheal swabs, for re-isolation of bacteria and PCR, in addition to blood samples for serological tests (RSA for MS and ELISA for ORT), were taken at the end of the trial in order to try to confirm the infection by ORT and MS.

Statistical analysis. The statistical analysis of the lesion scores was done using the Kruskal-Wallis one-way analysis of variance.

RESULTS

The lesion score results are shown in the table. Significant differences in score lesions ($P < 0.05$) were observed between group ORT/MS compared to group MS in air sac and lung lesions at seven days PI and between group ORT/MS compared to the uninoculated control group in all the organs evaluated at both times of necropsy. Infections were confirmed by bacterial re-isolation (100% for MS and 30% for ORT), PCR (100% for both agents) and serology (100% for both agents).

DISCUSSION

Although no interactions were observed between ORT and MS in turkeys under field conditions (7), a high synergism between both pathogens has been seen using the experimental conditions of the present study, in broilers. The use of the ND-IB vaccine and the pathogenicity of the MS field strain could be reasons for the results. The Argentinean MS field strain used has shown to be highly pathogenic for boilers (2). Further studies should be carried with different MS strains and different management conditions in order to determine possible results, according to the different scenarios, in the field.

REFERENCES

1. Marien, M., A. Decostere, A. Martel, K. Chiers, R. Froyman, and H. Nauwynck. Synergy between avian pneumovirus and *Ornithobacterium rhinotracheale* in turkeys. Avian Pathol. 34 (3), 204-211. 2005.

2. Meghan, M., S. Kleven, and D. Brown. Sialidase Activity in *Mycoplasma synoviae*. Avian Dis. 51:829–833. 2007.

3. Kleven, S. *Mycoplasma synoviae* infection. In: Diseases of Poultry (Calnek, B. W., Ed.) 10th edition, Iowa State University Press, Ames, Iowa, pp. 220–225. 1997.

4. van Empel, P. and H. Hafez. *Ornithobacterium rhinotracheale*: a review. Avian Pathol. 28. 217-227. 1999.

5. van Veen, L. Country report on The Netherlands. In: Aerosols, Newsl. World Vet. Poult. Assoc. p. 12. 1999.

6. van Veen, L., P. van Empel, and T. Fabri. *Ornithobacterium rhinotracheale*, a primary pathogen in broilers. Avian Dis. 44. 896-900. 2000.

7. Zorman-Rojs, O., I. Zdovc, D. Bencina, and I. Mrzel. Infection of Turkeys with *Ornithobacterium rhinotracheale* and *Mycoplasma synoviae*. Avian Dis. 44:1017-1022. 2000.

ACKNOWLEDGMENTS

We thank Adrian Mocket from ECO Animal Health for critical reading and comments on the manuscript. We also thank Andrew Pridmore for supplying the ORT strain and Fernando Marino and Daniel Gornatti for laboratory assistance. This study was supported by ECO Animal Health, UK.

Table 1: Postmortem lesion scores after seven and 14 days post-inoculation of ORT and MS.

Group	7 days PI			14 days PI		
	Air sacs	Lung	Trachea	Air sacs	Lung	Trachea
ORT/MS	5.4 (90.0)[b]	1.8 (90)[b]	1.8 (90)[b]	3.6 (60.0)[b]	1.4 (70)[b]	1.4 (70)[b]
MS	2 (33.3)[a]	0.2 (10)[a]	1 (50)[ab]	2.4 (40.0)[ab]	0.4 (20)[ab]	1.2 (60)[b]
ORT	2.2 (36.7)[ab]	0.6 (30)[ab]	0.8 (40)[ab]	2.2 (36.7)[ab]	0.6 (30)[ab]	1 (50)[ab]
Uninoculated	0.2 (3.3)[a]	0.2 (10)[a]	0.2 (10)[a]	0.4 (6.7)[a]	0.2 (10)[a]	0.2 (10)[a]

Scores are given as the maximum possible lesion scores in the group (percentage in brackets). Within columns, values with different lowercase superscripts are significantly different ($P < 0.05$).

PHENOTYPE AND FUNCTIONS OF T CELLS ISOLATED FROM MUCOSAL LININGS OF TURKEYS EXPOSED TO AVIAN METAPNEUMOVIRUS SUBTYPE C (AMPV/C)

Ra Mi Cha[A], Mahesh Khatri[B], and Jagdev Sharma[C]

[A]Department of Veterinary and Biomedical Sciences, University of Minnesota, 1971 Commonwealth Avenue, St. Paul, MN 55108,
[B]Current address: Department of Food Animal Health Research Program, Ohio Agricultural Research and Development Center, The Ohio State University, 1680 Madison Avenue, Wooster, OH 44691
[C]Current address: The Biodesign Institute, Arizona State University, 1001 S. McAllister Ave., Tempe, AZ 85287

SUMMARY

aMPV/C causes an acute, immunosuppressive respiratory tract infection in turkeys. The virus mainly replicates in the upper respiratory tract (URT). Two-week-old aMPV/C-free commercial turkeys were inoculated oculonasally with live aMPV/C. At five and seven days post inoculation (DPI), lymphocytes infiltrating the mucosal linings of the URT were isolated by enzymatic treatment. The proportions of T cell phenotype (CD4/CD8) in the turbinate cells were tested by FACS analysis. aMPV/C exposure increased the proportion of CD8+ cells but not of CD4+ cells. In addition, the gene expression of CD8 but not of CD4, was increased in the URT. Lymphoid cells isolated from the URT of virus-exposed turkeys were deficient in the proliferative response to a T cell mitogen. Cytokine gene expression in the URT was examined by quantitative RT-PCR. At five and seven DPI, RNA extracted from turbinate tissue had upregulated expression of genes of IL-10 and IFN-γ. These results indicated that aMPV/C stimulated mucosal cellular immunity in the URT.

ENTEROCOCCUS CECORUM OSTEOMYELITIS

B. R. Charlton, G. L. Cooper, S. Stoute, A. A. Bickford, and C.G. Senties-Cue

California Animal Health and Food Safety Laboratory System - Turlock Branch, University of California, Davis
P.O. Box 1522, Turlock, CA 95381

SUMMARY

Diseases caused by *Enterococcus* spp. in the California poultry industry are not too frequent or significant. They tend to be individual bird problems or secondary to some other flock problem. *Enterococcus* spp. are normal inhabitants of the intestinal flora of poultry and were commonly referred to as the "fecal streps." *E. faecalis* and *E. faecium* are the most prevalent of intestinal enterococci but *E. gallinarum*, *E. durans*, *E. hirae*, and *E. cecorum* are also present. Identification of the *Enterococcus* spp. to the species level is not frequently performed. Few disease conditions warrant such identification although exceptions may be made on a case by case basis or when pure growth is obtained from a specimen.

During the summer of 2008, a commercial broiler company began to notice several houses of a multi-house complex were experiencing leg weakness. The mortality was elevated and a number of birds were down and/or had difficulty in walking. The addition of vitamin D had no effect. An additional ranch from the same company also experienced a similar problem during this same time period. Several submissions to CAHFS-Turlock Branch showed predominately an osteomyelitis of the proximal tibiotarsus frequently with synovitis. An *Enterococcus cecorum* was isolated in pure culture from a number of these accessions. In addition to the proximal tibiotarsus, a few birds had similar lesions in the vertebra. These birds showed the classic clinical sign of kinky-back. Histopathology of the bones showed typical osteomyelitis with large areas of heterophilic inflammation and necrosis in the bone marrow.

The identification of *Enterococci* spp. is primarily based on gram-positive cocci which are catalase-negative, grow in 6.5% NaCl, and hydrolyze esculin in the presence of bile salts. Species identification can be accomplished primarily by sugar fermentation and other biochemical reactions (1,2). The CAHFS system also utilizes the API 20 STREP (bio-Mérieux) as an additional resource. In these particular cases in which pure cultures of *Enterococcus* spp. were obtained from bone, the identification of the cultures was somewhat problematic. Over a period of six months, seven isolations of *E. cecorum* were made. The only consistent reactions obtained were the gram stain and catalase tests. Following the CAHFS identification criteria for poultry *Enterococcus* spp. strains frequently narrowed the identification down to *E. hirae* or *E. cecorum.* Neither of these two species is in the API database, so this identification kit was not too beneficial. Partial sequencing of the 16S rRNA gene was performed on three separate isolates of *E. cecorum* and one isolate of *E. hirae* to confirm the identities of these organisms.

A ten year retrospective examination of poultry accessions in which *E. hirae* or *E. cecorum* were identified showed 80 accessions of *E. hirae* and only six accessions of *E. cecorum*. For *E. hirae*, birds less than a week of age were the most frequently involved (32% of cases), and the liver or yolk sac were the site of most isolations. The bone was a site of isolation in two cases. For the six accessions in which *E. cecorum* was identified, bone (including vertebrae and toe) was the most frequent site but isolations were also made from the blood, liver, and synovial fluid. A system wide search of accessions over the last 10 years showed two accessions from CAHFS – Fresno in 2007, both from the same producer.

E. cecorum osteomyelitis is not a new disease (3). The sudden appearance in multiple submissions over a short period of time suggests a common origin. Further investigation into the epidemiology of this condition will require cooperation among several segments of the poultry industry. Diagnosticians should consider complete bacterial identification when osteomyelitis is encountered.

REFERENCES

1. Thayer, S.G. and W.D. Waltman. Streptococcosis and enterococcoccus. In L. Dufour-Zavala, *et al*. (eds.). A laboratory manual for the isolation, identification and characterization of avian pathogens. 5th ed. Avian Association of Avian Pathologists, Jacksonville, FL. 44-46. 2008.

2. Devriese, L.A., J. Hommez, R. Wijfels, and F. Haesebrouck. Composition of the enterococcal and streptococcal intestinal flora of poultry. J Appl Bacteriol. 71:46-50. 1991.

3. Wood, A.M., *et al*. Isolation of *Enterococcus cecorum* from bone lesions in broiler chickens. Vet Rec. 150:27. 2002.

DEVELOPMENT OF SLIDE MICRO-AGGLUTINATION SYSTEM FOR THE RAPID DIAGNOSIS OF *SALMONELLA* INFECTION IN THE CHICKEN

R. Parvin, E. H. Chowdhury, M. Asaduzzaman, N. Jannat, M. G. Haider, and M. A. H. N. A. Khan

Department of Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh
emdad001@yahoo.com

ABSTRACT

The cultural method to identify avian *Salmonella* infections is laborious and expensive, thus a rapid, sensitive and cost-effective method for the diagnosis of salmonellosis is anticipated. *S. pullorum* was isolated and a stained *Salmonella* somatic antigen was prepared. The protein concentration of stained antigen was measured and adjusted to 3000 ng/µL. Preservative was used to increase its shelf life. Slide agglutination tests were carried out with un-diluted and diluted anti-sera having known ELISA titre and end point agglutination titre was determined. The standard curves were generated using 2- and 10-fold dilutions of sera to determine the titre of the unknown field sera. It was observed that the newly developed stained somatic antigen produced distinct tiny clumps with positive anti-sera of *Salmonella* spp. The present slide agglutination system was found to be easy, sensitive, reliable, cost and time effective and needs very small amount of antigen, sera and as well as accessories. This method may be used to screen the *Salmonella* infections in the poultry farms and to calculate the titre of the anti-salmonella antibody in the infected and vaccinated chickens under farm conditions. A test kit containing stained antigen along with positive and negative control sera may be prepared for commercial uses.

INTRODUCTION

Salmonellae including pullorum disease, fowl typhoid and other infections may cause varieties of clinical signs from acute systemic disease and gastrointestinal symptoms in poultry flocks to embryonic problem in hatchery (5). In recent years, diagnostic laboratories have been concerned with reducing the time required for diagnosis of *Salmonella* infections. The current standard laboratory procedure to culture and identify *Salmonella* takes approximately four to seven days. Even these methods are tedious, time consuming and confer little guarantee of sensitivity and species specificity. Tube agglutination test for pullorum disease was described by Jones's in 1913 (8) and subsequent introduction of a rapid plate serum agglutination (RSA) test (9) and a stained antigen for whole blood test (11) provided a practical basis. But none of these tests were found sensitive and was solved by the development of the enzyme-linked-immunosorbent assay (ELISA) (4) and its application to the measurement of antibody response to specific infections (13). The application of ELISA assays for *Salmonella enteritidis* have been described (3,7,12). However, ELISA test is expensive, time consuming, needs skilled manpower and not easy to perform in the field condition. The microagglutination (MA) system (microtest) had been adapted for a wide variety of serological procedures due to its better sensitivity (1,15). It has been cited for detecting agglutinations of several *Salmonella* serotypes (15) in the field condition as well as a routine diagnostic test for the detection of chronic carriers of *S. pullorum* and *S. gallinarum* (14). Antigen produce from local isolate is always more sensitive and will be economically cheaper than the imported one. Therefore, the present study was undertaken with the following objectives: Preparation of stained colored *Salmonella* antigen with a local isolate and development of slide microagglutination (MA) system for the rapid diagnosis of *Salmonella* infection in chickens in the field condition.

MATERIALS AND METHODS

Preparation of antigen. The locally isolated *S. pullorum* was used for production of the *Salmonella* colored antigen. Test tubes containing samples on nutrient broth were incubated for 24 h at 37°C. From the nutrient broth, subcultures were also made on Brilliant Green agar, Salmonella Shigella agar, MacConkey agar, EMB agar, TSI agar, LB agar, and nutrient agar, and incubated at 37°C for over night. On the basis of colony and staining characters, and biochemical tests the organisms were isolated and identified. The organism was further confirmed by PCR as described in Saha *et al*. (10). Single colony of *Salmonella pullorum* was inoculated into 50 mL of LB broth. Then the flasks were placed in incubation for 48 h. 50 mL broth culture was divided into two conical flasks containing each 25 mL. Then tetrazolium salt was added aseptically in the amount of 0.5% in each

broth culture and incubated one flask for 2 h and another flask for 24 h. After incubation, 0.5% phenol was added in each flask and was incubated for 1 h and 2 h, respectively. The stained broth suspension was filtered through sterile gauge and poured in to eppendorf tube. Afterwards these were centrifuged at 16000 rpm for 15 min, supernatant was decanted, and cells were suspended in 0.5% phenolized saline, 0.5% fomalized saline and 0.09% sodium azide. The suspension was vortex vigorously with a few sterile glass beats and transferred it into another eppendorf tube. Then the solution kept in 4°C as neotetrazolium stained antigen for future use. The total protein concentration of the stained antigen was measured by the Folin Phenol method of Lowry *et al.* (6). 20 µL of stained antigen and 20 µL chicken sera were placed on a sterile glass slide by a micropipette and mixed thoroughly by stirring with tips. The agglutinations titer of unknown sera were determined and compared with known ELISA titer. Correlation curve were prepared to determine the antibody titer of unknown field sera. Finally shelf life was determined for newly developed *Salmonella* antigen.

RESULTS AND DISCUSSION

Preparation of *Salmonella* colored antigen. In the present study a sensitive neotetrazolium stained *Salmonella pullorum* antigen was prepared from a local isolates and a micro-agglutination procedure for the detection of *S. pullorum* antibody was developed. The protein concentration of the colored antigen was adjusted to 3000 ng/µL with the help of BSA standard curve. A suitable preservatives 0.5% phenolized saline was chosen.

Determination of antibody titer. The collected known positive sera were diluted as 10-fold and 2-fold pattern and agglutination test was performed with the newly developed *Salmonella* colored antigen. In 10-fold dilution, the positive agglutination was recorded maximum up to 10^{-8} dilution with antibody having titer 11870, while it was up to 10^{-1} dilution when antibody titer was 1570. To check it further, 2-fold dilutions were made from a panel of antibodies having ELISA titer 335-2311. In case of 2-fold diluted sera the positive agglutination recorded maximum up to 2^{-7} dilution with antibody having titer 2311, while it was up to 2^{-2} dilution with antibody having titer 676 and no agglutination with antibodies having titer 335.

Standard curve. From the 10-fold and 2-fold dilution it was observed that the end point agglutination titer of serum sample having ELISA titer 1570 was found in 10^{-1}th dilution, while in 2-fold dilution end point agglutination titer was found in 2^{-5}th dilution with serum sample having ELISA titer 1630. This indicates a positive correlation between 2- and 10-fold dilution (Figure 1).

Validation with field sample. Among the seven flocks, the flock number 49 showed the higher prevalence as 35% confirmed case, 35% suspect and 30% negative. The flock number 50 and 51 also showed positive agglutination in 20% and 10% cases, respectively. The flock number 52, 53, 54, and 55 did not show any positive result but 30%, 10%, 15%, and 10% cases respectively, were found suspect. Detail of the results may be seen in Table 1. The agglutination percentage of total number of sera from seven flocks was also calculated. Among 180 sera it was shown that 13 sera were confirmed as positive, 31 suspect, and 76 negative. The ELISA titer of unknown sera were calculated from the standard curve that was almost similar to the instruction of the ELISA kit (GUILDHAY, UK).

Sensitivity and Specificity. The tetrazolium stained *Salmonella* antigen from a local isolate was successfully developed which could be used to screen the *Salmonella* infection in the poultry flocks at the farm premises. Although this antigen was developed from the local isolate of *Salmonella pullorum* it also reacts with antibodies of *Salmonella gallinarum* and other *Salmonella enteritidis*. The neotetrazolium stained *Salmonella* antigen showed enhanced sensitivity than the conventional agglutination test and the specificity of the stained antigen was high as it did not reacted with the negative serum and water control. The results are in agreement with a previous study (14).

This method also may be used to calculate the titer of the anti-*Salmonella* antibody in the infected and vaccinated chickens under farm conditions. Finally a kit was organized named "BAU-Path S Antigen Kit" contain 1mL of developed stained antigen that was sufficient for 50 test, positive serum, negative serum, and information sheet However, cautions have to be taken with the reading of the results. With increase time, the stained antigen may react with the non-specific antibody present in the serum. Therefore, results within one minute were suggested as suspected case of salmonellosis. The antigen must be shaken before use. Always keep away from the light.

REFERENCES

1. Anonymous. A selected bibliography of micro methods in microbiology with special emphasis on microtiter techniques. Cooke Engineering Co., Alexandria, Va. 1970.

2. Cheesbrough, M. District Laboratory Practice in Tropical Countries, part- 2. Cambridge low price edn. Cambridge University Press, UK. pp. 64-65. 2000.

3. Cooper, G.L., R.A. Nicholas, and C.D. Bracewel. Serological and bacteriological investigations of chickens from flocks naturally infected with *Salmonella enteritidis*. *Vet. Res.* 125: 567-572. 1989.

4. Engvall, E. and P. Perlman. Enzyme-linked immunosorbent assay (ELISA). Quantitative Assay of Immunoglobulin G. Immunochemistry, 8: 871-874. 1971.

5. Gast, R.K. Paratyphoid infections. In: Calnek, B.W., H.J. Barnes, C.W. Beard, L.R. McDoughand, and Y.M. Saif, (eds). *Diseases of Poultry*, 10th edn. Lowa State University Press. Ames, IA. Pp. 97-121. 1997.

6. Lowry O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. Protein measurement with the folin phenol reagent. *J Biol Chem; 193:265-75.* 1951.

7. Nicholas, R.A. and G.A. Cullen. Development and application of an ELISA for detecting antibodies to *Salmonella enteritidis* in chicken flocks. *Vet.Res.*128: 74-76. 1991.

8. Rettger, L.F. Septicaemia among young chickens. N.Y. *Med. J.* 71: 803-805. 1990.

9. Runnels, R.A., C.J. Coon, H. Farley, and F. Thorp. An application of the rapid-method agglutination test to the diagnosis of bacillary white diarrhoea infection. *J. An. Vet. Med. Ass.* 70: 660-662. 1927.

10. Saha S.S., E.H. Chowdhury, S.M. Rhaman, S. Sultana, M.G. Haider, and M.R. Islam. Detection of *Salmonella gallinarum* using polymerase chain reaction. Paper submitted to Bangladesh Veterinary Journal. 2007.

11. Schaffer, J.M., A.D. McDonald, W.J. Hall, and H. Bunyea. A stained antigen for the rapid whole blood test for pullorum disease. *J. Am. Vet. Med. Ass.*79: 236-240. 1931.

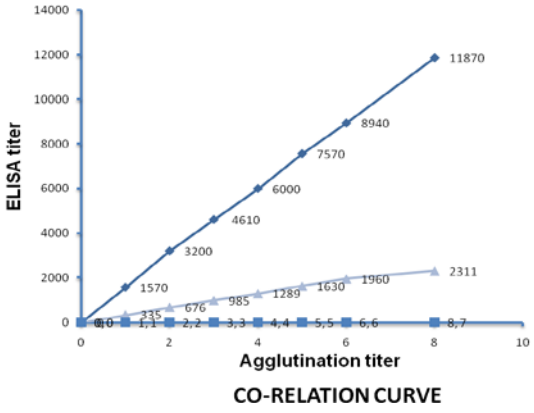
12. Timoney, J.F., N. Sikora, H.L. Shivaprasad, and M. Opitz. Detection of antibody to *Salmonella enteritidis* by a gm flagellin-based ELISA. *Vet. Res.* 127: 168-169. 1990.

13. Voller, A., D. Bidwell, and A. Bartlett. Microplate enzyme immunoassays for the immuno-diagnosis of virus infections. Manual of Clin. Immunol. ASM, Washington, D.C.: 506-512. 1976.

14. Williams, J.E. and A.D. Whitemore. Serological diagnosis of pullorum disease with the microagglutination system. *Applied Microbiol.* 21: 394-399. 1970.

15. Witlin, B. Detection of antibodies by microtitration techniques. *Mycopathol. Applied Microbiol.* 33: 41-257. 1967.

Figure 1. Standard curve between ELISA and agglutination titer (10-fold and 2-fold).



Legend:
Series 1: 10-fold agglutination titer and ELISA titer
Series 2: Dilution scale
Series 3: 2-fold agglutination titer and ELISA titer

Table 1. Field sample showing the agglutination result with newly developed stained antigen.

Flock no.	No. Of serum	Age in week	Agglutination result					
			Positive(+)		Suspect (±)		Negative(-)	
49	20	54.6	07	35%	07	35%	06	30%
50	20	54.6	04	20%	06	30%	10	50%
51	20	52.1	02	10%	05	25%	13	65%
52	30	42.2	0	0%	06	30%	24	80%
53	30	36.2	0	0%	02	10%	28	93.3%
54	30	36.2	0	0%	03	15%	27	90%
55	30	34.2	0	0%	02	10%	28	93.3%

INVESTIGATION ON OUTBREAKS OF LOW PATHOGENIC AVIAN INFLUENZA VIRUS IN POULTRY FARMS IN BANGLADESH

N. Jannat, E. H. Chowdhury, R. Parvin, M. A. H. N. A. Khan, and M. R. Islam

Department of Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh
emdad001@yahoo.com

ABSTRACT

Since March 2007, an epidemic of high and low pathogenic avian influenza occurred in backyard and commercial poultry in Bangladesh. The agents were pathotyped as highly pathogenic H5N1 and low pathogenic H9N2. This investigation reports outbreaks of low pathogenic H9 in commercial parent stock chickens. The morbidity and mortality were 50% and 3%, respectively during two to three months. The egg production decreased to 37% and hatchability reduced to 10%. The clinical signs include less feed and water intake, nasal and ocular discharges and severe respiratory distress. On postmortem, samples including larynx, trachea, and lungs were collected in 10% neutral buffered formalin and tracheal swabs, larynx, and tracheal tissues were collected in falcon tube containing 50% buffered glycerin with antibiotic. Histopathology was conducted using routine procedure. RNA extraction and RT-PCR was done using Qiagen RNA extraction and one step RT-PCR kits. Grossly, hemorrhagic nasal septum, sinuses, eyelids, larynx, and trachea were found. Lungs were congested. Histologically, in larynx and trachea loss of mucosal epithelium, hemorrhages and mononuclear cells infiltration in lamina propria, blood tinged exudates in laryngeal and tracheal lumen. In few cases, goblet cells were hypertrophied and mucosal glands were prominent that formed cystic spaces. RT-PCR confirmed the presence of 244 bp product of matrix protein gene and 488 bp fragment of H9 subtype specific hemagglutinin gene in the suspected samples. Cloning and sequencing of the virus is in progress.

INTRODUCTION

Avian influenza is an infectious disease of birds caused by influenza virus type A strains (2). It belongs to the *Orthomyxoviridae* family (9). Orthomyxoviruses are spherical or pleomorphic, enveloped and 80-120 nm in diameter (17). This is an RNA virus having the negative-sense segmented ssRNA and having eight segments. Wild aquatic birds, notably members of the orders *Anseriformes* and *Charadriiformes*, are carriers of the full variety of influenza virus A subtypes, and thus, most probably constitute the natural reservoir of all influenza A viruses (4,20). Serious problems have been reported in recent years associated with widespread outbreaks of viruses of H9N2 subtype, not only in Pakistan and Iran, but also in the Middle East and Asian countries through China. Influenza A viruses of subtype H9N2 are now considered to be widespread in poultry (16). H9N2 influenza viruses are endemic in many Asian countries (6). LPAI H9N2 appears to have spread across the whole of Asia in that time and has become endemic in poultry in many of the affected countries (1). Infections of domestic avian species with low pathogenic avian influenza (LPAI) viruses can be asymptomatic or cause a wide range of clinical signs varying from mild respiratory disease to more severe diseases affecting the respiratory and enteric systems. Details of clinicopathologic study of a naturally infected low pathogenic avian influenza virus (LPAI) is very limited. This study has reported the natural H9 infections in commercial chickens using RT-PCR and described the clinical, gross and histopathological findings.

MATERIALS AND METHODS

A parent stock farm was selected for this study. The history of the outbreak and clinical signs were recorded from the veterinarian of the farm. Routine necropsy was done and lesions were recorded. Tissue samples from larynx, trachea, and lungs were collected and preserved in 10% neutral buffered formalin immediately after postmortem for histopathology. Specimens of larynx, trachea, and lung tissues were processed and stained with hematoxylin and eosin (12). Tracheal swabs or tissues were collected in falcon tube containing sterile transport medium. Total RNA was isolated from the field samples using Qiagen RNeasy kit. One-step RT-PCR was done using Qiagen RT-PCR kit. RT-PCR products were analyzed by 1.5% agarose gel, stained with ethidium bromide and examined against UV light using an image documentation system. Appropriate primer sequences for RT-PCR were selected to amplify matrixprotein (MP) gene (4) and H9 hemagglutinin gene (11) of AI viruses. The primers targeting the NP genes are common for all

Type A influenza viruses, but the primers for H genes were designed for specific subtype (H9).

RESULTS AND DISCUSSION

The morbidity and mortality were 50% and 3%, respectively. The egg production decreased to 36% and hatchability decreased to 10%. Mild rales and gasping were observed in infected chickens. Less feed and water intake was observed. Hemorrhagic nasal septum, sinuses, eyelids were found. Hemorrhagic exudates in larynx and trachea with congested Lungs were found. Peritoneum, air sac, and pericardium were thickened and cloudy. In larynx and trachea, loss of mucosal epithelium, exudates containing desquamated, degenerated and necrotic epithelial cells and red blood cells in the lumen of the trachea and larynx, inflammatory cells were accumulated around the blood vessels in the sub-mucosa. No lesions were found in the lungs and air sacs. This type of histopathological lesions were supported by different authors for avian influenza (8,13,14,15). The hemagglutination (HA) test was performed to determine the presence of virus in allantoic fluid as AI virus has hemagglutinin. The suspected virus samples were serially diluted and agglutinated the chicken RBC up to 2^8 dilution. Results indicated that the suspected virus posses hemagglutinin antigens.

RT-PCR. The samples belong to H9 subtypes of AI viruses were first tested with RT-PCR for MP gene. Samples were tested positive and yielded a product of about 244 base pair (bp) as expected (Figure 1). The RT-PCR for H9 hemagglutinin gene worked perfectly as it amplified a product of about 488 bp from the field samples (Figure 2).

The one-step, reverse-transcriptase polymerase chain reaction (RT-PCR) was applied for the detection of avian influenza virus in field specimens by different authors (5,10,18,19). The paper confirms the LPAI infection in the parent stock farms in Bangladesh much before (September/06) the official declaration of HPAI outbreak in Bangladesh. However, N subtyping was not yet done, but presence of H9N2 virus has been already confirmed in the poultry farm in Bangladesh either as single or concurrent infection with H5N1. Continued surveillance of poultry for avian influenza infection is critical to minimize the magnitude of outbreak and thus limit the risk of human infection. H9 subtype AIV can infect human and thus this virus needs special attention. Detail molecular study like cloning and sequencing is necessary to understand the molecular epidemiology of the virus.

REFERENCES

1. Alexander, D.J. An overview of the epidemiology of avian influenza. *Vaccine.* 25:5637-44. 2007.

2. Ergin, S. Avian influenza as an emerging infection. Veteriner-Fakultesi-Dergisi-Istanbul. 32: 1-11. 2006.

3. Fouchier, R.A.M., T.M. Bestebroer, S. Herfst, L. van der Kemp, G.F. Rimmelzwaan, and A.D.M.E. Osterhaus. Detection of influenza A viruses from different species by PCR amplification of conserved sequences in the Matrix gene. *J. Clin. Microbiol.* 38: 4096-4101. 2000.

4. Fouchier, R.A.; B. Olsen, and T.M. Bestebroer. Influenza A virus surveillance in wild birds in Northern Europe in 1999 and 2000. *Avian Dis.* 47: 857-60. 2003.

5. Horimoto, T. and Y. Kawaoka. Molecular changes in virulent mutants arising from avirulent avian influenza viruses during replication in 14-day-old embryonated eggs. *Virology*. 206: 755-9. 1995.

6. Kim, J.A., S.H. Cho, H.S. Kim, and S.H. Seo. H9N2 influenza viruses isolated from poultry in Korean live bird markets continuously evolve and cause the severe clinical signs in layers. *Vet. Microbiol.* 118:169-76. 2006.

7. Krauss, S., D. Walker, S.P. Pryor, L. Nile, L. Chenghong, V.S. Hinshaw, and R.G. Webster. Influenza A viruses of migrating wild aquatic birds in North America. *Vector Borne Zoonotic Dis.* 4: 177-89. 2004.

8. Kwon, Y.K., Y.J. Lee, J.G. Choi, E.K. Lee, W.J. Jeon, O.M. Jeong, M.C. Kim, S.J. Joh, J.H. Kwon, and J.H. Kim. An outbreak of avian influenza subtype H9N8 among chickens in South Korea. *Avian Pathol*. 35: 443-447. 2006.

9. Lamb, R.A. and R.M. Krug, Orthomyxoviridae: the viruses and their replication. In: Fieldds, B.N., Knipe, D.M., Howley, P.M., Chanock, J.L., Melnick, R.M., Momath, T.P. and Roizman, B. (Eds.), *Fields. Virol*, 3rd ed., Lippincott-Raven, Philadelphia, PA. 1996.

10. Lee, C.W. and D.L. Suarez. Application of real-time RT-PCR for the quantitation and competitive replication study of H5 and H7 subtype avian influenza virus. *J Virol Methods.* 119: 151-8. 2004.

11. Lee, M.S., P.C. Chang, J.H Shien, M.C. Cheng, and H.K. Shieh. Identification and subtyping of avian influenza viruses by reverse transcription-PCR. *J. Virol. Methods*. 97: 13-22. 2001.

12. Luna, L.G. Manual of Histologic Staining Methods of the Armed Forced Institute of Pathology. 3rd edition. McGraw Hill Book Company, New York. 1968.

13. Nili, H. and K. Asas. Natural cases and an experimental study of H9N2 avian influenza in commercial broiler chickens of Iran. *Avian Pathol.* 31: 247-52. 2002.

14. Nili, H. and K. Asasi. Avian influenza (H9N2) outbreak in Iran. *Avian Dis.* 47: 828-31. 2003.

15. Nili, H., K. Asasi, H. Dadras, and M. Ebrahimi. Pathobiology of H9N2 avian influenza virus in Japanese quail (*Coturnix coturnix japonica*). *Avian Dis.* 51: 390-2. 2007.

16. Peiris, M., W.C. Yam, K.H. Chan, P. Ghose, and K.F. Shortridge. Influenza A H9N2: Aspects of Laboratory Diagnosis. *J. Clin. Microbiol.* 37:3426-3427. 1999.

17. Quinn, P.G., B.K. Markey, M.E. Carter, W.J. Donnelly, and F. C. Leonard. *Orthomyxoviridae.* In: Veterinary Microbiology and Microbial Disease by. Blackwell Science Ltd. Iowa State University Press. pp. 375-380. 2002.

18. Starick, E. and O. Werner. Detection of H7 avian influenza virus directly from poultry specimens. *Avian Dis.* 47: 1187-1189. 2003.

19. Starick, E., O.A. Roemer, and O. Werner. Type- and subtype-specific RT-PCR assays for avian influenza A viruses (AIV). *J. Vet. Med. Series B.* 47: 295-301. 2000.

20. Widjaja, L., S.L. Krauss, R.J. Webby, T. Xie, and R.G. Webster. Matrix gene of influenza a viruses isolated from wild aquatic birds: ecology and emergence of influenza a viruses. *J. Virol.* 78: 8771-9. 2004.

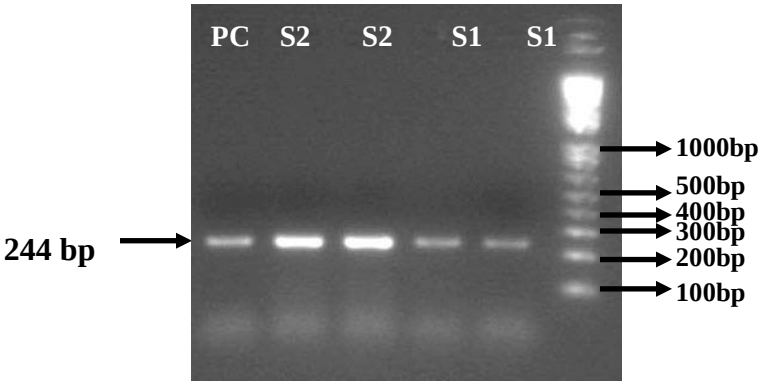


Figure 1. Amplification of the fragment of matrix protein gene of Type A influenza virus by RT-PCR using primers designed by Fouchier *et al.* (4). M = Marker, S1 = Field sample-1, S2 = Field sample-2, PC = Positive control (RNA extracted from inactivated H9 virus received from VLA, England).

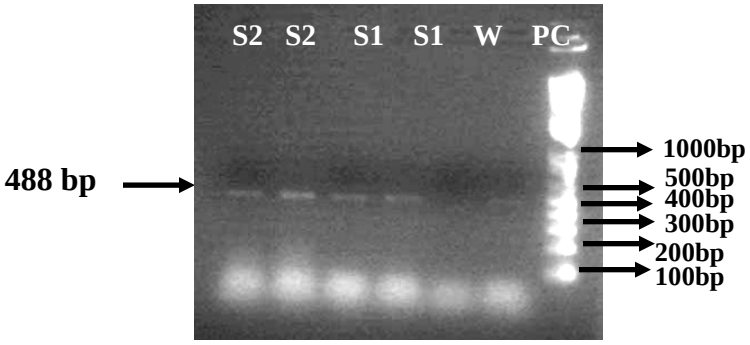


Figure 2. Amplification of the fragment of H9 gene by RT-PCR using primers of Lee *et al.* (11). M = Marker, PC = Positive control (RNA extracted from inactivated H9 virus received from VLA, England), W = Water control, S1 = Field sample-1, S2 = Field sample-2.

HOW INCUBATION OF TURKEY EGGS CAN AFFECT POULT QUALITY

Vern L. Christensen

North Carolina State University

The final destiny of the properly managed fertilized turkey egg within incubators in our modern management systems is a viable hatchling poult. Understanding how incubation can affect the viability and quality of modern turkey eggs is essential to the poultry industry because of limited profit margins and economies of scale. Modern-day turkey hatcheries generally have egg setting capacities for thousands of eggs. Fertilized turkey eggs require a 28 day developmental period so the scheduling of eggs settings and removal of hatched poults from the incubator requires careful planning and monitoring of incubation conditions. Sanitation is imperative as well because conditions for bacterial, mold and viral growth are ideal under incubation environments. In fact, human vaccine manufacture is still often accomplished using fertilized poultry eggs.

Our understanding of processes creating a hatchling that is characteristic of the species has improved over that last 30 years because of our understanding of basic biological principles that govern the growth and development of cleidoic eggs. Embryos within cleidoic eggs by definition are packaged with all the nutrients required to develop a new organism of that specie if the egg is treated properly to foster development. The outside elements that are required for development are oxygen and heat. We need to better understand how to use these two elements in the proper proportions.

Fick's First Law of Diffusion. The bird egg is a self-contained life-support system for the developing bird embryo. All the nutrients, minerals, energy sources and water utilized by the embryo during its incubation are already in the freshly laid egg so that the egg requires only warming by the parents and period turning to prevent the adhesion of the embryo to the shell membranes. Still the egg lacks one crucial requirement: oxygen, which drives the metabolic machinery of the embryonic cells so that they can execute the complex maneuvers of development. How does the egg breathe, taking up oxygen from the surrounding atmosphere and discharging carbon dioxide, the waste product of respiration? In the late 1970s the laboratory of Hermann Rahn in the dental school at the State University of New York in Buffalo described in detail how the egg accomplishes respiration. This knowledge suggested principles that can be used in our modern-day incubation systems to ensure poult quality.

Over the typical 28 days of incubation a typical turkey egg weighing 90 g will take about 7 L of oxygen and give off 20 L of carbon dioxide and 15 L of water vapor prior to the 25th day of development (8). The total communication channel or pore occupies only 0.024 of 1% of the total shell surface. The total functional pore area of a normal turkey eggs is only 2.2 mm^2. The embryo itself has no control over its respiration rates or its oxygen-driven metabolic rate. It is totally dependent upon the type of shell that is placed around it during egg formation. Little is known of the mechanisms in the turkey breeder hen that control this process (3). We do know that high altitude reduces the functional pore area of a turkey egg (10).

The movement of gas by diffusion through a permeable barrier depends on the random motion of the gas molecules and the concentration of the diffusing species on one side of the barrier and the concentrations on the other side. Since collisions among gas molecules are more frequent in a concentrated gas than in a dilute one, the molecules will tend to move from the side of higher concentration to the side of lower concentration.

A simplified version of Fick's law of diffusion states that the quantity of a given gas diffusing in a unit of time through the pores of an eggshell will be directly proportional to the area of the pores available for diffusion and to the difference between the concentration of the diffusing gas at one end of the pore and the concentration at the other end. On the other hand, the rate of diffusion will be inversely proportional to the length of the diffusion path (in this case the length of the pores through the eggshell). In other words, the gas conductance of the eggshell depends on the ratio of pore area to pore length. Doubling the area available for diffusion of a gas or doubling the concentration difference of that gas across the shell will double the rate of passage, whereas double the pore length will halve the rate of passage, all other factors remaining equal. Thus, if one could measure the flux of a gas and divide it by the concentration difference of that gas across the pores, one would be able to calculate the conductance of the shell for that gas (8). This calculation is used to estimate the functional properties of eggshells defined as eggshell conductance.

Eggshell conductance theory. Eggshell conductance is then the property of the eggshell that governs how an egg breathes. In an extension of that same idea, it is also the property that drives the metabolism and thereby the rate of growth and differentiation of a turkey embryo into a hatched poult (8). Eggshell conductance creates common characteristics at the time of hatching that are described as follows: 1) The total amount of oxygen that will have been consumed is about 100 mL per g of initial egg mass and equivalent to a caloric expenditure of .5 kcal per g of initial egg mass. 2) The oxygen concentration in the air cell shortly before pipping will have fallen from 21% initially to 14% while the carbon dioxide concentration will have reached a value of about 6%. 3) Eggs will have lost about 15% of their initial mass due to the loss of water vapor, yet their relative water content will still be the same as when they were laid. These common end results require a precise pore geometry of the shell or shell conductance, which in each species is matched to the egg mass, its metabolic rate and incubation time. Much of this information is readily available in a multi-authored review (9).

Egg shell conductance theory predicts a period of time in the development of precocial species when the metabolic rate or oxygen uptake of the embryo will exceed the functional ability of the shell to provide oxygen. This time has been defined as the plateau stage in oxygen consumption and occurs in the turkey embryo at 24 or 25 days of incubation (4). The plateau stage in oxygen consumption plays a major role in maturation of tissues required outside the shell as well as sets up mechanisms leading to successful thermoregulation. One lesser known function is to establish blood acid-base balance prior to emergence from the shell (7). The three conditions mentioned above in the prior paragraph must be met at the plateau stage in oxygen consumption in order to create a hatchling that has the characteristic maturity of the species. The plateau stage is not thought to occur in altricial species although some data suggest that it may (6).

Conductance constants. The third concept that is necessary to clarify how the incubation of eggs can affect poult quality is the conductance constant. Ar and Rahn (1) noted among 96 species of birds that three measurements were interrelated. The three factors were the egg weight, the eggshell conductance and the length of the incubation period. Ar defined the conductance constant as the ratio of the product of eggshell conductance and the length of the incubation period in days divided by the weight of the egg. For all species tested this was calculated to equal 5.13. Therefore, the constant varies directly with conductance and the length of the incubation period but inversely with egg weight. We can use the conductance constant to predict each of the components of the equation. For example, if we know the weight of an egg as well as its eggshell conductance prior to placement into an incubator, we can predict how long to incubate that egg to maximize the maturation of poults prior to hatching.

Practical considerations – hatching times. Conductance constants imply that for each egg weight and eggshell conductance value, there exists an appropriate incubation period. We control the length of the incubation period by the temperature of our incubation cabinets as well as the availability of oxygen (1). The reader is reminded that an egg must consume 100 mL of oxygen per g of initial egg mass to create a hatchling that has the maturity characteristic of the species (8). Thus, oxygen controls the growth of the embryo, but the temperature controls the rate of that growth (2). This is illustrated in Table 1.

These data suggest that when the incubation period is matched to the egg weight and the eggshell conductance that the “hatching window” is shorter. Most poults will hatch within a 36 hour time period. If the three egg variables are not matched, then the hatch window is much wider. This observation gives us an observable trait to create a good quality poult. Table 2 illustrates why this may be.

Overall the data suggest that short developmental periods require the embryo to remain at internal pipping in a hypoxic environment for a longer time. Extended time in hypoxia may demand more carbohydrate energy for activity than the hepatic and renal enzyme systems can recycle and shuttle back to critical tissues such as the heart and intestine. This may result in immature organ systems or in extreme cases embryo death.

Measure of poult quality affected by conductance constants. Table 3 and Table 4 illustrate how incubation can be used to improve poult quality. The initial table shows the effects of incubating eggs such that they hatch following a short and long incubation period. The first table illustrates eggs weighing nearly 100 g at setting in the incubator.

Table 4 illustrates the effect of eggshell conductance on poult quality from large eggs weighing nearly 100 g.

Eggshell conductance has a greater effect on poult quality of poults hatching from large eggs than does the length of the incubation period. Low eggshell conductance eggs of heavier weights obviously yield poults of reduced quality compared to Average and High conductance eggs.

Physiological and anatomical reasons why the conductance constant may affect poult quality are given in Table 5 and Table 6.

Although the length of the incubation period did not affect the growth of the poults (Table 3), it is clear

that longer incubation periods with heavy eggs resulted in differences in both intestinal and thyroid maturation.

CONCLUSIONS

Hatchery-related problems. Carver *et al*., (2002) concluded that the age of the breeder hen and the size of the egg she produced were factors in seven and 14 day poult mortality. Larger eggs produce better poults until late in the laying period when poult quality deteoriated. The data illustrated above suggest some ideas for troubleshooting these problems. The Hatching Window could be measured by doing 36 hour counts as well as the time of hatching. Hatchery-related factors considered and found no significant risks for mortality included age of egg, number of eggs set, percentage of fertile eggs, and percentage of hatched eggs, poult injection, toe clipping and beak trimming. Donaldson *et al*. (1995) indicated that elevated carbon dioxide (0.4%) caused metabolic effects in newly hatched poults that suggest hypercapnia is a stressor and an additional risk factor for early poult mortality. Poults may be routinely sexed, beak-trimmed, toe-trimmed, and desnooded before shipping. There is abundant evidence these hatchery poult-processing practices contribute to poult mortality (see Carver *et al*., 2002).

Watch for these ten hatchery-related problems. Don't blame your hatchery man for excessive early mortality - at least not always. The mortality could be the fault of poor brooder house management.

Having said that, however, there are 10 points for growers to watch for in hatchlings - hatchery related factors that could give producers problems with their poults. They are:

1. Dehydrated poults: They generally result from low humidity during incubation, and early hatch or excessively long holding periods in boxes before delivery. Dehydrated poults can be identified upon delivery at the farm by **examining the shanks and feeling the poults**. If a fairly large number of poults have shriveled legs or shanks and bodies feel hard and look angular, give the birds extra good care the first week. The poults should have "easy" access to water and feed and they should be kept comfortable. Do not chill or overheat the poults in the brooder house. **Always check a sample of poults (50 to 100 at random) upon delivery to determine the state of dehydration and then brood accordingly.**

2. Weak poults: Weak poults do not have to be dehydrated poults, and often are not. Weak poults usually result from higher than recommended temperatures during hatching, inadequate ventilation in the hatchers, over-fumigation at hatching time, infection, rough sexing or setting old eggs. Weak poults can be identified easily by pressing down on the poults in the boxes with the palm of the hand. If the poults are strong, they will offer considerable resistance to the pressure of the hand; if they are weak, they can be pushed down easily. **With a little practice (the touch of the master) you can detect weak poults upon delivery. Weak poults need better than average brooder house care.**

3. Large, soft-bodied poults: Large, sluggish poults usually are the result of high humidity during incubation and hatching. They often have a heavy abdomen and feel soft and full of moisture to the touch. They generally ship better when transported long distances. **These poults usually present no serious brooding problems except that they appear sluggish.**

4. Rough navels: The navels of poults always should be checked upon delivery to the farm. A rough or open navel makes the chick more susceptible to infections. Rough or unhealed navels result when the hatch is late (more than 28 days), incubation temperature has been variable and high, or when excessively high humidity was used during hatching. **Poults with rough navels upon delivery probably should receive a broad-spectrum antibiotic in the feed or water for the first week to minimize the possibility of infection and morbidity.**

5. Omphalitis (navel infection): Omphalitis is the result of filth in the hatchers and/or contaminated poult boxes and poult box pads. *E. coli*, *Pseudomonas*, *Proteus*, or occasionally a *Staphylococcus* usually causes it. Sometimes the yolk sac is involved in addition to the navel. Yolk sac contents change from a yellow-green material to a caseous material or to a yellow-brown watery material when contaminated with *E. coli.* The navel opening often has an offensive odor. **Mortality and morbidity will be high with a high percentage of runts among the surviving poults.**

An omphalitis infection means that the hatchery must change its clean-up and egg and hatchery sanitation programs immediately. **A broad-spectrum antibiotic or a nitrofuran may help reduce morbidity and the percentage of runts.** The type of organism involved and drug resistance will affect the type of response one gets to treatment.

6. Poult delivery. Errors in the chick delivery system can injure potentially strong, healthy poults. Damage can occur in several ways; namely, overheating in the delivery van, chilling in the van, poor van ventilation resulting in overheating, chilling, or CO_2 poisoning. Assuming good judgement in programming the load, the driver becomes the "key" to a successful delivery of undamaged poults. Some truck drivers have no feel for the product being delivered. **The salvage process at the brooder house consists of ample feed and water, and a comfortable brooding temperature, along with a tremendous amount of**

care and attention. The amount of loss depends on the damage done to the chicks in transit, and the amount of care given them during brooding.

7. Improper toe trimming, wing clipping, snood removal, poultry injection and rough handling during sexing: Quality control and sanitation are the greatest problems a hatchery has with these operations - getting the hatchery personnel to do their jobs properly and uniformly. Improper wing clipping or toe trimming can leave a chronic sore. Many years ago poultrymen recommended wing clipping or toe trimming through a joint taking into account the proper angle, and using a modified beak trimming unit to do the job.

The injection site and injection process should be kept as sterile as possible. Poults should be injected according to directions - meaning proper equipment and needles, recommended dosage of antibiotic mixture, injection in the upper portion of the neck and subcutaneously. If the antibiotic mixture is of the type that puts the chicks to sleep, delivery should be delayed until **all** poults have recovered from the injection.

There is little that farm management can do with poults that have been improperly processed except to talk to hatchery management.

8. Poult grading. All malformed, straddle-legged, and weak chicks should be culled at the hatchery before delivery. A high percentage of abnormal poults will die or be morbid. Most hatcheries do a good job of removing the abnormal poults.

9. Nutritional deficiencies. Breeder rations that are marginal in certain vitamins and/or trace minerals can result in hatched chicks that are weak and marginal in vitamin and/or mineral reserves. Those chicks should be fed a prestarter well fortified with vitamins and minerals. Often when young breeder hens have been on a poorly vitamin and mineral-fortified holding ration, the first two or three hatches of chicks will not start and live as well the first week of brooding as later hatches.

Some of the vitamins and minerals which could be deficient in the breeder ration and which could reflect themselves in the young poult are E, K, riboflavin, biotin, folic acid, pantothenic acid and B_{12}. Some of the minerals would be iodine, potassium, manganese and cobalt. Check to see whether the breeder hens are receiving an adequate diet, if not, feed a prestarter. **A prestarter will have more protein, higher vitamin and mineral fortification and higher levels of growth promotant than the regular starter.**

10. Irregular-sized poults: Irregular-sized chicks result from different age of breeder flocks and age size, variations in incubator temperature and humidity. **If the poults are from a healthy flock, there is little worry since hatching weight is poorly correlated with market weight.**

REFERENCES

1. Ar, A., and H. Rahn. Interdependence of gas conductance, incubation length, and weight of the avian egg. In: Respiratory Function in Birds, Adult and Embryonic, J. Piiper, ed., Springer Verlag, Berlin. Pages 227-236. 1978.
2. Christensen, V.L., L.G. Bagley, J. Prestwich, T. Olson, M. Wineland, and D.T. Ort. Length of the developmental period of turkey eggs affects cardiac physiology and subsequent embryo survival. Int. J. Poult. Sci. 6:95-101. 2007.
3. Christensen, V.L., and G.S. Davis. Maternal dietary iodide influences turkey embryonic thyroid function. Poultry Sci. 80:1286-1292. 2001.
4. Christensen, V.L., W.E. Donaldson, and K.E. Nestor. Embryonic viability and metabolism in turkey lines selected for egg production or growth. Poultry Sci. 72:829-838. 1993.
5. Christensen, V.L., D.T. Ort, K.E. Nestor, G.B. Havenstein, and S.G. Velleman. Genetic control of embryonic cardiac growth and functional maturation in turkeys. Poult. Sci. 87:858-877. 2008.
6. Dietz, M.W., M. van Kampen, M.J.M. van Griensven, and S. van Mourik. Daily energy budgets of avian embryos: The paradox of the plateau phase in egg metabolic rate. Physiol. Zool. 71:147-156. 1998.
7. Erasmus, B. deW., and H. Rahn. Ontogeny of acid-base balance in the bullfrog and chicken. Respir. Physiol. 11:46-53. 1970/71.
8. Rahn, H. Gas exchange of avian eggs with special reference to turkey eggs. Poult. Sci. 60:1971-1980. 1981.
9. Rahn, H., A. Ar, and C.V. Paganelli. How bird eggs breathe. Sci. Amer. 240:46-55. 1979.
10. Rahn, H., C. Carey, K. Balmas, B. Bhatia, and C.V. Paganelli. Reduction of pore area of avian eggshell as an adaptation to altitude. Proc. Natl. Acad. Sci. 74:3095-3098. 1977.

Table 1. Time of hatching (h of incubation) of turkey embryos developing in eggs at three different temperatures.

	Hours of Incubation				
Temperature	648	654	660	666	672
37.1°C	2.7b	9.0c	39.7c	59.9c	100.0
37.3°C	3.4b	25.0b	44.5b	78.7b	100.0
37.5°C	11.3a	46.2a	65.5a	83.1a	100.0
Mean ± SEM	5.8 ± 0.4	26.7 ± 0.9	49.9 ± 1.0	73.9 ± 0.9	NA
Probability	0.0001	0.0001	0.0001	0.0001	

[a,b,c] Columnar means followed by a different superscript differ significantly ($P < 0.01$). [1]Overall mean ±SEM of pooled data from three trials.

Table 2. Times (h) to attain a stage of development and the time remaining at that stage of poult embryos incubated at three different temperatures.

	Internal pip		**External pip**		**Hatched**
Temperature	Attain	At	Attain	At	Attain
37.1°C	628	8.1c	636	20.1a	657a
37.3°C	626	11.4b	637	18.1a	655ab
37.5°C	624	14.7a	639	14.1b	653b
Mean ± SEM	626 ± 6	11.4 ± 0.5	638 ± 8	17.4 ± 0.1	655 ± 3
Probability	0.1887	0.0001	0.4917	0.0315	0.0568

[a,b,c] Columnar means followed by a different superscript differ significantly ($P < 0.01$). [1]Overall mean ±SEM of pooled data from three trials.

Table 3. Effects of different incubation temperature profiles on growth of poults (large eggs).

	HP	**LP**	**Overall SE**	**P value**
Time to feed (min)	104	144	97.56	NS
Weight d 1(g)	60.6	60.0	8.2	NS
Weight d 3 (g)	94.2	91.1	23.4	0.0200
Weight d 7 (g)	123	125	12.6	NS
Feed per gain	1.28	1.19	0.01	NS

HP = poults from eggs incubated at high temperature profile; LP = poults from eggs incubated at low temperature profile.

Table 4. Effect of different eggshell conductance values on growth of poults hatching from large eggs.

	Low	**Average**	**High**	**Overall SEM**	**P value**
Time to feed (min)	163[a]	129[b]	81[c]	9.8	0.0387
Weight d 1 (g)	59	60	61	8.2	NS
Weight d 3 (g)	90[b]	93[a]	95[a]	2.3	0.0120
Weight d 7 (g)	118[b]	123[a]	130[a]	4.3	0.0071
Feed per gain	1.33[a]	1.26[ab]	1.12[b]	0.06	0.0387

Low = low eggshell conductance; Average = average eggshell conductance; High = high eggshell conductance.

Table 5. Effect of different incubation profiles on anatomic and physiologic factors determining maturity of poults hatching from large eggs.

	HP	LP	Overall SEM	P value
Body weight at hatching (g)	52.4	53.3	1.81	NS
BW without yolk (g)	48.2	49.3	1.49	NS
Yolk sac weight (g)	4.2	4.2	0.15	NS
Jejunal length (cm)	12.96	13.44	0.80	NS
Jejunal weight (g)	0.455	0.482	0.0009	NS
Relative jejunum weight	0.9	0.10	0.00003	NS
Total maltase activity	327.8[b]	367.5[a]	2.0	0.0393
Total ALP activity	18,113	18,466	7408	NS
Specific Maltase activity	17.39	18.63	2.98	NS
Specific ALP activity	0.960	0.890	0.01	NS
Plasma T_3 (ng/mL)	3.41[b]	3.76[a]	0.09	NS
Plasma T_4 (ng/mL)	10.60[b]	12.75[a]	1.53	0.0151
Ratio T_3:T_4	0.344[a]	0.302[b]	0.001	0.0508

HP = poults from eggs incubated at high temperature profile.
LP = poults from eggs incubated at low temperature profile.

Table 6. Effects of different eggshell conductance values on anatomic and physiologic factors determining maturity of poults at hatching from large eggs.

	Low	Average	High	Overall SEM	P value
Body weight (g)	61.3	62.2	61.6	3.39	NS
BW without yolk (g)	50.9	53.8	53.2	2.63	NS
Yolk sac weight (g)	10.33[a]	8.44[b]	8.41[b]	0.60	0.0024
Jejunal length (cm)	14.64	14.34	14.06	0.66	NS
Jejunal weight (mg)	466	464	465	0.001	NS
Relative jejunum wt	0.93	0.86	0.88	0.0003	NS
Total maltase activity	416	387	398	80.47	NS
Total ALP activity	20,182[b]	26,590[a]	21,457[ab]	1,884	0.0019
Specific Maltase activity	16.61	15.26	16.18	2.30	NS
Specific ALP activity	0.799[b]	1,016[a]	0.844[ab]	0.01	0.0019
Plasma T_3 (ng/mL)	2.93	3.22	2.92	0.04	NS
Plasma T_4 (ng/mL)	10.40	10.95	9.46	0.88	0.0500
Ratio T_3:T_4	0.290	0.210	0.330	0.001	0.0007

Low = low eggshell conductance; Average = average eggshell conductance; High = high eggshell conductance.

CROSSPROTECTION STUDY OF A MODIFIED LIVE *E. COLI* VACCINE AGAINST THREE HETEROLOGOUS APEC SEROTYPES IN COMMERCIAL BROILER CHICKENS

Kalen Cookson[A], Steve Davis[B], and Lisa Nolan[C]

[A]Fort Dodge Animal Health, Overland Park, Kansas
[B]Colorado Quality Research, Wellington, Colorado
[C]Dept. of Vet Microbiology & Preventive Medicine, Iowa State University

INTRODUCTION

E. coli induced chronic respiratory disease is a leading cause of broiler mortality and condemnations. Recent *E. coli* field surveys have revealed two interesting findings: 1) there is tremendous diversity in O serotypes within even a limited geographic region (4) and 2) most *E. coli* isolates coming from diseased flocks contain a preponderance of virulence genes, as measured by PCR analysis, while commensal fecal isolates tend to contain very few (4,5). We have reported previously on a live *E. coli* vaccine's ability to protect commercial broilers against different O78 avian pathogenic *E. coli*, or APEC (1,3). Because of the diversity of APEC serotypes in the field, this study was conducted to measure that live *E. coli* vaccine's ability to cross-protect against three of the more common, heterologous (non-O78) APEC isolates.

MATERIALS AND METHODS

A pilot challenge study was first performed using ten broiler APEC isolates from a total of six different serotypes (2). All ten APECs caused significant levels of airsacculitis (30 to 83%) and varied in their ability to cause mortality (0 to 50%). Three of these isolates (#1, #9 and #8) were chosen for this study based on their serotypes (O1, O2 and O18, respectively) and their ability to cause a high incidence of respiratory disease.

At day of hatch 1,280 commercial, straight-run broilers were divided equally into two treatments. All birds were coarse sprayed with a modified live Newcastle/infectious bronchitis vaccine while half also received Poulvac® E. coli. After 30 minutes the two vaccine treatments (No Vax and D1 Vax) were allocated to a total of 64 pens of 20 birds each. Birds were raised on wood shavings and fed a typical broiler ration *ad libitum* throughout the course of the study. At 18 days of age, half of each of the day one treatments were vaccinated by coarse spray with Poulvac E. coli, resulting in four *E. coli* vaccine treatment groups: 1) No Vax, 2) D18, 3) D1 and 4) D1&18. At 42 days of age all four vaccine treatments were pared down equally to 18 birds per pen and further divided into four different challenge groups: a) No challenge, b) APEC O1, c) APEC O2 and d) APEC O18. Challenge inocula were administered intratracheally (IT) at a dose of 2.0, 2.0 and 2.1 x 10^7 CFU/bird, respectively. Dead and moribund birds were removed daily until termination of the study at 47 days, when all birds were weighed and necropsied. Lesions of colibacillosis were recorded and airsacculitis was scored using the following scale: 0) none, 1) mild suds, 2) moderate suds or multifocal exudate and 3) heavy suds or severe, profuse exudate.

RESULTS

Control Groups. None of the unchallenged vaccine treatments suffered from mortality from 42-47 days of age (see Table) and they had similar, low levels of airsacculitis (rate of 4.2-9.9% and mean scores ranging from 0.25 to 0.43). This was considered within normal limits considering the time of year of the study (March). In contrast, the unvaccinated challenge controls had mortality ranging from 0.0 to 13.9% and significant levels of airsacculitis (rate of 80.6-93.1% and mean scores ranging from 2.17 to 2.54). Similar to the pilot challenge study, APECs O1 and O2 were similar in virulence while the O18 isolate was capable of causing significantly higher mortality (13.9%).

Vaccination/Challenge Groups. Except against the O18 challenge in the Day 18 vaccine treatment, all other vaccine treatments had significantly lower mean airsac lesion scores than the challenge controls. In fact, the Day 1&18 treatment mean lesions after O1 and O2 challenge were not different from the negative controls. All vaccine treatment groups had a significantly lower percentage of challenged birds showing airsacculitis. Again, the Day 1&18 treatment saw the greatest reductions, with no significant differences versus the negative controls against O1 and O2 challenge. None of the vaccine treatments was able to significantly reduce mortality from the O18 challenge; however, the rate in the Day 1&18 group was low enough that it was not statistically different from the negative controls.

DISCUSSION

Recent field surveys of diseased broiler flocks have supported previous findings of Lisa Nolan and her associates (4,5). While there is tremendous diversity and no apparent pattern in O serotypes within a given geographic location, a common thread is that the vast majority of these isolates are not commensal, avian fecal *E. coli* (AFEC) but isolates loaded with virulence genes and capable of causing a high level of disease and mortality. Because Poulvac E. coli types as an O78 serotype, the goal of this study was to see if this vaccine would also protect against heterologous *E. coli* serotypes. In case the standard broiler application (day of age, coarse spray) would not provide adequate cross-protection, two other vaccination regimens were employed. As it turned out, the order of protection in the day-of-age treatments was comparable to protection levels seen against O78 APECs (1,3).

While all three vaccination strategies gave significant protection against the three serotypes tested, there were at least subtle differences between them. The two-time vaccination program resulted in numerically greater reductions in airsac lesion scores over the Day 1 strategy, regardless of the challenge isolate, and was significantly more successful than Day 18 vaccination. The Day 18 vaccination program performed about as well as the Day 1 program in this (late-challenge) model, but it seems plausible that there may be advantages to hatchery vaccination—in uniformity of application and immunizing chicks prior to a potentially early field exposure—in a real-life setting. The additional level of reductions in the two-time vaccination group, especially against mortality from the more virulent O18 isolate, suggests that certain high-challenge situations might further benefit from a booster vaccination.

REFERENCES

1. Cookson, K. and S. Davis. *E. coli* challenge study in commercial broilers by either respiratory or skin route of exposure and the effect of prior vaccination with a live attenuated (aro-A) *E. coli*. Abstract 4457. 144th AVMA Annual Convention, Washington, D.C. July 2007.

2. Cookson, K., S. Davis and L. Nolan. Comparison of ten avian pathogenic E. coli strains in commercial broiler chickens. Proceedings of the 57th Western Poultry Disease Conference. Puerto Vallarta, Mexico. pp 180-182. April 2008.

3. Cookson, K., K. Macklin and J. Giambrone. The efficacy of a novel live E. coli vaccine using a broiler skin challenge model. Abstract 1568. Proceedings of the 23rd World's Poultry Congress. Brisbane, Australia. July 2008.

4. Cookson, K., L. Nolan and C. Gustafson. The characterization of several avian pathogenic E. coli (APEC) strains from commercial broilers using PCR analysis of key virulence genotypes. Abstract 6027. 145th AVMA Annual Convention, New Orleans, La. July 2008.

5. Rodriguez-Siek, K.E., C.W. Giddings, C. Doetkott, T.J. Johnson, and L.K. Nolan. Characterizing the APEC pathotype. Vet Res. 36: 241-256. 2005.

Table 1. Summary of mortality and *E. coli* lesions related to each APEC challenge isolate.

***E. coli* Vaccine Treatment**	**APEC Challenge Treatment**	**Mean Airsac Lesion Score**		**% Airsacculitis >1**		**% Mortality**	
		Value	Statistic *	Value	Statistic	Value	Statistic
None	None	0.43	A	6.94	A	0.00	A
	O1	2.17	F	80.56	E	0.00	A
	O2	2.20	F	82.86	E	5.71	ABC
	O18	2.54	F	93.06	E	13.89	C
Day 18	None	0.31	A	9.86	A	0.00	A
	O1	1.32	CDE	44.44	BCD	0.00	A
	O2	1.57	DE	51.39	D	1.39	AB
	O18	1.68	EF	54.17	D	9.72	BC
Day 1	None	0.31	A	4.23	A	0.00	A
	O1	1.17	BCDE	40.28	BCD	0.00	A
	O2	1.31	CDE	46.48	CD	0.00	A
	O18	1.23	CDE	42.25	BCD	9.86	BC
Day 1&18	None	0.25	A	7.04	A	0.00	A
	O1	0.66	AB	23.29	AB	1.37	AB
	O2	0.80	ABC	26.76	ABC	2.82	AB
	O18	1.04	BCD	36.11	BCD	5.56	ABC

*Groups sharing a letter are not statistically different, based on Tukey's test (HSD; $P < 0.05$).

BC POULTRY INDUSTRY BIOSECURITY INITIATIVE: THE EVOLUTION OF A "NECESSARY EVIL"

Bill Cox

Canadian Animal Health Management Services Ltd

INTRODUCTION

The poultry industry in British Columbia is as one of the most densely populated in Canada, in spite of its serving a relatively small local market. Compounding the density is the mixed population of hatching egg, broiler, turkey, and layer flocks, each of which is, for the most part, a small independently owned farm. With proximity, common service activity, and common social activity considered, the industry is extremely vulnerable to the incursion and spread of a serious infectious disease. In February, 2004, the inevitable happened and the Fraser Valley poultry industry was hit with highly pathogenic avian influenza H7N3.

BACKGROUND

In Canada, the conventional poultry industry, including broiler breeders, broiler chicken, layers, and turkeys, operates under a supply managed system. Under the oversight of federal boards, a marketing board for each commodity functions in each province to adjust product supply based upon expected local demand. Price is also regulated on a cost-of-production basis. As a consequence of this system, poultry production matches population closely for each province and there is minimal export. Concentrations of production, therefore, are located where it is most advantageous within each province.

In British Columbia, the poultry industry is concentrated in the Fraser Valley, east of Vancouver. The Fraser Valley is located in the southwest corner of British Columbia and agriculture extends from the eastern margins of the Metro Vancouver area eastward to the transition of the Fraser Valley to the Fraser Canyon, a distance of little more than 100 km (60 miles). The north-south scope of the area is no more than 20 km at its widest point, bounded on the north by the Coast Mountains and on the south by the Canada-U.S. border. The greatest concentration of poultry production is contained within 20 km of the city of Abbotsford. This situation has evolved over the last 100 years and has resulted in a number of serious challenges including carrying capacity of the land, urban encroachment, the presence of the major Pacific flyway for wild waterfowl, and, of course, biosecurity.

On February 18, the first case of avian influenza in the 2004 BC outbreak was identified and control measures were activated on February 19. Following depopulation and cleaning and disinfection of the premises, the Emergency Operations Centre was shut down on March 3, 15 days after identification of the index case. The situation was considered to be under control. Six days later, however, a second case was suspected, and confirmed the following day, to be AI on a farm 1.5 km (1 mile) away from the index farm. Emergency operations were recommenced on this confirmation. Thirty-one days after the index case, the third case was identified and by 12 days following that, five commercial premises within the 5 km High Risk Region (HRR) were declared to be positive. At the request of the industry, on April 5, day 48 of the event, the entire Fraser Valley was declared a Control Area. By day 55, 25 commercial flocks were now positive for H7 AI. Emergency activities continued to expand as new High Risk Regions were declared within the Control Area and it was not until May 18, 92 days since the index case was identified, that the 42nd and last premises was detected. Cleaning and disinfection was complete on the last infected premises on June 18, day 123, and repopulation of layer and breeder flocks was allowed to commence on July 9 (day 143). The response effort was officially concluded on August 18, 182 days after the index case and after over 90% of commercial poultry barns in the Fraser Valley were emptied.

POST EVENT REVIEW

In October, 2004, an industry forum was held to review the entire outbreak event. Gathering together leaders from all sectors of the poultry industry, veterinarians, Canadian Food Inspection Agency (CFIA) and British Columbia Ministry of Agriculture and Lands (BCMAL), a detailed assessment was conducted. From that process, a number of gaps were identified and recommendations made to move forward. It was recognized that, at least initially, the response was not well organized and many mistakes occurred, in handling destruction on infected premises. There was no formal industry emergency response plan in place. Biosecurity breaches, both within industry and responder groups, significantly enabled the spread of the disease. Lack of readily available information, especially farm locations and population details, seriously hampered sampling and eradication efforts.

These observations, among others, led to a series of recommendations for action 1.

Recommendations emerging from the event review included measures to address public health, biosecurity, emergency response, and recovery. A list of action items was written with the commitment of all parties to work together to ensure their successful implementation. Among the 15 specific recommendations were the proposals for the industry to lead in implementing mandatory biosecurity standards and an Emergency Response Plan, with technical assistance from government agencies. Within the context of an Emergency Response Plan was the identified need for a premises identification program.

EVOLUTION OF BIOSECRUITY INITIATIVE

The industry group designated to lead the biosecurity initiative was the BC Poultry Association (BCPA), representing the four regulated commodities (hatching egg, chicken, table egg, and turkey) and the non-regulated specialty bird group. The BCPA was the formalized successor to its more loosely structured predecessor, simply referred to as the Poultry Association. The first task of the BCPA was to spearhead the compilation of a biosecurity manual beginning in the fall of 2004. With Dr. Victoria Bowes as lead author, Angela Ryder from industry as editor, and with input from other veterinarians, industry experts, and leading producers, work began on preparing a manual for voluntary compliance by producers. Bound in a red 3-ring binder, the first Biosecurity Manual became commonly known simply as the "Red Binder"2 and was launched in February, 2005. Analysing the various risks encountered during normal farm operation, specific mitigating procedures that would address each risk were listed. For example, vehicles, especially those that visit multiple farms, are recognized as a potential platform for pathogen spread. So, procedures were described that either prevent vehicle entry or require a cleaning and disinfection procedure prior to entry. These intuitive steps expanded into a set of 27 standards. Within the manual was an accompanying self-assessment guide for producers. This allowed each farmer to evaluate his or her own biosecurity practices against the standards and develop a framework for improvement. The Red Binder ultimately served as the framework for the formal BC Poultry Industry Biosecurity Program and its Mandatory Biosecurity Standards.

With the trilateral (industry, provincial government, federal government) agreement to enable the development and delivery of an industry-led biosecurity program, a pool of money was committed by Agriculture and Agrifood Canada (AAFC) and BCMAL to help pay for the program and its delivery. The money was administered through the Investment Agriculture Foundation, a not-for-profit organization that manages and distributes government funds in support of innovative projects that benefit agriculture in BC. While the supply managed system and its regulations allow for enforcement of programs and their auditing for compliance, a system of encouragement was recognized as being more likely to succeed. Consequently, financial assistance was made available, allowing producers to not only comply but also make improvements that they might not otherwise do, provided they related to the basic biosecurity standards or enhanced standards. Additionally, success with their farm plan was recognized through certification that could be promoted by the farm.

The development of a set of mandatory biosecurity standards was the first technical challenge to be tackled. The supply managed system of marketing chicken, turkey, and eggs present in Canada provided the mechanism through which mainstream commercial production could be subjected to mandatory biosecurity standards. Once written into the board orders, biosecurity plans detailing implementation of mandatory standards became a requirement for all supply managed poultry farms. A Biosecurity Committee led by the newly-formed BC Poultry Association was convened to steer the process and, from that, a technical subcommittee struck to develop, organize, and detail the program. Following a selection process from a "Request for Proposals," a contractor was hired to coordinate the writing of the standards and their guidelines, develop the auditing process, and deliver the program.

Development of the Biosecurity Program started in summer of 2005, more than a year after the start of the AI outbreak. The first program goal was to compile a set of practical but effective standards that would be consistent with existing plans, including the On Farm Food Safety Programs and the BC Environmental Farm Plan3 (EFP). The On Farm Food Safety Programs were initiated among the four feather groups – hatching eggs, broiler chicken, table eggs, and turkeys – beginning as a national initiative. The programs were implemented on a province by province basis. Structured on a HACCP model, the food safety programs focus on biosecurity as it relates to the transmission of food-borne contaminants such as *Salmonella*, with little emphasis on bird health. The Environmental Farm Plan concept initiated in 2003 and the program launched to farmers in 2005. Improved land stewardship and environmental sustainability were the ultimate goals of this program. Delivery of the program is through the BC Agriculture Council. Each farm's individual plan was based on a set of auditable standards contained in the EFP. It was the structure of

the EFP upon which the BC Poultry Biosecurity Program was modelled.

From the outset, it was recognized that the mandatory standards to be developed would have to be practical, effective, and auditable. Using the Red Binder as the foundation, its 27 biosecurity standards or procedures were closely reviewed and revised by the Biosecurity Technical Subcommittee. All principles were retained, but some were combined into a single standard, while others became steps needed to accomplish other standards. The final 18 mandatory standards were listed in 2006 (Table 1) and organization into a written manual began in earnest. The 18 standards were grouped into four broad categories: Farm Access (5 standards), Barn Access (4 standards), Flock Health Management (2 standards), and Farm Management (7 standards). Standards covered elements from procedures for entering and leaving a premises or its barns to required flock observations and responses when anomalies were seen. The Biosecurity Standards can be reviewed in detail at the website given in the References 5.

It was recognized during the HPAI 2004 response that the poultry industry had a key collaborative role in an avian Foreign Animal Disease response and this highlighted the need for an industry-specific plan. The Poultry Industry Emergency Response Plan (ERP) 4 was developed in a manner similar to the Biosecurity Program, with an Emergency Response Committee to steer the process and ad hoc subcommittees to deal with specific details. The ERP was designed to be entirely complementary with the Biosecurity Plan. A response triggered by flock health observations, as detailed by procedures outlined in the Biosecurity Plan, would potentially activate the Industry Emergency Response Plan. As a complementary project to the Biosecurity Standards and the Emergency Response Plan, a Premises Identification Program was initiated. The goal of this program was to have the capability of rapidly locating all farms and collate real-time population details to aid any emergency response effort by identifying all farms within a high risk zone and predicting surveillance needs or, in the event of infected premises, for predicting euthanasia and disposal needs for those farms. This program was to be an important part of the Industry Emergency Response Plan.

Interaction of the Biosecurity Program with the Emergency Response Plan was essential, and was covered through prescribed procedures activated under specific circumstances. For example, flock health standards require that a Standard Operating Procedure is in place that prescribes situations in which veterinary or laboratory diagnoses are required. This improves the chances of picking up a serious disease problem early in its course. A self quarantine guideline is provided to assist the producer in following appropriate steps should a serious infectious disease be suspected. The self quarantine procedures outline steps that will increase the ability of the farmer to contain a disease. A farm log is required that tracks all visitors and activity, facilitating any trace-back or trace-forward that might be required for an infectious disease response. These activities would potentially trigger the Industry Emergency Response Plan and activate the first steps required of all industry in the event of an animal disease emergency.

The first edition of the Biosecurity Program was released in early 2007 and consisted of a Planning Guide and a Reference Guide. The actual delivery of the program to the farms was the next challenge. Following the lead of the Environmental Farm Plan, a team of planning advisors and auditors was trained in biosecurity in general and the Biosecurity Standards in particular. Each farmer was responsible for developing their own farm's specific plan and the planning advisor's role was to meet personally with farmers and assist in its development. It was the farmer's responsibility to implement the plan. Once the plan was in place and the farmer was ready, an audit was requested. An auditor attended the farm and reviewed the producer's plan, checking for the specific critical points to assure that compliance was realized. The farmer was left with a checklist that detailed the audit results and the elements that required corrective action. Identified deficiencies were corrected by the farmer prior to a follow-up audit. Once complete to the satisfaction of the auditor, the farm was certified under the BC Poultry Industry Biosecurity Program.

The goal was to have the plan rolled out to all farms by September 30, 2008, first audits complete by October 31, 2008, and certification complete on all farms by December 30, 2008. The plan has now been successfully implemented on 99% of the regulated poultry farms in British Columbia. Only a handful of farms having difficulty meeting some standards or refusing to cooperate remain to be certified.

CHALLENGES

Many challenges were encountered throughout the life of the Biosecurity Program. Most were resolved, though not necessarily to everyone's satisfaction. Some, however, remain contentious. A number of identified weaknesses among contemporary practices meant that some cultural changes were required. Among these were details such as increased documentation, tighter control over people entering the premises, and prescribed procedures for entering a premises and its barns.

There was a certain amount of industry push-back on the whole process, with most producers feeling that

they had sufficient biosecurity to keep themselves from risk. The most acute division was between those producers in the zone that was depopulated during AI 2004 and those outside the zone. The trauma of experiencing depopulation was apparently a major motivator for the implementation of biosecurity. Educating farmers about the benefits of biosecurity when they have not, in fact, experienced a significant disease event is a difficult but important part of implementing an industry-wide program.

One of the greatest challenges met during the development of the standards was the issue of wording. While auditable standards were required, the need for flexibility was highlighted by the variation in farm types, not just among commodity groups but also among farms within each commodity group. This resulted in a great deal of time spent debating over the wording of standards and requirements to meet those standards. As a simple example, appropriate use of the words "will," "must," or "should" was argued extensively. To deal with this problem at least to some degree requires that the technical committee has a good understanding of the structure and variation within the industry for which the program is being designed. A "cookie-cutter" approach will not work and a program for one area cannot be easily fit into another unrelated area.

To help deal with the issues of variance from a fixed standard, a risk analysis procedure was put into place. This procedure allowed for a variance from the standard provided a valid reason was given and a plan was provided to reduce the risk that might be associated with the variance. The requirement for gates to be closed was a hotly contested standard; while people, equipment, and vehicles were recognized as important vectors for disease agents, the standard requiring gates to be kept closed was challenged on many fronts. Several good arguments were brought forward in an attempt to soften this standard, including matters of safety and practicality. The risk analysis procedure helped to answer some of these issues on a case-by-case basis, but the controversy still rages.

The use of an anteroom as a transition area from the Controlled Access Zone to the Restricted Access Zone (the bird housing area) created significant controversy as this meant a major cultural change for some producers. Funding was available to assist in making changes where required in certain circumstances and, eventually, the matter was resolved. This, however, did not occur without some foot-dragging in some quarters.

While the conventional poultry sectors in BC was well engaged in the biosecurity process, there was a large fifth player that could not be as readily included – the unregulated specialty bird producers. This group included principally producers of waterfowl and game birds. Because they did not fall under a supply managed system, there was no way for costs to be built in to their prices to account for the changes that would be required to bring their farms into compliance. Many of such producers viewed the efforts that would be required by them to be of benefit only to the conventional poultry sectors. Furthermore, without oversight by any board, there was no way that auditable standards could be enforced. The specialty bird sector remains to be brought under the biosecurity umbrella, and solutions to some of the issues are still being sought.

No plan, particularly one promoted by government or regulatory agencies, is without its fierce opponents. A handful of producers resisted the implementation of a plan and auditing of their premises. These holdouts eventually saw value in their participation in the program when it became clear that penalties in the form of lost quota would be dealt to those that were not certified by the deadline of December 31, 2008.

CONCLUSION

While most farmers would view anything that is mandatory is an evil thing, the necessity of mandatory standards was recognized after the costly avian influenza outbreak of 2004. Once that recognition was clear, the road to a concrete biosecurity plan was open, but it was definitely not without its bumps and detours. After much debate and many challenges, the BC Poultry Biosecurity Program became a reality and raised the bar significantly for a very vulnerable industry. With this plan in place, poultry production in BC should be just a bit more biosecure.

REFERENCES

1. Report on the Canadian Poultry Industry Forum, http://www.bcac.bc.ca/documents/CPIF-Dec15withlinks.pdf.
2. The BC Poultry Industry Biosecurity Initiative, http://www.bcac.bc.ca/documents/BC%20Poultry%20Association%20Biosecurity%20Initiative%20Version%201.0%2005.02.16.pdf.
3. The Canada – British Columbia Environmental Farm Plan Program, http://www.bcac.bc.ca/EFP_pages/documents/index.html.
4. The BC Poultry Industry Emergency Response Plan, http://www.bcac.bc.ca/bio_emergency.htm.
5. The BC Poultry Biosecurity Program, http://www.bcac.bc.ca/bio_program.htm.

Table 1. The BC Poultry Industry Biosecurity Standards.

Standard #	Standard
Farm Access Standards	
1	A secure barrier that restricts vehicle entry must be present at all primary and secondary accesses to the Controlled Access Zone.
2	Approved biosecurity signage must be clearly displayed at all primary and secondary accesses.
3	All primary accesses to the Controlled Access Zone must be constructed of hard surface or gravel that prevents any persistent accumulation of pooled water.
4	All primary accesses to the Controlled Access Zone must have an approved Cleaning and Decontamination site for vehicles and personnel.
5	The Controlled Access Zone must be maintained clean and free of organic debris at all times.
Barn Access Standards	
6	All poultry barn entrances shall remain locked at all times that the barn is unoccupied by farm personnel.
7	Approved restricted access signs shall be posted at all barn entrances.
8	All poultry barns must have an anteroom at all primary entrances that allow personnel to comply with the farm biosecurity procedures during entry and exit.
9	Barn entryways and anterooms must be maintained clean and free of debris at all times.
Flock Health Management Standards	
10	Individual flock health records must be maintained
11	Poultry mortalities and cull eggs must be handled and disposed of in an approved manner.
Farm Management Standards	
12	An effective pest control program must be in place.
13	A management program that prevents the contamination of feed and water sources must be in place.
14	All equipment and materials related to the production of poultry that enter or leave the Controlled Access Zone, regardless of size or use, must be clean and decontaminated.
15	All farms must have a documented manure management strategy.
16	On-farm biosecurity training is required for all producers and farm employees.
17	Standard operating procedures (SOP) for on-farm biosecurity must be available.
18	An activity log book for the premises that records visitors and daily on-farm activities relevant to the biosecurity standard operating procedures must be maintained.

ISOLATION AND DISTRIBUTION OF WEST NILE VIRUS IN EMBRYONATED CHICKEN EGGS

R. Crespo, H.L. Shivaprasad, and P.R. Woolcock

California Animal Health and Food Safety Laboratory System, University of California Davis, Fresno Branch, 2789 South Orange Avenue, Fresno, CA 93725, USA

Cell culture followed by indirect immuno-florescent antibody staining, reverse transcription-polymerase chain reaction (RT-PCR), or *in situ* enzyme immunoassay (EIA) are the only methods described in the literature for the isolation and identification of West Nile virus (WNV). RT-PCR directly from the tissue is the preferred method for WNV detection, because of the rapid turnaround time and lower biosafety risks. However, virus isolation may be requested as other viruses can be detected as well, including newly emerging viruses. Furthermore, many avian laboratories maintain a limited number of cell lines and most isolations are performed by inoculation of embryonated chicken eggs.

Eight psittacines (seven Rosellas, one Princess of Wales Parakeet) and one Red tail hawk submitted between 2004-08 to the California Animal Health and Food Safety Laboratory System were included in this study. The birds died suddenly or after a brief onset of loss of weight and occasionally neurologic signs. In one of the rosellas, Pacheco's disease was suspected. Histologically all birds had multifocal necrosis and lymphocytic infiltration in most organs, including heart, kidney, and liver. The red tail hawk also had non-suppurative perivascular cuffing of the brain.

Tissue pool consisting of heart, kidney and or liver alone were homogenized and inoculated into embryonating chicken eggs via the chorioallantoic membrane, chorioallantoic sac, and yolk sac. Virus particles in the range of 40 to 45 nm suggestive of flavivirus were identified from the allantoic fluid by negative staining electron microscopy, in all cases except in one Rosella. From this Rosella the liver inoculated into choriollantoic membrane was positive for WNV by RT-PCR. From all the cases, sections of embryo including various organs, their chorioallantoic membrane, and yolk sac were collected and processed for immunohistochemistry (IHC) for WNV. The tissues showing the most intense and widely distributed IHC staining was the chorioallantoic membrane (CAM). Furthermore, the CAM was more intensively stained if the first passage had been through the chorioallantoic sac (CAS). No virus could be detected in the yolk sac by IHC. In the embryo, the WNV antigen was best detected in the muscles (both skeletal and smooth), followed by skin. Additionally, there were a few positive foci in the turbinates. WNV was not detected in the viscera of the embryo.

In conclusion, this study demonstrates that WNV may be isolated through inoculation of embryonated chicken egg embryos as occurs with other arboviruses. The best route of inoculation is CAS, and the virus can be best demonstrated by IHC in the CAM.

(The full-length article will be published in the *Journal of Veterinary Diagnostic Investigation*.)

ENTERIC VIRUS STATUS OF TURKEY FLOCKS OVER TIME: MOLECULAR DIAGNOSTIC STUDIES BEGINNING ON THE DAY OF PLACEMENT

J. Michael Day[A], Laszlo Zsak[A], and H. John Barnes[B]

[A]USDA/ARS Southeast Poultry Research Laboratory, 934 College Station Road, Athens, GA 30605
[B]College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606

Despite considerable evidence of the presence of certain enteric viruses in the poultry population in the United States (4,8), little is known concerning the etiology of the recurring enteric disease syndromes such as poult enteritis complex (PEC) in young turkeys and runting-stunting syndrome in broiler chickens (RSS). There is evidence that the age at virus exposure may play a part in enteric disease progression and severity in turkey poults (2,12,13). Analysis of intestinal contents from flocks showing signs of enteric disease—and from otherwise healthy flocks—often reveals infections with numerous suspect viruses. The complex nature of these enteric syndromes makes laboratory diagnosis and field management of affected flocks difficult. Recent regional and national enteric virus surveys of commercial turkey and chicken flocks has revealed the ongoing presence of avian reoviruses, rotaviruses, astroviruses and parvoviruses in flocks, with the viruses often detected in combination (4,8). The turkey astroviruses, particularly turkey astrovirus type 2, appear to be ubiquitous in U.S. flocks, where they may affect the performance of commercial turkeys (5,9). Several turkey-origin reoviruses have recently been isolated and described at the molecular level and in pathogenesis studies, but they do not appear to be the sole causative agent in syndromes such as PEC and PEMS (1-3,7,10,11). A recent RT-PCR test targeting the NSP4 gene of turkey-origin rotavirus has revealed the presence of rotavirus in numerous field samples, including in poults prior to placement on farms (8). Avian parvovirus has also recently been described from turkeys and chickens, and its role in poultry enteric disease is currently being investigated (14). For the present study, we utilized molecular tests for several enteric viruses to assess the colonization of six turkey flocks over time. One of these flocks was placed at a Teaching Animal Unit (TAU) farm at North Carolina State University (NCSU), which was monitored for enteric viruses from the day of placement and periodically thereafter. The remaining five flocks were commercial turkey flocks placed with a turkey integrator in North Carolina, USA, one of which was a "sister flock" to the TAU flock, with poults from the same hatching being placed at the TAU farm and the commercial farm housing the sister flock.

During the early fall of 2007, in cooperation with poultry industry representatives and university researchers, we received intestinal and composite fecal samples collected from commercial and university "teaching" turkey flocks in North Carolina, USA. The barn housing the poults at the NCSU TAU had undergone a complete barn cleaning and three months of down time prior to placing the poults on fresh litter. Over the course of five weeks, beginning the week of placement, entire intestinal tracts were collected weekly from five poults on each of five commercial farms and shipped overnight on ice packs to Southeast Poultry Research Laboratory (SEPRL) in Athens, GA. Further, composite fecal samples were collected from the day of placement through day 63 post-placement from the TAU flock at NCSU. The composite fecal samples were collected every three days except for days 12 through 21, when daily composite samples were collected. The fecal samples were shipped in batches on wet ice to SEPRL. Upon receipt at SEPRL, all samples were held at 4°C and processed within 24 hours. Intestinal and fecal samples were processed into 10% homogenates in sterile phosphate buffered saline, clarified via centrifugation, and total RNA was extracted using TRIZOL reagent (Invitrogen) and the MagMax RNA extraction kit (Ambion), and total DNA was extracted using the DNeasy blood and tissue kit (Qiagen). Samples were tested via RT-PCR or PCR for the presence of the avian astrovirus polymerase (Pol) gene, avian rotavirus NSP4 gene (viral enterotoxin) the avian reovirus σNS gene, and avian parvovirus NS gene (4,8,14).

All five flocks placed on the commercial farms were positive via RT-PCR for avian astrovirus during the first week after placement, and three of the five flocks remained positive for avian astrovirus through the fifth week after placement. Avian astrovirus was not detected in the TAU flock until day 31 following placement, and was detected intermittently through day 52 following placement. Sequencing of selected amplicons revealed the presence of turkey astrovirus type 2 in all cases (9). Avian rotavirus was detected in all flocks during the sampling period, with three of the five commercial flocks testing positive for rotavirus during the first week following placement, including

the sister flock. The TAU flock tested positive for rotavirus on day 17 following placement and intermittently through day 63 following placement. Parvovirus was detected in all of the commercial flocks by the fourth week following placement, with one commercial flock testing positive during the second week. Turkey-origin avian reovirus was detected intermittently in four of the five commercial flocks and only once, on day 14 following placement, in the TAU flock. Observations of flock performance taken in the field noted non-specific poult enteric disease in three of the five commercial flocks by day ten following placement; one of the commercial flocks with observed enteric disease was the sister flock to the TAU flock. The observation of enteric disease in the commercial flocks correlated with the detection of rotavirus in those flocks in the first week following placement. No enteric disease was noted in the TAU flock during the sampling period. The poults that were placed at the TAU facility and in the commercial sister flock were also colonized with *Escherichia coli* prior to placement.

The results of this study are generally consistent with other, earlier longitudinal studies which have followed the enteric virus status of poultry, in that the simple presence of enteric viruses alone or in combination is not necessarily correlated with the onset or eventual occurrence of enteric disease. Interestingly, the detection of avian rotavirus during the first week following placement in the commercially-reared poults did correlate with a field diagnosis of enteric disease in those flocks at ten days. Poults from the same hatching did not develop enteric disease during grow out on the TAU farm, despite the appearance of rotavirus at day 17 following placement. The early appearance of astrovirus in all of the commercial flocks and the eventual appearance of parvovirus in all flocks did not correlate with the diagnosis of enteric disease. Turkey astrovirus can cause enteric signs in young poults and can have an effect on poult body weights (6). Little is known about the pathogenesis of parvovirus in turkeys. The intermittent detection of turkey-origin avian reovirus did not correlate with enteric disease. The turkey-origin avian reoviruses probably do not play a major role in enteric disease signs, although they can result in immune dysfunction in young poults (2,7,11). Further research should concentrate on the role of avian rotavirus in turkeys, particularly in birds concomitantly infected with bacteria. This study also suggests that management techniques and farm location may play significant roles in the development of enteric disease in turkeys infected with combinations of enteric viruses.

REFERENCES

1. Day, J., M. Pantin-Jackwood, and E. Spackman. Sequence and phylogenetic analysis of the S1 genome segment of turkey-origin reoviruses. Virus Genes. 2007.

2. Day, J.M., E. Spackman, and M. Pantin-Jackwood. Turkey origin reovirus induced immune dysfunction in specific pathogen free and commercial turkey poults. Avian Dis 52:387-391. 2008.

3. Kapczynski, D.R., H.S. Sellers, V. Simmons, and S. Schultz-Cherry. Sequence analysis of the S3 gene from a turkey reovirus. Virus Genes 25:95-100. 2002.

4. Pantin-Jackwood, M., J.M. Day, M.W. Jackwood, and E. Spackman. Enteric viruses detected by molecular methods in commercial chicken and turkey flocks in the United States between 2005 and 2006. Avian Dis 52:235-244. 2008.

5. Pantin-Jackwood, M., E. Spackman, and P. Woolcock. Phylogenetic Analysis of Turkey Astroviruses Reveals Evidence of Recombination. Virus Genes 32:187-192. 2006.

6. Pantin-Jackwood, M.J., E. Spackman, and J.M. Day. Pathogenesis of type 2 turkey astroviruses with variant capsid genes in 2-day-old specific pathogen free poults. Avian Pathol 37:193-201. 2008.

7. Pantin-Jackwood, M.J., E. Spackman, and J.M. Day. Pathology and virus tissue distribution of Turkey origin reoviruses in experimentally infected Turkey poults. Vet Pathol 44:185-195. 2007.

8. Pantin-Jackwood, M.J., E. Spackman, J.M. Day, and D. Rives. Periodic monitoring of commercial turkeys for enteric viruses indicates continuous presence of astrovirus and rotavirus on the farms. Avian Dis 51:674-680. 2007.

9. Pantin-Jackwood, M.J., E. Spackman, and P.R. Woolcock. Molecular characterization and typing of chicken and turkey astroviruses circulating in the United States: implications for diagnostics. Avian Dis 50:397-404. 2006.

10. Sellers, H.S., E.G. Linnemann, L. Pereira, and D.R. Kapczynski. Phylogenetic analysis of the sigma 2 protein gene of turkey reoviruses. Avian Dis 48:651-657. 2004.

11. Spackman, E., M. Pantin-Jackwood, J. Day, and H. Sellers. The pathogenesis of turkey origin reoviruses in turkeys and chickens. Avian Pathology 34:291-296. 2005.

12. Yason, C.V., and K.A. Schat. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: clinical signs and virology. Am J Vet Res 48:977-983. 1987.

13. Yason, C.V., B.A. Summers, and K.A. Schat. Pathogenesis of rotavirus infection in various age

groups of chickens and turkeys: pathology. Am J Vet Res 48:927-938. 1987.

14. Zsak, L., K.O. Strother, and J. Kisary. Partial genome sequence analysis of parvoviruses associated with enteric disease in poultry. Avian Pathol 37. 2008.

EXAMINATION OF *CAMPYLOBACTER JEJUNI* PUTATIVE ADHESINS IN HOST CELL BINDING AND CHICKEN COLONIZATION

A. Singh Dhillon and Michael E. Konkel

Washington State University, Avian Health and Food Safety Laboratory, 2607 W. Pioneer, Puyallup, WA 98371

Campylobacter jejuni colonization of chickens is dependent upon surface exposed proteins termed adhesins. Putative *C. jejuni* adhesins include CadF, CapA, JlpA, MOMP, PEB1, Cj1279c, and Cj1349c. We examined the genetic relatedness of ninety-seven *C. jejuni* isolates recovered from human, poultry, bovine, swine, ovine, and canine sources by multilocus sequence typing (MLST) and examined their profile of putative adhesin-encoding genes by dot blot hybridization. To assess the individual contribution of each protein in bacteria-host cell adherence, the *C. jejuni* genes encoding the putative adhesins were disrupted by insertional mutagenesis. The phenotype of each mutant was judged by performing *in vitro* cell adherence assays with chicken LMH hepatocellular carcinoma epithelial cells and *in vivo* colonization assays with broiler chicks. MLST analysis indicated that the *C. jejuni* isolates utilized in this study were genetically diverse. Dot blot hybridization revealed that the *C. jejuni* genes encoding the putative adhesins, with the exception of *capA*, were conserved among isolates. The *C. jejuni* CadF, CapA, Cj1279c, and Cj1349c proteins were found to play a significant role in the bacterium's *in vitro* adherence to chicken epithelial cells, while CadF, PEB1, and Cj1279c were determined to play a significant role in the bacterium's *in vivo* colonization of broiler chicks. Because Cj1279c promotes the binding of *C. jejuni* to host cells, plays a significant role in *C. jejuni* colonization of chickens, and harbors fibronectin Type III domains, we have termed the product encoded by the *Cj1279c* gene FlpA for Fibronectin-like protein A.

MECHANISMS OF INTESTINAL BARRIER FAILURE IN SUBCLINICAL ENTERITIS

J.J. Dibner, M.A. Quiroz, and C.D. Knight

Novus International, Inc., 20 Research Park Drive, Missouri Research Park, St. Charles, Missouri 63304

SUMMARY

One common consequence of enteritis, regardless of its cause, is a breakdown in the barrier function that normally protects the animal against invasion by commensal and pathogenic gut microbiota. The objective of this review is to describe the structure and function of the intestinal barrier that prevents invasion of the host, to summarize the evidence that loss of barrier function accompanies oxidative stress associated with enteritis, and to discuss possible consequences of the resulting bacterial translocation.

INTRODUCTION

Subclinical enteritis is an increasing problem in the poultry industry. It can be caused by a number of factors, including any of a variety of relatively mild parasitic, bacterial and viral infections as well as transient toxic challenges (1). As the name implies, it rarely causes death but can be associated with poor feed digestibility and absorption, resulting in poor performance. It is also becoming clear that activation of the innate immune system and the associated generation of reactive oxygen metabolites (ROM) plays a role in the normal host response to its microbiota (2) and is essential in the development of homeostatic balance between the host and its microbiota early in life. It has been suggested that the ROM response can exceed the host's ability to control it, resulting in oxidative stress that itself can become a pathogenic factor in the further development of chronic intestinal inflammation (3).

The gastrointestinal tract is the primary site of entry for any orally administered compound, including dietary ingredients. The functions of this organ system include digestion, absorption, and protection, and the structure of the gut is well adapted to perform these functions. The mucosa of the gut is the first tissue to encounter dietary ingredients and contaminants, and studies of its macroscopic and microscopic structure have been used to clarify the initial response of the animal to these materials. Research has demonstrated the enormous changes in the structure and function of this organ system during the first week after hatching and in its adaptation to environmental and dietary changes later in life (4). Results suggest that the more mature gut is still responsive to microbial changes, environmental conditions, toxins and dietary factors, and that the initial response to these various events is general subclinical enteritis, i.e. intestinal inflammation. Thus, studying the consequences of intestinal inflammation is to some extent independent of the causative factors. This review will focus on barrier failure as a general effect of enteritis itself rather than the factors that can initiate intestinal inflammation.

OXIDATIVE STRESS

Animal nutritionists typically include antioxidants in their finished feed formulations to prevent oxidative damage to the fat portion of the feed, including fat soluble vitamins and pigments. Controlling oxidation in the feed is important, but it should be recognized that there are endogenous sources of ROM. First, the active metabolism of gut epithelium is itself a source of ROM, associated with activity of the electron transport chain (5). The reactive species produced include the superoxide anion (O_2^-), and hydrogen peroxide (H_2O_2). These are considered to be an inevitable result of oxidative phosphorylation by mitochondria (6). Another endogenous source of oxidative stress includes the nitric oxide (NO) generated by the gut innate and acquired immune systems as they react to the numerous commensal and pathological microbial species that are inevitably introduced during ingestion of feed and water.

Cells are protected from damage by these ROM through the action of endogenous antioxidant defenses such as mitochondrial-Mn-dependent superoxide dismutase, Cu-Zn SOD and glutathione (3). These systems can be overwhelmed, however, if the oxidant stress and antioxidant capacity become unbalanced, and under such conditions tissue damage can be extensive (7). If O_2^-, H_2O_2, and NO are not rapidly detoxified they can generate more damaging free radicals such as the hydroxyl radical (·OH). This species is more dangerous because there is no enzymatic path to detoxify it. Oxidative stressors are additive, therefore ingestion of oxidized feed ingredients can tip the oxidative balance in the intestine. It is clear then that supplemental antioxidants preserve the feed but can also reduce the damage associated with constitutive and metabolic sources of ROM.

INTESTINAL BARRIER STRUCTURE AND FUNCTION

The gut provides a barrier to the invasion of the commensal and opportunistic microflora. The physical barrier consists of mucin overlying the gut epithelium, the epithelial cells themselves with their impermeable cell to cell tight junctions and toll-like receptors (TLR), intraepithelial leukocytes, and the basal lamina. For the dissemination of bacteria to other organs, these barriers, as well as those represented by the innate and adaptive immune cells of the lamina propria must be overcome.

The first structural layer of the barrier is extracellular mucin. Mucin is a mixture of high molecular weight proteoglycans that forms a continuous layer overlying and connected to the gut epithelial cells. The role of the mucin in barrier preservation is to restrict interaction between the microbiota and the gut epithelium itself, reducing the likelihood of attachment and subsequent activation of the innate immune response mediated by the TLR of the gut epithelium (8).

Figure 1 shows another component of the barrier, the gut epithelial cells. This epithelium consists of a single layer of columnar epithelial cells. These cells are tightly adherent to one another by virtue of cell to cell junctions at the apical and basolateral regions. The junctions consist of several parts: the apical tight junction (zonula occludens) is the most important in regulating paracellular permeability. Tight junctions consist of a continuous band of branching cytoskeletal proteins that form a perijunctional actin ring of integral membrane proteins (9). The function of these continuous interepithelial tight junctions is to control the movement of water, solutes, and electrolytes, in addition to members of the microbiota.

Oxidative stress in the GI system, regardless of cause, is associated with loss of barrier function. This can be demonstrated using inhibitors of inducible nitric oxide synthase (10). The mechanisms responsible for this loss of barrier function may include denaturation of the protein component of the enterocyte junctional complexes (11) resulting in failure of both cell-cell adhesion and apical membrane integrity (11). The importance of the association of barrier failure and oxidative stress in the gut is that it could be a cause of dissemination of CP or other opportunistic pathogens into other organs that does not necessarily require pathogen invasion or toxic damage to occur. Other contributors to oxidative stress, including oxidized dietary ingredients could also be involved. Thus, the maintenance of balance between total oxidative stress in the gut and total antioxidant capacity can be augmented with the use of dietary antioxidants. Because enteritis and barrier failure result in bad performance, lack of skin pigmentation and has the potential of killing the animal, the relationship of them to oxidative stress is an important additional reason to include dietary antioxidants in all diets, even those supplemented with fresh fat.

CONCLUSIONS

Barrier function failure has numerous causes, but oxidative stress should not be one of them. There are two very important ways to reduce the likelihood of systemic disease associated with bacterial translocation. First is the control of gut oxidative stress. The association between it and barrier failure means that antioxidant supplementation should always be a part of the formulation, even in diets supplemented with stabilized or fresh fat sources. The second way is to reduce the likelihood that a barrier failure will result in translocation of such potentially deadly anaerobes as CP. This means that homeostasis of the gut microbial populations needs to be protected. Addition of organic acids or enzymes that improve nutrient availability can have benefits beyond performance; it can reduce the acid-sensitive CP population, reducing the likelihood that it will be disseminated by gut barrier failure from any cause, including subclinical enteritis.

REFERENCES

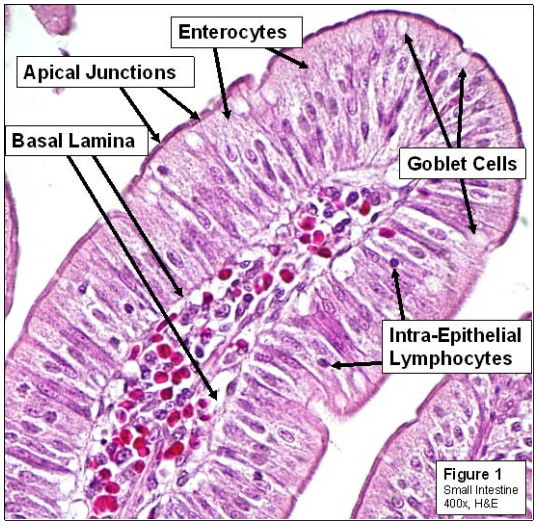
1. Hoerr, F. Pathogenesis of enteric diseases. Poult. Sci. 77: 1150-1155. 1998.
2. Rumbo, M., and Schiffrin, E. Ontogeny of intestinal epithelium immune functions: Developmental and environmental regulation. Cell. Mol. Life Sci. 62: 1288-1296. 2005.
3. Aw, T. Molecular and cellular responses to oxidative stress and changes in oxidation-reduction imbalance in the intestine. Am. J. Clin. Nut. 70: 557-565. 1999.
4. Dibner, J., and Knight, C. Early feeding and nutritional programming in hatchling poultry. In: Proceedings Arkansas Nutrition Conference, pp. 1-9. Fayetteville, AK. 2001.
5. Ojano-Dirain, C., Tinsley, N., Wing, T., Cooper, M., and Bottje, W. Membrane potential and H_2O_2 production in duodenal mitochondria from broiler chickens with low and high feed efficiency. Comp. Biochem. Physiol. Part A. 147: 934-941. 2007.
6. Chance, B., Sies, H., and Boveris, A. Hydroxide metabolism in mammalian organs. Physiol. Rev. 59: 527-609. 1979.
7. Weiss, S. Tissue destruction by neutrophils. N. Engl. J. Med. 3220: 365-376. 1989.
8. Swank, G., and Deitch, E. Role of the gut in multiple organ failure: Bacterial translocation and permeability changes. World J. Surg. 20: 411-417. 1996.
9. Gonzalez-Mariscal, L., Betanzos, A., and Avila-Flores, A. MAGUK proteins: Structure and role in

the tight junction. Seminars Cell Devel. Biol. 11: 315-324. 2000.

10. Wingler, K., Muller, C., Schmehl, K., Florian, S., and Brigelius-Flohe, R. Gastrointestinal glutathione peroxidase prevents transport of lipid hydroperoxides in CaCo-2 cells. Gastroenterology. 119: 420-430. 2000.

11. Blikslager, A., Moeser, A., Gookin, J., Jones, S., and Odle, J. Restoration of barrier function in injured intestinal mucosa. Physiol. Rev. 87: 545-564. 2007.

Figure 1.



INTERESTING DIAGNOSTIC CASES FROM PENNSYLVANIA

P. A. Dunn[A], E. A. Wallner-Pendleton[A], S. E. Myers[A], S. Kariyawasam[A], H. Lu[A], D. J. Hampson[B], A. Murdoch[C] , and C. DebRoy[A]

[A]Animal Diagnostic Laboratory, Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA 16802
[B]Murdoch University Veterinary School, Murdoch, WA, Australia
[C]Spirogene Pty Ltd, Murdoch University, Murdoch, WA, Australia

Descriptions of several cases of different poultry species and production types submitted to the Pennsylvania State University Animal Diagnostic Laboratory of the Pennsylvania Animal Diagnostic Laboratory System (PADLS) will be presented.

CASE REPORTS

Broiler: A series of clinically similar cases were submitted from one broiler company during the fall and winter of 2008. The company consists of feed mill and grow-out operations, but does not include breeder, hatchery or processing plant facilities. Most chicks are from one hatchery source. Part of the production is raised without antibiotics and part is on conventional feed and medication programs, with a different genetic strain of bird for each. The common complaint was increased culls due to lameness. Typically the lameness was first noticed in a small percentage of the birds at 14 to 16 days, and weight loss, stunting and dehydration of affected birds would follow. Low level incidence would continue throughout the grow-out, even with consistent culling. Poor culling efforts in some flocks resulted in large numbers of birds left by the catch crew. In one example, ~400 birds in a ~30,000 bird flock were left in the house to be killed by the grower. Flock livability rates were typically decreased by 2 to 4% when compared to normal baselines for this company. Performance at the processing plant was not reported as significantly different in most cases. Flocks in both antibiotic free (abf) and conventional programs were affected. *In ovo* administration of antibiotics at the hatchery was used in the conventional program, but had recently been discontinued in the abf program. Progeny from one breeder flock raised on the abf program were more often affected, but there was no specific breeder flock association identified for the other strain on the conventional program. The occurrence of the problem in a flock did not appear to correlate with first week mortality rates, type of house, litter source, feed, lights, geography, flock supervisor, feed deliveries or other traffic. Routine flock monitoring during this time period for "gut health" and bursa scores revealed adequate (better than usual) coccidiosis control and normal bursas.

Bird ages in the submitted cases ranged from 18 to 42 days. The most consistent lesions were osteochondritis, most often in the proximal tibiotarsus, and synovitis of various leg joints. Other lesions included pericarditis, splenomegaly, and hepatomegaly. The most consistent bacteria isolated by routine culture of swabs from the lesions (bone, joint, spleen, liver, pericardium/heart) were gram positive cocci that were positive for the bile esculin reaction, but negative for growth in the presence of 6.5% sodium chloride. In every case, the isolates were identified as *Streptococcus suis* by the automated identification system routinely used in our laboratory. However, results of DNA sequencing of the 16S ribosomal RNA gene of six representative isolates matched 97 to 99% with *Enterococcus cecorum*. The results showed that the isolates were not related to *Streptococcus* spp. including *Streptococcus suis*. Attempts at isolating this bacterium from different sources/inputs for this company, including one- and two-day-old chicks (yolk sac, liver, pericardium, joint, brain), litter, feed truck, and feed, were negative. During the course of this investigation in 2008, *Enterococcus cecorum* infection was confirmed by culture in submissions from seven different farms, and was suspected in seven others. Retrospectively, "*Streptococcus suis*" was isolated from bone, joint, liver, and heart from two similar cases from this company in 2005. Two of the involved growers (one from a 2005 case and one from a 2008 case) also raise swine, and the initial identification of the bacterium led to speculation that transmission of a specific swine pathogen to chickens may have occurred. Identification at the genetic level suggests otherwise. An underlying cause (change in bacteria, host or environment) for this problem has not been determined.

Broiler Breeder: Cases from three broiler breeder flocks of 22 to 28 weeks of age from one company were submitted. Increased culls that were underweight and not maturing into breeding condition compared to flock mates were evident within a few weeks after placement in the breeder houses. Both females and males were affected. The manager

suspected that some of the affected birds were blind. Gross eye lesions consisting of bilateral cloudy areas centrally and/or at the margins of the iris were present in several birds, and bilateral buphthalmos was present in a few. Corneal ulcers were not present. Cataracts and anterior uveitis of variable severity were confirmed microscopically as the main lesions. After affected breeders were culled, the flocks achieved acceptable production statistics for the rest of the lay period. The problem was not seen in any other breeder flocks in this company.

History revealed that these birds were from pullet/cockerel flocks in which a confirmed diagnosis of hypoglycemia-spiking mortality syndrome (HSMS) was made at about nine to 14 days of age, and that other causes of chick mortality and neurologic disease had been ruled out at that time. Although cataract formation has not previously been reported as a sequela to HSMS in chickens, both hypoglycemia and hyperglycemia are known initiators of lenticular degeneration in other species. Most confirmed cases of HSMS have been in broilers that have insufficient life span for detection of slowly developing lesions.

Layer: Several cases of layers were submitted for increased numbers of eggs with stained shells, wet droppings, soiled vents, and in a few instances, mildly decreased egg production. In the most extreme case, 6 to 8% of the eggs were sorted out as "dirties." A large layer company reported consistent economic losses from downgraded eggs partly due to "dirties." For example, a 1.2 million bird complex had 3 to 4% stained eggs compared to 1% or less on other farms. At the time, this represented a loss of $0.30 per dozen or $5500 per week on this complex. In most of the cases, feed, water, equipment and management factors had been ruled out as contributors. The most common finding was detection of spirochete-type bacteria in the cecal mucosa, and lack of detection of other enteric and urinary tract pathogens. PCR tests on some of these samples most often identified the spirochete as *Brachyspira intermedia* (*BI*). In a few cases, a presumptive identification of *Brachyspira pilosicoli* (*BP*) was made by histopathology based on the colonization pattern considered typical for this species (mats of spirochetes aligned in parallel to each other and perpendicular to the cecal mucosal epithelium). Small challenge studies in our lab with a *BI* isolate from a field case were not successful at reproducing the condition reported in the field, but bird numbers were quite low and other confounders were present.

In the summer and fall of 2008, in collaboration with veterinary scientists in Australia, a survey was done to determine the presence of certain *Brachypira* spp. in Pennsylvania (PA) layer flocks and possible correlation with production problems. A series of fecal sample collections and flock manager questionnaires from 20 different commercial layer flocks were completed. Flock ages ranged from 46 weeks to two years. A variety of management styles, housing, breeds, feeds, antibiotic usage, flock sizes and locations were represented. Fifty fresh fecal samples from each flock were tested by PCR for *BI*, *BP*, and, in some cases, other *Brachyspira* spp. Preliminary data on *BI* and *BP* show that 13 flocks were positive for *BI* only (in 10 to 100% of samples), one flock was positive for *BP* only (64% of samples), four flocks were positive for both *BI* (58 – 100% of samples) and *BP* (8- 82% of samples), and two flocks were negative for both. Of the 11 flocks for which the managers noted increased dirty eggs on the survey, nine had *BI* (in 70% or more of samples) and no *BP*, one had *BP* (in 64% of samples) and no *BI*, and one had neither. Of the seven flocks for which the managers reported antibiotic use within three months prior to the sampling date, six were positive for *BI* and negative for *BP*, and one was positive for both. The results show high rates of infection by two potentially pathogenic intestinal spirochetes in older layer flocks in our state. More studies are needed in North America to further define epidemiology and possible clinical, subclinical and economic significance in poultry.

Layer Breeder: A flock of 7,000 57-week-old leghorn breeders was visited in response to a complaint of a sudden onset of depression, respiratory signs, swollen eyes and face, decreased feed and water consumption and decreased egg production. Inflamed nasal passages and infraorbital sinuses (unilateral and bilateral) containing copious turbid mucoid to caseous exudates were present in most birds examined, and caseous peritonitis was also present in several hens. *Avibacterium paragallinarum* (serovar C, Modesto strain) was isolated from sinus cultures, and *E. coli* was isolated from most peritoneal/air sac swabs. Infectious bronchitis virus (IBV) (Mass serotype) was isolated from pooled cecal tonsils, but no IBV was recovered from pooled tracheas. Avian influenza, avian paramyxovirus infection, infectious laryngotracheitis, and mycoplasmosis were ruled out.

Additional history revealed that the problem began two days after many of the males, that had been moved off site for about one week, were reintroduced into the flock. Over the ~ 10 day clinical course, morbidity was nearly 100%, mortality was ~10%, and egg production decreased by ~75%. The flock was buried on the farm at 58 weeks. No other flocks in the vicinity were affected. Infectious coryza had not been diagnosed in a commercial flock in PA for many years, and the specific source of the infection could not be identified in this case.

Turkey: A series of cases with common findings have been submitted by one commercial turkey company over the past several years. Early onset of

"leg problems" and stunting were noted in the field. Increased first week mortality, decreased overall flock livability, decreased slaughter weights, and increased whole bird condemnations were also reported in affected flocks. Various medications and vitamin supplementations had been ineffective. Nutritional problems were not found, and correlation with better versus worse flock management could not be made. This company received poults from a breeder/hatchery operation in another state, and poult placement information suggested that those houses with a high percentage of progeny of certain breeder flocks were more likely to be affected than others.

Most submitted birds ranged in age from three to 26 days, and both toms and hens were represented. In general, younger birds (two to 10 days) had slightly increased clear or mildly cloudy joint fluid in hock joints (most bilateral) and mild splenomegaly and older poults (two to four weeks) were small for age and had shortened long bones and widened hocks and other joints of legs and feet. Microscopic lesions included synovitis, perichondritis and chondritis in legs, and diffuse lymphoid depletion and reticuloendothelial cell hyperplasia in spleens. Bacteria were not isolated from most swabs taken of joint and bone lesions and spleens by routine aerobic culture, nor detected in gram-stained impression smears of synovial fluid. *Mycoplasma* spp. culture attempts were also negative in our laboratory. By PCR, some joint swabs were positive for *Mycoplasma* spp., but negative for *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. Swab pools from most cases were sent to University of Georgia Poultry Diagnostic and Research Center for PCR for *Mycoplasma iowae* (*MI*), culture for *MI* or both. Tests for *Mycoplasma meleagridis* (*MM*) were also requested in some cases, but all results were negative. In 17 of these cases to date, one or more pools were positive for *MI*. Twelve other cases were considered suspect for *MI* because of findings consistent with the pattern, although *MI* was not detected. In 2008, one submission of legs from 16-day-old toms from an out of state company that receives poults from the same hatchery source was also positive for *MI* by PCR. In our laboratory, *MI* infection was diagnosed in very young poults with high mortality from an independent turkey farm in the early 1990s. No cases had been confirmed in PA before that or in the ~14 year interval between then and the onset of the currently described cases.

Game Bird: A single case consisting of three two-year-old chukar partridge breeders (one male and two females) from a breeder/hatchery operation was submitted in the winter of 2008 with a flock history of lethargy, weight loss, and increased mortality. The flock was kept on raised wire floors in an enclosed house. The house was described as "used for many years" and ventilation was reported as "not the best." The owner reported some problems with mice in the house, but no problems with wild birds. The birds were fed a commercial game bird breeder ration. Production during last year's breeding season was considered normal by the owner.

Gross lesions included unkempt plumage (two birds), overgrown beaks (three birds), decreased body weight (two birds), multiple tan or gray nodular masses in lungs (all birds) and gizzard and intestines (two birds), firm and mottled liver (two birds), mild splenomegaly (one bird), and exudate in conjunctiva and sinuses (one bird). Tests for mycoplasmosis and fungal infections were negative. Microscopic examination of lung, proventriculus, ventriculus, intestine, liver, spleen, nose, and sinus showed focal or multifocal granulomas. Acid fast-stained sections of these tissues were positive for acid fast rod-shaped bacteria which were more numerous in lung, intestine and gizzard than in liver and spleen. *Mycobacterium avium* subspecies *avium* was isolated and confirmed by DNA probe at the National Veterinary Services Laboratory.

The remaining breeders were depopulated, and the owner's intentions were to thoroughly clean and disinfect the house, and to keep breeders through one breeding season only. Although avian mycobacteriosis has been diagnosed sporadically in aviary and hobby collections of several species in our laboratory, it had not been diagnosed in any game bird farm submissions in memory.

All Species – Avian Influenza (AI): The most interesting and appreciated aspect of AI in our state is the absence of "significant" outbreaks during the past 7+ years. ("Significant" in this context is defined as H5 or H7 AI virus (AIV) in multiple flocks of commercial-size flocks of gallinaceous birds.) The only notifiable AI detected in our laboratory this year was in an out of state submission of young pheasants in a mixed species game bird operation from which H5N8 low pathogenicity (LP) AIV, waterfowl strain, was isolated. The four significant AI outbreaks in PA in the past 26 years have all involved H5 or H7 viruses that matched the predominant strains circulating in the northeast urban live bird markets (lbm) concurrently, and that had likely adapted from waterfowl strains to gallinaceous strains under multispecies selection pressure over time. During the past eight to nine years, a more rigorous, multifactorial approach has been taken to decrease the likelihood of AIV introduction into and AIV persistence within the lbm system. The most recent, persistently resident strain in the Northeast lbm (H7N2 LPAIV) appears to be eliminated from the system at this point. From the perspectives of supply states such as ours, the major market states of New York and New Jersey, and the commercial poultry

industry at large, it is hoped that these efforts continue to be successful.

(The full length article on *Brachyspira* spp. in Pennsylvania will be submitted for publication in *Avian Diseases*.)

A LOOK AT SEVERAL LIVE COCCIDIA VACCINES

Steve H. Fitz-Coy

Poultry Pathologist-Parasitologist, Intervet/Schering-Plough Animal Health
steve.fitz-coy@sp.intervet.com

SUMMARY

The *Eimeria tenella* was apparent in all vaccines; this was confirmed by the bloody dropping pans. The *E. maxima* in vaccine D appeared to be relatively pathogenic or the number of *E. maxima* oocysts per dose was high; this was based on the pathological signs exhibited by affected birds. The coccidia organisms in vaccine A were more sensitive to the drugs tested than the coccidia isolates in the other vaccines. Of the live coccidia vaccines tested, the organisms in product A was less pathogenic to the host.

INTRODUCTION

Coccidia are very prolific, hardy, ubiquitous, and highly antigenic, but susceptible to drying. For the coccidia susceptible host, these agents can be pathogenic leading to weight loss, impaired feed utilization, poor pigmentation, and even mortality. The coccidia life cycle follows an intricate but yet direct pathway. It is during these developmental pathways of the parasites that they may destroy vast numbers of intestinal cells in the affected birds. The level of damage in the host may be related to several factors: (a) the number of coccidia ingested that have completed their life cycle in the host; (b) the species of coccidia ingested, for example, *E. maxima* is more pathogenic than *E. acervulina*; and (c) pathogenicity of the isolate, some isolates with in a species may be more pathogenic than others.

Maintaining good bird health and productivity is dependent on good coccidia control. A low parasite burden in the host is necessary, this is particularly important during the critical periods of the bird's growing curve. Judicious use of anticoccidial drugs and managing anticoccidial drug responsiveness is essential in maintaining effective long-term coccidiosis control. During the 1960s and 1970s, there was one approved live coccidia vaccine. By the 1980s there were two live coccidia vaccines and in 2009 there are five approved products available for broiler chickens in the US.

MATERIALS AND METHODS

Commercial broiler chickens obtained from a local hatchery were used in these studies; birds were six to 10 days before being placed in experimental pens. The coccidiosis vaccines were coded A, B, C, D, and E. The dose levels were 0, 20x and 33x doses per bird via oral inoculation. Parameters measured were growth, feed utilization (FCR), consistency of feces, lesion scores, parasite burden, and oocysts output. The drugs tested were those commonly used by the broiler industry and at the levels recommended.

RESULTS AND DISCUSSION

Between 117 and 121 h post-inoculation (pi), *E. acervulina* type oocysts were seen in samples from birds that had received the vaccines. Feces were normal for the control birds and those given vaccine A. But the feces for those birds that got D were bloody, watery and mucoid. Feces produced by the birds given B were bloody and watery. By 129 h pi, *E. acervulina* type seen in the samples from birds given vaccines A, C and D; *E. tenella* seen in fecal samples from birds given vaccine D. By 141 h pi, *E. acervulina* type seen in sample from birds given vaccine A. *E. acervulina* type and *E. tenella* seen in feces from birds given vaccine C. *E. acervulina* type, but *E. maxima* and *E. tenella* seen with vaccine D. Feces were normal for the control birds, those birds given vaccine A had watery and bloody feces. The birds given vaccine B had watery feces and those given D had watery and mucoid feces. By 164 h pi, *E. acervulina* type, *E. tenella* and *E. maxima* were seen in all the fecal samples.

Birds given vaccine A or C grew at rates comparable to that of the controls when measured between 96-120 h pi, 35 g, 33 g, or 30 g per bird, respectively. However, birds given vaccine D grew at a rate of only 6 g per bird during the same period. When growth was measured between 120-144 h pi, the controls grew at a rate of 34 g per bird, whereas the birds given vaccine A or C grew at rates of 27 g and 29 g, respectively. But the birds that were given vaccine D grew at a rate of 14 g during the same period. Growth rate for the controls between 144-164 h pi was 62 g and the growth rate for those birds given vaccine A or C grained 47 g. The growth rate of the birds given D had lowest gain during that period, 28 g per bird. The control group had the lowest FCR, followed by those given vaccines A, C and D, respectively (1.59, 1.64, 1.69, and 1.96).

The coccidia in vaccine A were sensitive to the anticoccidial drugs tested. Those organisms from

vaccine B were relatively sensitive and those from vaccines C and D showed signs of drug tolerance. Vaccine D appeared to be the most pathogenic among the vaccines tested; this vaccine caused severe growth suppression, impaired FCR, and relatively high parasite burden. The organisms in vaccine C appeared to be the most prolific as measured by oocysts produced per dose; followed by vaccines D and A, respectively.

EVALUATION OF PRODUCTIVE PARAMETERS AFTER USING AN O78 *E. COLI* VACCINE IN BROILERS

Veronica Flamenco

Fort Dodge Animal Health, San Salvador, El Salvador

INTRODUCTION

Escherichia coli is recognized as an inhabitant of the intestines of healthy birds. Sometimes, due to stressing factors, some *E. coli* strains can become opportunistic causing cellulitis and airsacculitis as secondary infections. Other strains, known as APEC (avian pathogenic *E. coli*) are considered highly pathogenic and affect birds by elevating mortality, increasing the percentage of condemned broilers at the slaughter-house, and generally, by affecting productive parameters.

The objective of this study is to evaluate productive parameters in broilers after using an O78 *E. coli* vaccine, as well as the percentage of condemned carcasses due to *E. coli* at the slaughter-house.

MATERIALS AND METHODS

E. coli isolates were sent to the Wiley Laboratory at The Pennsylvania State University for serotyping. These came from a poultry farm located in El Salvador that has problems related to *E. coli*. The serotypes isolated were O5, O79, and O143. Three houses containing 26,000, 25,000 and 13,000 broilers respectively, were vaccinated with one dose of an O78 modified live *E. coli* vaccine at the third day of age via spray. Another house of 13,000 broilers was left as a control group. All of these were housed on the same farm. At the hatchery, they received their normal vaccination program which included a live and inactivated ND vaccine, plus BI and AI. In the field, their vaccination program consisted of one dose of IBD and ND at day eight and at day 18. The flocks were monitored throughout the growing period and at the slaughter-house. Parameters were recorded as well were necropsies done on daily mortality. Since other variables influenced the productive parameters, it was decided to focus the trial on the percentage of clinical signs related to mortality, plus the percentage of condemned birds at the slaughter-house due to *E. coli*. The results shown here are from the second round of flocks using the O78 *E. coli* vaccine, from the same houses and farm.

RESULTS

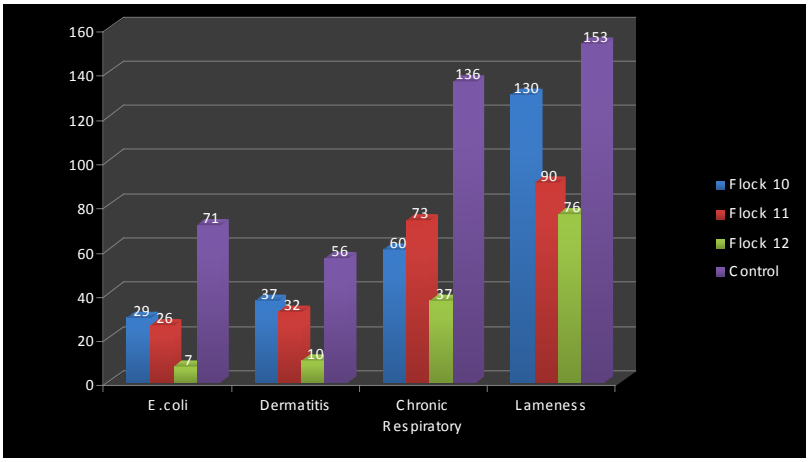
Chart 1 shows that the vaccinated flocks presented less *E. coli*-related mortality due to chronic respiratory disease and dermatitis versus the control group. The vaccinated flocks also had a lower incidence of lameness than the control group.

Chart 2 shows the number of birds condemned at the slaughter house due to dermatitis and airsacculitis, resulting in a lower number of condemned broilers from the vaccinated groups versus the control group.

CONCLUSIONS

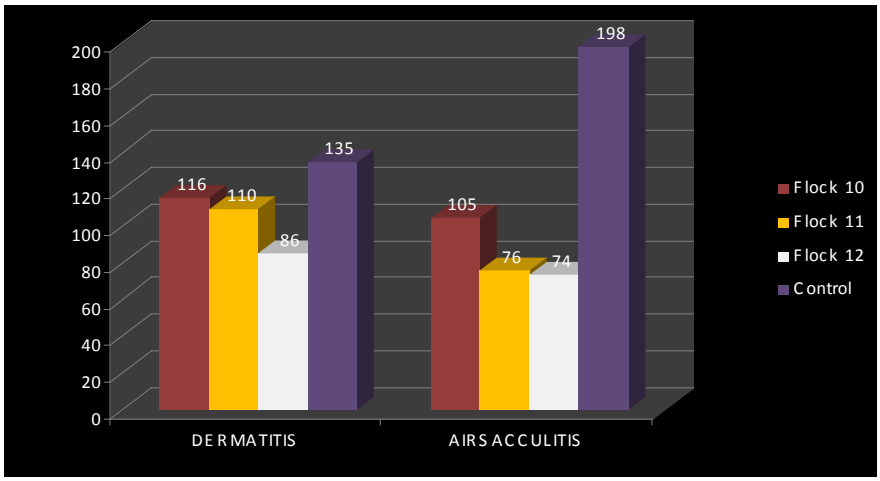
- Productive parameters such as body weight increased a 14% in the vaccinated flocks.
- The vaccine worked as an aid by reducing the number of condemned birds, and therefore, increasing profits. The vaccinated group had 32% less airsacculitis than the control group and 30% less dermatitis than the control group.
- The O78 vaccine demonstrated cross-protection against the O5, O79, and O143 serotypes from this farm.

Chart 1. *E. coli*-related mortality in vaccinated vs. control flocks.



	# of Birds
Flock 10	26000
Flock 11	25000
Flock 12	13000
Control	13000

Chart 2. Condemned birds at slaughter – vaccinated vs. control groups.



FIBRINONECROTIC TYPHLITIS IN TURKEY POULTS IN CALIFORNIA

M. Franca[A], R. Crespo[A], R. Chin[A], P. Woolcock[A], G. Senties-Cue[AB], and H.L. Shivaprasad[A]

[A]California Animal Health and Food Safety Laboratory System – Fresno
University of California, Davis, 2789 S. Orange Avenue, Fresno, CA 93725
[B]Present address: California Animal Health and Food Safety Laboratory System, Turlock
1550 N. Soderquist, Turlock, CA 95381

Fibrinonecrotic typhlitis of unknown etiology in turkey poults have been seen in California as well as in other parts of USA. Turkey poults from 32 cases were submitted to the California Animal Health and Food Safety Laboratory System, Fresno Branch, between 1991 and 2008. The age of the affected birds with typhlitis ranged from four days to one week in 30 cases. In one case, the birds were nine days old and in another case the birds were 25 days old. Both toms and hens were affected. Clinical history reported included increased mortality in 26 cases and decreased feed consumption in 13 cases. Grossly, isolated birds had mild to severely distended ceca with fibrinonecrotic cores in the lumen. Occasionally serosal and mucosal hemorrhages were observed in three cases. Microscopically, various degrees of necrosis of the mucosa with fibrin exudation and inflammation were seen. There were large numbers of bacteria within the fibrinonecrotic debris in 19 cases. *Salmonella arizonae* was isolated from the intestine in three cases. Other *Salmonella* species were isolated from the intestine in 12 cases. Culture for anaerobic bacteria was performed in 11 cases and for *Campylobacter* spp. in three cases and yielded isolation of *Clostridium perfringens* in four cases and *Campylobacter jejuni* in one case. Direct electron microscopy in portions of the small and large intestines and its contents was performed in 24 cases which revealed the presence of Rotavirus-like particles in five cases and 25 to 30 nm viral particles in four cases. *Blastocysts* spp. was seen in association with the lesion in six cases and coccidia of *Eimeria* spp. in two cases.

The exact cause of the fibrinonecrotic typhlitis in turkey poults is still unknown but it appears to be due to multifactorial etiologies including bacteria, enteric viruses, parasites, and perhaps others.

(A full-length article will be published in *Avian Diseases*.)

UNUSUAL AVIAN MYCOBACTERIOSIS IN COMMERCIAL BROWN LAYERS

D. Gallazzi, G. Manarolla, T. Rampin, and G. Sironi

Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria – Sezione di Anatomia Patologica Veterinaria e Patologia Aviare – Università degli Studi di Milano –via Celoria 10 - 20133 Milano – Italy

ABSTRACT

An unusual outbreak of avian mycobacteriosis in 46-week-old commercial brown layers is described. Reduced feed intake, gradual drop in egg production and increased mortality rate were observed in the flock housed in Northern Italy. Eight birds were humanely euthanatized and necropsied. The birds were in poor body condition, with pale, shrivelled combs, and principally presented prominent orbital lesions. Grossly, multiple, variable-sized nodular lesions were found in the intestinal wall, liver, spleen, lungs, bone marrow, and conjunctiva. Histologically, both visceral and orbital lesions displayed granulomatous nature with variable numbers of acid fast bacilli. Polymerase chain reaction confirmed *Mycobacterium avium* as the causative agent. The peculiarity of this outbreak of mycobacteriosis regards type and age of the affected birds and the uncommon involvement of the soft orbital tissues.

INTRODUCTION

Avian mycobacteriosis is a well known disease with a world-wide distribution. In poultry, the disease is usually termed tuberculosis and is principally caused by *Mycobacterium avium*. This disease is chronic and affected flocks are characterized by unthriftiness, decreased egg production, and finally death. Although tuberculosis in commercial poultry is now rare, it still occurs sporadically in backyard poultry and game birds. In poultry, the onset of mycobacterial infections is usually confined to the gastro-intestinal tract (2,6). Here we describe an unusual avian mycobacteriosis in commercial brown layers.

MATERIALS AND METHODS

Animals. Eight 46-week-old commercial brown layers out of a flock with egg drop and increased mortality were submitted for diagnosis. The animals were humanly euthanized and immediately necropsied.

Histopathology. Samples of periocular tissues, eyes, heart, lungs, spleen, liver and intestine were fixed in 10% buffered formalin and routinely included for histopathology. Four-micron sections were stained with hematoxylin and eosin and with Zeehl-Neelsen (ZN). Additional samples of conjunctiva were store at -20°C until use.

PCR. DNA was extracted from frozen tissues using a DNA minikit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The identification of the mycobacterial species was based on the amplification and sequence analysis of a hypervariable region of the 16S rRNA gene as previously described (1,3,4). A 5 µL sample of the PCR products was electrophoresed in 2% agarose (Promega, Milan, Italy) and stained with ethidium bromide (Euroclone, Milan, Italy). PCR products of the expected length (555 bp) were purified from agarose gel slices using a QIAquick PCR purification kit (Qiagen) and sequenced using an ABI Prism 310 genetic analyser (Applied Biosystems, Foster City, California, USA). The sequence was submitted to the GenBank database (FJ639163). The percentage of similarity with reference sequences was evaluated by BLAST search in the NCBI website.

RESULTS

Animals. All the birds were in poor body condition with atresic ovary and oviduct. Three layers showed monolateral, moderate to extremely severe enlargement and thickening of the soft periocular tissues with chemotic conjunctivitis and subconjunctival nodular lesions. Disseminated nodular lesions of varying dimensions were also observed in intestine, spleen, liver, lungs bone marrow of all the eight birds.

Histopathology. The sections of both orbital and visceral nodular lesions revealed granulomatous nature characterized by numerous hystiocytes and multinucleated giant cells surrounded by variable numbers of lymphocytes and lesser numbers of plasma cells and viable heterophils. Larger granulomas were centred on necrotic areas. The sections of orbital tissues showed the granulomas were immediately beneath the conjunctiva which was diffusely hyperplastic and infiltrated by large numbers of lymphocytes and plasma cells. Erosion of conjunctival epithelium was associated with larger granulomas which merged inwardly with massive necrosis. Mild uveitis was observed in the eye sections. ZN stain revealed scarce numbers of acid fast bacilli (AFB)

within the visceral granulomas whereas AFB were extremely rare in the orbital granulomas.

PCR and sequencing. Molecular analysis revealed *Mycobacterium avium* as the causative agent with a percentage of similarity of 99%.

DISCUSSION

As in other countries around the world, *Mycobacterium avium* infections are occasionally reported in commercial Italian poultry whereas they are still diagnosed in backyard and game birds. Typically, clinical signs appear in the second year of age and are characterized by disseminated granulomas associated with numerous AFB. Here we describe an outbreak of tuberculosis caused by *Mycobacterium avium* in 46-week old commercial layers with peculiar involvement of the orbital tissues and scarce AFB within the lesions. It was not possible to ascertain the source of infection and no follow-up data were available. It is possible to hypothesize that the precocious onset of the disease with exuberant paucibacillary granulomas was due to a hyperergic response as well-known in mammals (5). As mycobacteria are usually shed via faeces from intestinal lesions, the uncommon orbital involvement can be explained with superinfection via aerosolized bacteria in the environmental dust. Extreme paucibacillarity of the orbital lesions and the low numbers of birds with these signs seems to sustain a secondary involvement of the periocular tissues.

REFERENCES

1. Dumonceau, J.M., P.A. Fonteyne, L. Realini, A. van Gossum, J.P. van vVoren, and F. Portaels. Species-specific Mycobacterium genavense DNA in intestinal tissues of individuals not infected with Human Immunodeficiency Virus. J. Clin 33, 2514-2515. 1995.
2. Fulton, R.M and C.O. Thoen. Other bacterial diseases. Tuberculosis. In: Diseases of poultry, 11th ed. Y.M. Saif, H.J. Barnes, A.M. Fadly, J.R. Glisson, L.R. McDougald, and D.E. Swayne, eds. Iowa State University Press, Ames, Iowa, USA. pp 836-844. 2003.
3. Hughes, M.M., G. James, N. Ball, M. Scally, R. Malik, D.I. Wigney, P. Martin, S. Chen, D. Mitchell, and D.N. Love. Identification by 16S rRNA gene analyses of a potential novel Mycobacterial species as an etiological agent of canine leproid granuloma syndrome. J. Clin. Microbiol. 38, 953-959. 2000.
4. Manarolla G., E. Liandris, G. Pisoni, D. Sassera, G. Grilli, D. Gallazzi, G. Sironi, P. Moroni, R. Piccinini, and T. Rampin. Avian mycobacteriosis in companion birds: 20-year survey. Vet. Microbiol. 133: 323-327. 2009.
5. Snyder, P.W. Diseases of Immunity. In: Pathologic basis of veterinary disease, M.D. Mc Gavin and J.F. Zachary, eds. Mosby Elsevier, St. Luis, Missouri, USA. pp 193-251. 2003.
6. Tell, L.A., L. Woods, and R.L. Cromie. Mycobacteriosis in birds. Rev. Sci. Tech. Ser. Sci. Hum. (International Office of Epizootics). 20, 180-203. 2001.

PATHOGENICITY MARKERS OF *CLOSTRIDIUM* SPP. IN COMMERCIAL TURKEYS

M. Saita[A], L. Bano[B], G. Grilli[A], and D.Gallazzi[A]

[A]Department of Veterinary Pathology, Hygiene and Public Health, University of Milan, Via Celoria 10, 20133 Milano, Italy, E-mail: daniele.gallazzi@unimi.it
[B]Istituto Zooprofilattico Sperimentale delle Venezie, Laboratorio di Treviso, Italy

SUMMARY

Since the growth promoter ban in Europe, enteritis of different etiologies (virus, bacteria, and protozoa) are increasingly becoming the main cause of economic loss in commercial turkeys production. This study is focused on typing of *Clostridium* spp. isolated from samples of jejunum and ileum of 82 birds out of 17 turkey flocks. The birds were six-day to 104-day old, both male and female, with enteric disorders. The presence of toxin NetB was investigated. Multiplex PCR to detect cpa, cpb1, cpetx, cp1, cpb2 and cpe toxin genes were used for *Clostridium* typing. No lesions of necrotic enteritis were observed. *Clostridium perfringens* type A was isolated from 25 enteric samples, *Clostridium difficile* was found in four cases and *Clostridium sordelli* in one case. *Clostridium perfringens* was present from six to 104 days of age indicating its possible role in the enteric disorders of commercial turkeys. NetB toxin was found in no sample. Three out of four isolates of *Clostridium difficile* were characterized by the presence of toxin genes.

INTRODUCTION

The main clostridia responsible for a wide range of diseases in avian species are: *Clostridium colinum*, *C. botulinum*, *C. septicum*, and *C. perfringens*, *C. fallax*, *C. novyi*, *C. sporogenes*, and *C. difficile* (1).

Pathological signs are caused by the different toxins but in many cases cofactors such as dietary ingredients or changes, severe stress, coccidiosis, and other protozoal diseases of the intestinal tract or immunosuppressive infections can enhance the disease (1). *Clostridium perfringens* (CP) is often isolated from the intestinal tract of healthy birds but can also cause outbreaks of disease in poultry, and especially in broiler and turkey flocks. CP is a gram-positive, spore forming, and anaerobic bacterium responsible for a wide range of diseases in humans and animals. Its pathogenicity is associated with the production of 17 toxins, of which α, β, ε, and ι are the major lethal ones (2). A commonly used classification scheme divides CP isolates into five types (A-E) on the basis of their capability to produce the major lethal toxins (2). Some CP strains, in addition to α toxin, produce β2 and enterotoxin: two toxins that have been proposed as being important in the pathogenesis of intestinal disorders in animals and humans respectively (3,4,5).

Clostridiosis occurs as acute or subclinical disease. The acute clinical disease is characterized by necrotic enteritis (NE). Intestinal focal necrosis and hepatitis are typical signs frequently associated with subclinical clostridiosis (6). The role of CP toxin types in the pathogenesis of NE in poultry is still not clear. Studies conducted in Finland, Sweden, Belgium, and Denmark demonstrated that CP isolated from chickens affected by NE belong to toxin type A (6,7,8,9,10), and demonstrated that α toxin is not essential in causing NE in broilers. Very few studies are focused on turkeys, although since the growth promoters ban in Europe in 2006, it has become a pathology of major concern. Recently, NetB, a novel toxin that is associated with broiler NE, has been described (10). The toxin was identified using screens for proteins from the supernatant of *C. perfringens* cultures that were cytotoxic for chicken hepatocellular carcinoma cells (LMH) *in vitro*.

The aim of this study was to perform toxin genotyping of CP field strains collected from the intestines of diseased turkeys by multiplex PCR for detection of α, β, ε, ι, β2, NetB, and enterotoxin genes.

MATERIALS AND METHODS

Birds. Eighty-two birds from 17 commercial turkey flocks showing enteric disorders were humanly euthanized and necropsied. The turkeys were six-day to 104-day old, both male and female.

Strains and growth conditions. All strains were obtained streaking on Perfringens Agar Base (Oxoid) 0.1 mL of 24 h broth (Cooked Meat Medium, Difco) culture of jejunum and ileum fragments (5 cm back and 3 cm after the Merkel's diverticulum) collected from sick commercial turkeys. CP ATCC 27324 (toxin-type E+enterotoxin), CCUG 2036 (toxin-type C), CCUG 2037 (toxin-type D), ATCC 10543 (toxin-type A + β2) were used as reference strains. All strains were incubated in anaerobic conditions at 37°C for 48 hours.

DNA extraction. Colonies of each CP strain were recovered from the agar plate and the DNA was

extracted with DNeasy Blood & Tissue Kit (Qiagen) according to manufacturer's instructions.

Toxin coding gene detection. One multiplex PCR for cpa, cpb1, cpetx , and cpi genes and three single PCR for cpb2 (2), cpe (11) and NetB (10) genes detection were used. PCR primers and fragment length are listed in table 1. The sequencing of the amplified product confirmed that the targeted netB gene was indeed amplified with the PCR assay.

Parasitological examination. Intestinal mucosa of all chickens was scraped in different districts and observed by optic microscope searching for protozoa and helminths (eggs and worms).

RESULTS

At necropsy, all 82 turkeys showed enteric lesions. In younger subjects (one to three weeks) intestinal lesions were consistent with viral enteritis, a common finding in Italian flocks. In older turkeys (three to six weeks of age) coccidiosis was diagnosed. Twenty-five (30.48%) out of 82, aged from six to 104 days old, were positive for *C. perfringens* type A. All strains resulted positive for α toxin gene (toxin-type A) and only one (1.2%) of these was also positive for β2 toxin (toxin-type A + β2). No CP cpe-positive or NetB positive strains were detected. Four (4.8%) turkeys were positive for *C. difficile*. Among these, one was negative for both toxin genes while two were positive for TcdA and TcdB, and one was positive only for TcdB. One (1.2%) was positive for *C. sordelli*.

DISCUSSION

The data highlight that the CP isolates included in the study were of toxin type A and a relatively low percentage of isolates carried the β2 toxin gene, irrespective of enteric lesions. No CP toxin type C was found also in birds affected by NE. Our findings confirm the most recent results reported from different countries, and the data suggest that the role of CP type C should be revaluated in the pathogenesis of NE. The presence of *Clostridium* spp. was often associated with other pathogens, such as viral enteritis in the first three weeks, coccidiosis between three and five weeks and hemorrhagic enteritis between six and 12 weeks of age.

These observations underline the importance of predisposing factors (nutrition, drug treatments, concomitant diseases) in poultry clostridiosis. It must be kept in mind that the presence of CP type A already exists in six-day-old turkeys. The role of this pathogen at such a young age must be clearly understood, but surely it could play an important role in developing enteric disorders. After the growth promoter ban in 2006, enteric imbalances are a main concern. The lack of NetB positive findings, which seems to play a major role in NE of chickens, is an important result as there is no data available for this toxin in turkeys. Moreover, the presence of *C. difficile* in four samples – three of them toxin genes positive – is quite interesting because of its potential zoonotic role.

REFERENCES

1. Barnes E.M., C.S. Impey, and D.M. Cooper. Manipulation of the crop and intestinal flora of newly hatched chick, Am. J. Clin. Nutr. 33: 2426-2433. 1980.
2. Meer, R.R. and J. Songer. Multiplex polymerase chain reaction assay for genotyping *Clostridium perfringens*. Am. J. Vet. Res. 58:702-705. 1997.
3. Sarker, M.R., R.J. Carman, and B.A. McClane. Inactivation of the gene (*cpe*) encoding *Clostridium perfringens* enterotoxin eliminates the ability of two cpe-positive *C. perfringens* type A human gastrointestinal disease isolates to affect rabbit ileal loops. Mol. Microbiol. 33:946-958. 1999.
4. Thiede, S., R. Goethe, and G. Amtsberg. Prevalence of β2 toxin gene of *C. perfringens* type A from diarrhoeic dogs. Vet. Rec. 149:276-274. 2001.
5. Manteca, C., G. Daube, T. Jauniaux, A. Linden, V. Prison, J. Detileux, A. Ginter, P. Coppe, A. Kaeckenbeeck, and J.G. Mainil. A role for the *Clostridium perfringens* β2 toxin in bovine enterotoxaemia? Vet. Microbiol. 86: 191-202. 2002.
6. Engström, B.E., C. Fermér, A. Lindberg, E. Saarinen, V. Båverud, and A. Gunnarsson. Molecular typing of isolates of *Clostridium perfringens* from healthy and disease poultry. Vet. Microbiol. 94: 225-235. 2003.
7. Nauerby, B., K. Pedersen, and M. Madsen. Analysis by pulsed-field gel electrophoresis of the genetic diversity among *C. perfringens* isolates from chickens. Vet. Microbiol. 94:257-266. 2003.
8. Heikinheimo, A. and H. Korkeala. Multiplex PCR assay for toxinotyping *Clostridium perfringens* isolates obtained from Finnish broiler chickens. Lett. Appl. Microbiol. 40:407-411. 2005.
9. Gholamiandekhordi, A.R., R. Ducatelle, M. Heyndrickx, F. Haesebrouck, and F. Van Immerseel. Molecular and phenotypical characterization of *Clostridium perfringens* isolates from poultry flocks with different disease status. Vet. Microbiol. 113:146-152. 2006.
10. Keyburn, A.L., S.A. Sheedy, M.E. Ford, M.M. Williamson, M.M. Awad, J.I. Rood, and R.J. Moore. Alpha-toxin of *Clostridium perfringens* is not an essential virulence factor in necrotic enteritis in chickens. Infect. Immun. 74:6496-6500. 2006.

11. Baums, C.G., U. Schotte, G. Amtsberg, and R. Goethe. Diagnostic multiplex PCR for toxin genotyping of *Clostridium perfringens* isolates. Vet. Microbiol. 100:11-16. 2004.

Table 1. Primers used to detect *C. perfringens* toxin coding genes.

GENE	Primers	Sequence (5'-3')	Fragment length
cpa	cpa_F	GTT GAT AGC GCA GGA CAT GTT AAG	402
	cpa_R	CAT GTA GTC ATC TGT TCC AGC ATC	
cpb	cpb_F	ACT ATA CAG ACA GAT CAT TCA ACC	236
	cpb_R	TTA GGA GCA GTT AGA ACT ACA GAC	
cpetx	etx_F	ACT GCA ACT ACT ACT CAT ACT GTG	541
	etx_R	CTG GTG CCT TAA TAG AAA GAC TCC	
cpi	cpi_F	GCG ATG AAA AGC CTA CAC CAC TAC	317
	cpi_R	GCG ATG AAA AGC CTA CAC CAC TAC	
cpe	cpe_F	GGG GAA CCC TCA GTA GTT TCA	506
	cpe_R	ACC AGC TGG ATT TGA GTT TAA TG	
cpb2	cpb2_F	AGA TTT TAA ATA TGA TCC TAA CC	567
	cpb2_R	CAA TAC CCT TCA CCA AAT ACT C	
NetB	AKP78_F	GCT GGT GCT GGA ATA AAT GC	384
	AKP79_R	TCG CCA TTG AGT AGT TTC CC	

COMPARISON BETWEEN AN ANTICOCCIDIAL TREATMENT AND AN IMMUNOGLOBULIN Y-BASED TREATMENT IN BROILERS CHALLENGED WITH *E. TENELLA*

Donají García López, Ruth I. Larios Castillejos, J. Andrés Morales Garzón, and Eduardo Lucio Decanini

Investigación Aplicada, SA de CV., 7 Norte No 416, Colonia Centro, Tehuacán, Puebla, CP 75700, México
E-mail: dgacia@grupoidisa.com

SUMMARY

The goal was compare the effect of an immunoglobulin Y product (Supracox®), liquid and powder, obtained from hyperimmunization of hens with multiple species of *Eimeria* oocysts vs. liquid anticoccidial with toltrazuril 2.5% like active ingredient (TZ) face to challenged of *Eimeria tenella* (Et). All immunoglobulin Y-based groups reduced fecal oocyst shedding in comparison with positive control group. TZ group fecal oocyst shedding was not constant and some values were higher than positive control. Better group in score lesions was powder treatment B. We conclude that immunoglobulin Y-based treatment is an effective option against coccidia challenge infection.

RESUMEN

Comparación entre un tratamiento a base de inmunoglobulinas-Y y un anticoccidiano, al ser administrados en un desafío con *Eimeria tenella*. Se comparó el efecto de las inmunoglobulinas Y (Supracox®), tanto líquidas como en polvo, obtenidas de la hiperinmunización de aves con oocistos de diferentes cepas de *Eimeria*, contra un anticoccidiano líquido que contenía como principio activo el toltrazuril al 2.5% (TZ) ante un brote de *Eimeria tenella* (Et), Las dosis para Supracox fueron de de 0.02 g/ave en el tratamiento en polvo y de 2 mL/ave en el tratamiento líquido, mientras que para el toltrazuril se siguieron las recomendaciones del fabricante. El 100% de los grupos tratados con Supracox presentaron una disminución en la excreción de oocistos, por debajo del control positivo. El grupo tratado con TZ tuvo una excreción de oocistos variable y en ocasiones mayor a la del control positivo. El 66.7% de los grupos tratados con Supracox tuvieron mayor ganancia de peso que el grupo tratado con TZ. De los tres tratamientos de Supracox el denominado 2 (producto en polvo) fue el que mejor resultados brindo comparándolo con todos los grupos, ya que fue el que obtuvo la mayor ganancia de peso y su calificación de lesiones fue de 1.

INTRODUCTION

Poultry coccidiosis is a widespread, economically important disease characterized by poor weight gain or feed conversion, depigmentation, and a slight increase in mortality. The higher economic effects are on the applied control methods, such as drug treatment or vaccination strategies. The occurrence of resistance in different *Eimeria* species (3) and the authorities restrictions give us the opportunity of researching new options (1). The use of egg yolk derived immunoglobulin like as an alternative to antibiotic treatment for control in several animal species has been proved (2).

MATERIAL AND METHODS

Chickens and experimental design. Seventy male one-day-old commercial broilers were randomly assigned to six groups (11 birds/group) and were fed throughout the trial with standard diet without anticoccidials. Each bird was vaccinated with NDV at first day of age. Group 1 and 2 were A and B immunoglobulin-Y powder, group 3 was liquid immunoglobulins, group 4 was toltrazuril; groups 5 and 6 were positive and negative controls. The animals were necropsied at eight days post challenge; the rest of each group until 13 days post challenge to continue the fecal oocyst counts.

***Eimeria* and challenged.** Animals were orally inoculated with 4.5×10^4 sporulated wild-type strain of *E. tenella* oocysts, 13 days post-challenge; chickens were necropsied and checked for characteristic *E. tenella* cecal lesions.

Inmunoglobulin Y-based treatment: was supplemented for three days, from five to seven day-post infection. Powder A and B refers two different schedules of hyperimmunization.

Anticoccidial product. Liquid with toltrazuril 2.5% like active ingredient observing the dose rates and specifications recommended for maker lab.

Parameters. Fecal oocyst counts from three d post infection to 13 d. Table 1 shows the counts of oocyst per g, and scores lesions using the Johnson and Reid method.

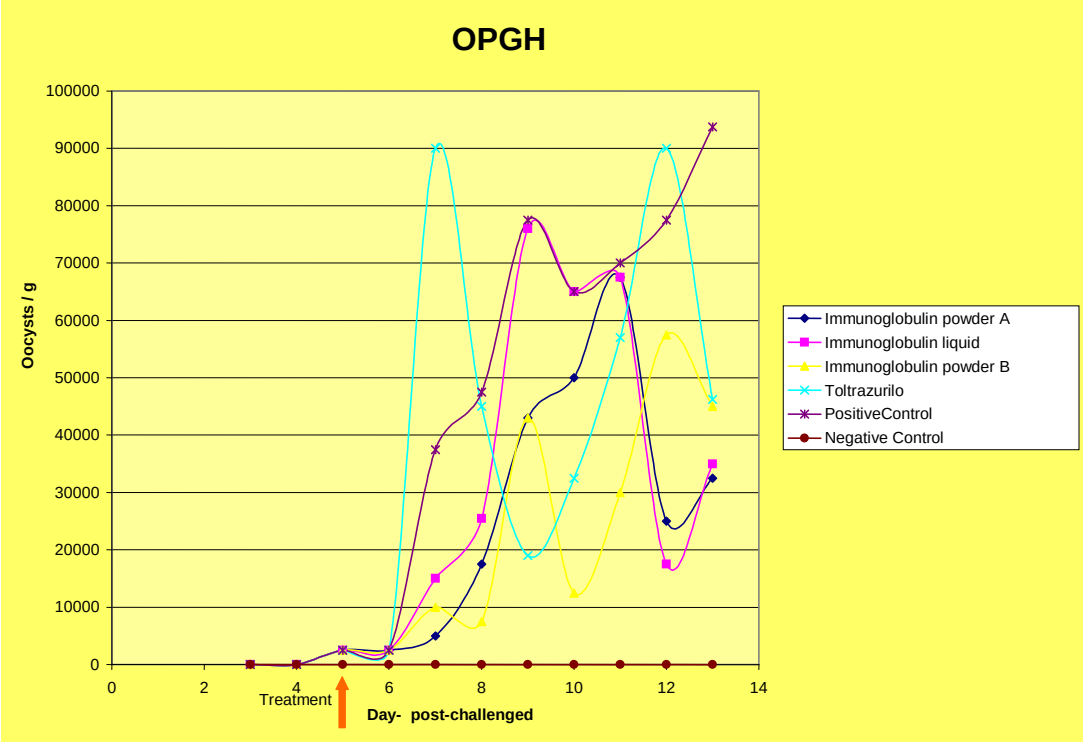
RESULTS AND DISCUSSION

All groups began to shed oocysts from five d post infection; immunoglobulin Y-based groups reduced fecal oocyst shedding in compare with positive control group. TZ group fecal oocyst shedding was not constant, and some values were higher than positive control (Figure 1). Better group in lesion scores was powder treatment B. In the present trial, best protection was afforded by the powder immunoglobulin B; this one reduced fecal oocyst shedding compare with all groups. Powdered product had no statistical difference in lesion score or fecal oocyst counts with the control group (Tukey test), although a numerical difference existed. The probable reason was that the animals were in battery units and did not get reinfected. Passive immunization of chickens with specific Ig-Y is a good alternative against coccidiosis control.

REFERENCES

1. Dallaoul, R.A. and H.S. Lillehoj. Recent advances in immunomodulation and vaccination strategies against coccidiosis. Avian Dis. 49: 1-8. 2005.
2. Ikemori,Y., M. Kuroki, R.C. Peralta, H. Yokohama, and Y. Kodama. Protection of neonatal calves against fatal enteric colibacillosis by administration of egg yolk powder from hens immunized with K-99- piliated enterotoxigenic Escherichia coli. Am. J. Vet. Res. 53:2005-2008. 1992.
3. Vertomen, M.H., H.W. Peek, and A. van der Laan. Efficacy of toltrazuril in broilers and development a laboratory model for sensitivity testing of Eimeria field isolated. Vet Q. 12: 183-192 .1990.

Figure 1. Fecal oocyst counts.



ORGANIC ACIDS AND ESSENTIAL OILS: A VIABLE ALTERNATIVE TO ANTIBIOTIC GROWTH PROMOTERS IN POULTRY PRODUCTION

R. Gauthier

Jefo Nutrition Inc., 5020 Avenue Jefo, St-Hyacinthe, Quebec, Canada, J2S 7B6
rgauthier@jefo.ca

SUMMARY

A better understanding of the mode of action of organic acids and essential oils on bacteria and the discovery of a strong synergy between those two families of compounds, coupled with an appropriate processing technology adapted to the anatomy and physiology of poultry, has given rise to a new and more efficacious alternative to antibiotic growth promoters. In order to evaluate the real efficacy of this strategy, *in vitro* and *in vivo* experiments were conducted showing that by applying the microencapsulation technology, the dosage of both organic acids and essential oils could be reduced substantially.

INTRODUCTION

In the late 80s and early 90s, strong regulatory actions have removed most of the antibiotic growth promoters from the European Union market; the last ones have been withdrawn in January 2006. The adjustments following the withdrawal of these products in animal production have been difficult at times, and many replacement solutions have been proposed by the feed additive industry. It is not easy to replace products that have proven to be generally efficacious for the last 50 years. A consensus seems to develop among the scientific community concerned by this subject (9); and one approach is definitely standing out for its efficacy, technological, and economical feasibility: the organic acids. Another option is, under the generic name of "botanicals", essential oils (plant extracts or related compounds).

ORGANIC ACIDS

Organic acids have been used successfully in pig production for more than 30 years and continue to be the alternative of choice. Even if much less work has been done in poultry (1), we can now confirm that the organic acids are very efficacious provided their use is adapted to the physiology and anatomy of poultry. Organic acids (C1-C7) are widely distributed in nature as normal constituents of plants or animal tissues. They are also formed through microbial fermentation of carbohydrates mainly in the large intestine (6). They are also found in their sodium, potassium, or calcium form. Over the years, it was thought that a pH reduction of the gastrointestinal tract content was the mode of action; research has proven differently. The key basic principle on the mode of action of organic acids on bacteria is that non-dissociated (non-ionized) organic acids can penetrate the bacterial cell wall and disrupt the normal physiology of certain types of bacteria that are called "pH sensitive" meaning that they cannot tolerate a wide internal and external pH gradient. Among those bacteria we have *E. coli*, *Salmonella* spp., *C. perfringens*, *Listeria monocytogenes*, and *Campylobacter* spp.

Upon passive diffusion of organic acids into the bacteria where the pH is near of above neutrality, the acids will dissociate and lower the bacteria internal pH, triggering mechanisms that will impair or stop the growth of bacteria. On the other hand, the anionic part of the organic acids that cannot escape the bacteria in its dissociated form will accumulate within the bacteria and disrupt many metabolic functions and lead to osmotic pressure increase, incompatible with the survival of the bacteria. It has been well demonstrated that the state of the organic acids (undissociated or dissociated) is extremely important to define their capacity to inhibit the growth of bacteria. As a general rule, we need more than ten to one hundred times the level of dissociated acids to reach the same inhibition capacity of bacteria, compared to undissociated acids (8).

Too often, *in vitro* assays showing the antibacterial capacity of organic acids are done at a low pH, to avoid the dissociation of the acids. At a pH below 3.0 to 3.5, almost all organic acids are very efficacious in controlling bacteria growth. This does not reflect at all what is happening in the gastrointestinal tract of poultry. Logically, organic acids added to feeds, should be protected to avoid their dissociation in the crop and in the intestine (high pH segments) and reach far into the gastrointestinal tract, where the bulk of the bacteria population is located. More likely, the organic acids in poultry might play a direct role on the intestinal bacteria population, reducing the level of some pathogenic bacteria (ex. *C.*

perfringens) and mainly controlling the population of certain types of bacteria that compete with the birds for nutrients (3).

ESSENTIAL OILS

Essential oils are any of a class of volatile oils obtained from plants, possessing the odor and other characteristic properties of the plant, used chiefly in the manufacture of perfumes, flavors, and pharmaceuticals (extracts after hydro-distillation).

Essential oils or plant extracts can be used as appetite stimulant, aroma, stimulant of saliva production, gastric and pancreatic juices production enhancer, and antioxidant. However there is no clear demonstration of the importance of these factors on the chicken performance.

Plants contain hundreds of substances having different properties but essential oils composed mainly of nine groups (and many sub-groups) of molecules are of interest to us. There are many chemical constituents but no two oils are alike in their structure and effect. One must make a difference between non purified plant extracts containing numerous different molecules interacting and pure active compounds, either extracted from plants or synthesized (nature identical). According to the plant chosen, one or more active compounds are dominant and the quantity found will differ according to factors like plant variety, soil, moisture, climate, time of harvest, etc. Almost all essential oils (EO) are based on isoprene (5C) frame.

Nutritionally, metabolically, and toxicologically, we have a clear interest in using as low as possible levels of essential oils in animal nutrition. Essential oils are extremely potent substances; they can lead to feed intake reduction, gastrointestinal tract microflora disturbance, accumulation in animal tissues and products.

Most essential oils are GRAS (generally recognized as safe) but they must be used cautiously because they can be toxic (allergens) and potent sensitizers and their odor/taste may contribute to feed refusal (2,5). They are also very volatile and will evaporate (sublimate) rapidly, leading to a large variation in concentration in the finished feeds. Encapsulation of essential oils could solve the problem (5).

It is extremely difficult to generalize on the mode of action of essential oils on bacteria and yeasts because each essential oil has different properties and each type of microorganism has a different sensitivity. Generally, gram-positive bacteria are considered more sensitive to essential oils than gram-negative bacteria (2) because of their less complex membrane structure.

The consensus on the mode of action of essential oils on bacteria is now that these compounds influence the biological membranes of bacteria. The cytoplasmic membrane of bacteria has two principal functions (10); a barrier function and energy transduction, which allow the membrane to form ion gradients that can be used to drive various processes and the formation of a matrix for membrane-embedded proteins influencing the ATP-synthase complex.

A VIABLE ALTERNATIVE

In our own experiments with organic acids, we have experienced very consistent results, both under research station and field conditions; our rate of positive response exceeded 90% for weight gain and feed conversion using a blend of protected organic acids. Not only protected organic acids can act as growth promoter but also play a role in the prevention of necrotic enteritis and in the reduction of intestinal *Salmonella* spp. It appears that the amplitude of the response is often related to the level of contamination or intestinal disease challenge in the flock.

More and more, the concept of combining essential oils and organic acids is proving to be efficacious (13) because there appears to be a synergy between the two concepts (11,12). Our own experiments in field trials, or when using a chicken necrotic enteritis challenge model, have shown a strong synergy between essential oils and organic acids.

TECHNICAL PROBLEMS WITH ORGANIC ACIDS AND ESSENTIAL OILS

The use of organic acids and essential oils in the feed industry is often a source of problems, such as corrosion, worker's safety, handling, vitamin stability in premixes, environmental concern, and stability of products.

It has been demonstrated that when both organic acids and essential oils are protected in a triglyceride matrix, the quantity required to achieve maximum performance in poultry can be reduced drastically. The active ingredients can be delivered into the intestine, directly where the bulk of gastrointestinal bacteria are located (7). Without protection, organic acids are readily dissociated in the first part of the chicken gastrointestinal tract and are rendered useless (1). Essential oils are very rapidly absorbed in the duodenum and cannot interact with the microflora (4).

CONCLUSION

There is a general consensus on the efficacy of organic acids as the best alternative to antibiotic growth promoters.

Essential oils have a limited effect as a replacement of antibiotic growth promoters but they

can act in synergy with organic acids both for their growth promoting effect and prevention of specific intestinal diseases. Now there is an encapsulation technology that enhances the efficacy of organic acids and essential oils, at a low inclusion level.

REFERENCES

1. Dibner, J.J. and P. Butin. *Journal of Applied Poultry Research*. 11:453-463. 2002.
2. Lambert, R.J.W., P.N. Skandamis, P.J. Coote, and G.J.E. Nychas. *Journal of Applied Microbiology*, 91:453-462. 2001.
3. Lee, M.D. Molecular basis for AGP effects in animals. *Antimicrobial Growth Promoters: Worldwide Ban on the Horizon*. Noordwijk aan Zee, The Netherlands, p. 37-38. 2005.
4. Lee, K.W., H. Everts, and A.C. Beynen. *International Journal of Poultry Science*. 3:738-752. 2004.
5. Lis-Balchin, M. Feed additives as alternatives to antibiotic growth promoters: botanicals. *Proceedings of the 9th International Symposium on Digestive Physiology in Pigs*. University of Alberta, Canada, publisher. 1:333-352. 2003.
6. Partanen, K.H. and Z. Mroz. *Nutrition Research Reviews* 12:117-145. 1999.
7. Piva, A. and M. Tedeschi. Composition for use in animal nutrition comprising a controlled release lipid matrix, method for preparing the composition and the method for the treatment of monogastric animals. United States Patent Application Publication. Pub. No.: US 2004/0009206 A1; EU patent EP1391155B1. 2004.
8. Presser, K.A., D.A. Ratkowsky, and T. Ross. *Applied & Environmental Microbiology*. 63:2335-2360. 1997.
9. Rosen, G. Setting and meeting standards for the efficient replacement of pronutrients antibiotics in poultry and pig nutrition. *Antimicrobial Growth Promoters: Worldwide Ban on the Horizon*. Noordwijk aan Zee, The Netherlands, p. 66. 2005.
10. Ultee A., E.P.W. Kets, and E.J. Smid. *Applied and Environmental Microbiology* 65:4606-4610. 1999.
11. van Dam, J.T.P., M.A.M. Vente-Spreeuwenberg, and H.P.T. Kleuskens. Combination of medium chain fatty acid and organic acids provides a cost-effective alternative to AGP in pig nutrition. *Antimicrobial Growth Promoters: Worldwide Ban on the Horizon*. Noordwijk aan Zee, The Netherlands, p. 5. 2005.
12. van Kol, E.M.R. Organic acids and essential oils in AGP free diets. *Antimicrobial Growth Promoters: Worldwide Ban on the Horizon*. Noordwijk aan Zee, The Netherlands, p. 7. 2005.
13. van Wesel, A.A.M., H.B. Perdok, and D.J. Langhout. Phasing out antimicrobial growth promoters. *II Congresso Latino Americano De Suinicultura*, Foz do Iguaçu, Brasil, 20-22 Outubro, p. 141-144. 2004.

Table 1. Effect of essential oils and essential oils-organic acid combination on production parameters, no challenge farm trial, broiler chickens, 28 days of age, $P < 0.05$ (Jefo Nutrition internal data).

	# of chickens	Body weight (g)	DWG (g)	Feed conversion	% mortality
Negative control	1198	1.406[a]	48.70[a]	1.605[a]	3.83
Essential oils (2)	1200	1.367[b]	47.31[b]	1.621[a]	3.00
EO-OA (1)	1198	1.436[c]	49.79[c]	1.557[b]	2.59
EO-OA (2)	1200	1.467[d]	50.90[d]	1.560[b]	3.67

EFFICACY STUDY OF A LIVE *E. COLI* VACCINE IN COMMERCIAL TURKEYS RAISED IN FLOOR PENS

Cheryl Gustafson[A], Stephen W. Davis[B], and Kalen Cookson[A]

[A]Fort Dodge Animal Health, Overland Park, KS 66210
[B]Colorado Quality Research, Inc., Wellington, CO 80549

ABSTRACT

A recently licensed live *Escherichia coli* vaccine has proved to reduce losses due to *E. coli* infections and provide added protection for chickens in the field. It is the purpose of this report to present the results of a floor pen study demonstrating the efficacy of this live *E. coli* vaccine in commercial turkeys. Two vaccination programs were tested, one at three days of age and the second treatment group at three days of age and three weeks of age. Birds were challenged at six weeks of age with a virulent strain of *E. coli.* Protection was evaluated by necropsy of the birds one week following challenge. These results show that both vaccination programs provide protection in turkeys against *E. coli* infection.

INTRODUCTION

E. coli infections cause severe losses both in chickens and turkeys. *E. coli* was first isolated in chickens in the late 1800s and over a century later is still causing mortality and disease in not only chickens but turkeys as well. It is recognized as one of the more damaging and economically important disease causing bacteria in the poultry industry. Colibacillosis can involve either localized or systemic infections of *E. coli*. It is the disease causing *E. coli*, known as avian pathogenic *E. coli* (APEC) that appear to be increasing in virulence and resistance (1,2), keeping this age-old bacterium an ever increasing concern to the poultry industry.

MATERIALS AND METHODS

The poults (Nicholas toms) were raised on wood shavings and housed at 36 birds per floor pen. Feed and water was provided *ad libitum.* Each treatment group consisted of 4 repetitions (pens) for a total of 144 birds per group. Treatment group B received the live *E. coli* test vaccine (*E. coli* aroA - attenuated strain) at three days and three weeks of age. Treatment group C received the test vaccine at three weeks of age. The test vaccine was administered by coarse spray using the ShurFlo Backpack sprayer with the XR TeeJet nozzle at 40psi. Groups A and D did not receive the test vaccine. All birds received live Newcastle disease B1, B1 vaccine on days 14 and 35 via drinking water and HE (hemorrhagic enteritis) vaccine on day 28. No other treatments or medications were given during the study.

Groups A, B, and C were challenged and group D was the negative control. At 42 days of age the designated birds were challenged according to the established model for intratracheal (IT) inoculation with a virulent (APEC) *E. coli* serotype O78 isolate. Titer determined at time of challenge was 1.1×10^9 CFU/bird. All birds were observed daily for clinical signs and mortality. Dead or morbid birds were removed and a post-mortem examination was done to determine the cause of morbidity/mortality. At 49 days of age, seven days post-challenge, all remaining birds were posted and lesions recorded.

RESULTS

The mortality for this study before challenge was 0.7 to 3.3%. The two control groups had mortality greater than 1% and the two vaccinated groups fell below 1% mortality prior to challenge. The three-day vaccinated group was the only group without mortality in the first two weeks. The mortality for the challenged treatment groups ranged from 24 to 51%. The unvaccinated challenged group suffered 51% mortality, while the vaccinated groups were at 24% and 33% mortality. Percent severe airsacculitis was also less in the vaccinated group compared to the unvaccinated challenged group which was 94%. Negative controls suffered no severe airsacculitis or mortality.

DISCUSSION

The APEC challenge in this study was very severe with over 50% mortality and over 90% severe airsacculitis in the unvaccinated control group. Despite this strong challenge the live *E. coli* vaccine did provide protection and decreased mortality and incidence of severe airsacculitis. Prior to challenge, it was noted that the vaccinated groups had better livability. This may suggest added protection from low level infection. Both treatment groups provided protection against the six-week challenge. With *E. coli* being a ubiquitous bacterium, early vaccination may be beneficial, especially when early challenge is imminent.

REFERENCES

1. Nolan, L.K. Emergence of avian pathogenic *Escherichia coli* with enhanced resistance and disease causing capacity. In: Iowa Egg Symposium Proceedings. Iowa State University, Ames, IA. 2007.

2. Rodríguez-Siek, K.E., *et al.* Characterizing the APEC pathotype. Vet. Res. 36:241-256. 2005.

EFFICACY OF DIFFERENT DOSES OF PAROMOMYCIN IN THE FEED AGAINST *HISTOMONAS MELEAGRIDIS* IN EXPERIMENTALLY CHALLENGED TURKEY POULTS

H. M. Hafez[A], A. Lotfi[A], R. Hauck[A], and K. De Gussem[B]

[A]Institute of Poultry Diseases, Faculty of Veterinary Medicine, Free University Berlin,
Königsweg 63, 14163 Berlin, Germany
E-mail: hafez@vetmed.fu-berlin.de
[B]Huvepharma, Uitbreidingstraat 80, 2600 Antwerp, Belgium
E-mail: Koen.degussem@huvepharma.com

SUMMARY

The protozoan parasite *Histomonas meleagridis* is capable of causing an enterohepatitis in gallinaceous birds. In the last years outbreaks of histomoniasis in turkey flocks have caused considerable economic losses, since currently no prophylactic or therapeutic drugs against the parasite are licensed in the European Union due to food safety concerns (1).

Paromomycin is an aminoglycoside antibiotic with activity against most gram-negative and many gram-positive bacteria, some protozoa and many cestodes. Currently, paromomycin is registered for all food producing animal species, but not for use in animals from which milk or eggs are produced for human consumption.

According to Lindquist (3) the application of 1000 to 2000 ppm paromomycin sulfate in the feed reduced mortality after experimental infection with *H. meleagridis* significantly (3). *In vitro* paromomycin sulfate reduced the growth of parasites at 10 µg/mL, and was completely effective at 100 µg/mL. However, 200 or 400 ppm paromomycin sulfate given in the feed starting one day before infection did not reduce lesions in ceca and livers of experimentally infected chickens (2).

In the present study we evaluated the efficacy of different doses of paromomycin in the feed against *H. meleagridis* in experimentally challenged turkey poults.

To that end 130 male one-day-old turkey poults were divided in four groups of 30 birds each (groups IC, T100, T200, T400) and an additional group (NC) consisting of 10 birds. Groups NC (non-infected-non-treated, negative control) and IC (infected non-treated, positive control) received feed without anti-histomonal drugs. Groups T100, T200, and T400 were given feed with 100, 200 and 400 ppm paromomycin (histoBloc®, Huvepharma, Antwerp, Belgium) respectively starting on day one through day 42 (end of the experiment). On day 21, each bird in groups IC, T100, T200 and T400 received 1 mL Dwyer's medium containing 150,000 histomonads intracloacally. After the challenge, the birds were observed for three weeks post infection, and daily mortality, feed and water consumption, as well as the feed conversion rate were recorded. After three weeks the surviving birds were euthanized. All birds were examined for pathological changes. Lesions in ceca and liver were scored.

Before the challenge there was no significant difference between untreated and treated groups in regard to daily weight gain, feed and water consumption, as well as feed conversion rate.

There was no mortality in group NC during the entire experiment. In the infected groups mortality started around day nine post infection and ended between day 15 and 20 post-infection. Mortality was 80% in group IC (infected non-treated). In the treated groups the mortality rate was: 73.3% in T100, 43.3% in T200, and 20% in T400 group. In all treated groups mortality was significantly less than in group IC.

The mean lesion scores in liver and ceca were significantly lower in groups T200 and T400 than in groups IC and T100. No histomonal DNA was found in ceca and livers of the surviving birds.

The mean weight of the surviving birds was not significantly different from the birds of the uninfected control. The feed conversion rate as well as European production efficiency factor was significantly better in groups T200 and T 400 than in groups T100 and IC.

In conclusion, a prophylactic application of histoBloc in the feed was effective against the challenge with *H. meleagridis*. In the field it might prevent the losses of whole turkey flocks due to outbreaks of histomoniasis.

REFERENCES

1. Hafez, H.M., R. Hauck, D. Lüschow, and L.R. McDougald. Comparison of the specificity and sensitivity of PCR, nested PCR, and real-time PCR for the diagnosis of histomoniasis. Avian Dis.49:366–370. 2005.

2. Hu, J. and L.R. McDougald. The efficacy of some drugs with known antiprotozoal activity against *Histomonas meleagridis* in chickens. Vet Parasitol 121:233-238. 2004.

3. Lindquist, W.D. Some effects of paromomycin sulfate on blackhead in turkeys. Am J Vet Res 23:1053-1056. 1962.

MOLECULAR TYPING OF INFECTIOUS BRONCHITIS VIRUS

H. M. Hafez, V. L. deQuadros, and D. Lüschow

Institute of Poultry Diseases, Faculty of Veterinary Medicine
Freie Universität Berlin, Königsweg 63, 14163 Berlin, Germany

SUMMARY

Infectious bronchitis (IB) is an acute, rapidly spreading disease of chickens characterized by respiratory signs, drop in egg production, and poor egg quality or nephritis/nephrosis. The causative agent, infectious bronchitis virus (IBV) is a member of the genus *Coronavirus*, within the family *Coronaviridae*. The genome of IBV consists of single-stranded positive sense RNA coding for four structural proteins: the nucleocapsid protein – N, the membrane protein – M, the small membrane protein – E, and the spike protein – S which consists of the two subunits S1 and S2. The high genetic variation within the S1 protein is responsible for multiple serotypes and variant strains which have been identified throughout the world. The occurrence of IBV serotypes and strains vary from country to country as well as from region to region.

In the last years, molecular biological methods like polymerase chain reaction (PCR) systems have become increasingly important for a fast and sensitive diagnosis of IB. The use of IBV universal primers allows the general diagnosis of the infection, the various serotypes and variant strains can be differentiated by IBV type specific primers or rather by restriction enzyme analysis of PCR products or sequence analysis of selected genome regions. The objective of the present study was the development of a molecular system for detection and typing of IBV field strains circulating in German poultry flocks. For this reason a system composed of three different steps was established:

1. The general diagnosis of IBV infection is carried out by a RT-PCR using a universal primer pair which amplified a fragment within the conserved N protein gene of IBV (3) and which is able to detect all circulating serotypes and variant strains (Fig. 1). In addition, IBV positive samples are investigated by a subtype specific RT-PCR for detection of subtype 4/91 IBV with primers located within the S1 gene region (3) and which allowed an exclusive hybridization with Subtype 4/91 IBV RNA (Fig. 1).

2. For further molecular typing of almost all serotypes and variant strains of IBV a RT-PCR system combined with restriction enzyme analysis (REA) or sequence analysis of PCR products was established. For this purpose a primer pair was chosen, which was localised on the one hand within relative conserved parts of the S1 gene and enclosed on the other hand the S1 gene hypervariable region (4,6). The subsequent restriction enzyme analysis of amplified PCR products using at least two different enzymes should allow an easy and fast differentiation. Initial investigations revealed that selected primers were able to amplify a fragment of the expected size from all available reference and variant strains. These strains were differentiated by the use of two different restriction enzymes (Fig. 2).

However, analysis of IBV universal PCR positive field samples showed with the used primer pair in some cases only very weak or negative results. So, in consequence of the high sequence variation of some IBV strains the system was expanded by a further primer pair and a set of nested primers for amplification of some field strains (1,2) and subsequent sequence analysis of the amplified fragment. Up to now different viruses of the Massachusetts type as well as 4/91, D1466, V1397, and QX-like IBV were detected and differentiated with the established system.

3. Recent investigations reported about the incidence of the new subtype QX-like IBV in Europe (5). In this view we have developed a subtype specific RT-PCR for fast and sensitive detection of QX-like IBV. The designed primers which amplified a fragment of about 220 bp within the S1 gene region revealed only positive results for this subtype (Fig. 3).

Using this PCR a retrospective study from 2004 to 2008 of IBV positive samples showed a dramatic increase of QX-like IBV in German poultry flocks. In 2008 QX-like IBV was detected in more than 60% of IBV positive samples, mainly in samples derived from broiler flocks.

Altogether, the established system composed of three different steps has shown to be a reliable method for diagnosis and monitoring of IBV infection.

REFERENCES

1. Bochkov, Y.A., G. Tosi, P. Massi, and V.V. Drygin. Phylogenetic analysis of partial S1 and N gene sequences of infectious bronchitis virus isolates from Italy revealed genetic diversity and recombination. Virus Genes 35:65-71. 2007.

2. Dolz, R., J. Pujols, G. Ordonez, R. Porta, and N. Majo. Antigenic and molecular characterization of

isolates of the Italy 02 infectious bronchitis virus genotype. Avian Pathology 35:77-85. 2006.

3. Handberg, K.J., O.L. Nielsen, M.W. Pedersen, and P.H. Jorgensen. Detection and strain differentiation of infectious bronchitis virus in tracheal tissues from experimentally infected chickens by reverse transcription-polymerase chain reaction. Comparison with an immunohistochemical technique. Avian Pathology 28:327– 335. 1999.

4. Kwon, H.M., M.W. Jackwood, and J. Gelb, Jr. Differentiation of infectious bronchitis virus serotypes using polymerase chain reaction and restriction fragment length polymorphism analysis. Avian Diseases 37:194-202. 1993.

5. Worthington, K.J., R.J. Currie, and R.C. Jones. A reverse transcriptase-polymerase chain reaction survey of infectious bronchitis virus genotypes in Western Europe from 2002 to 2006. Avian Pathology 37:247-257. 2008.

6. Yu, L., Z. Wang, Y. Jiang, S. Low, and J. Kwang. Molecular epidemiology of infectious bronchitis virus isolates from China and Southeast Asia. Avian Diseases 45:201-209. 2001.

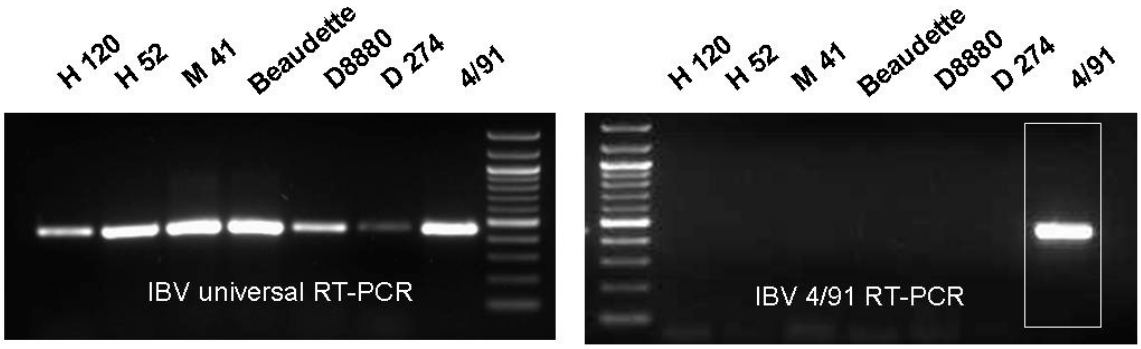


Fig. 1 Specificity of selected primer pairs of the IBV universal and IBV 4/91 RT-PCR

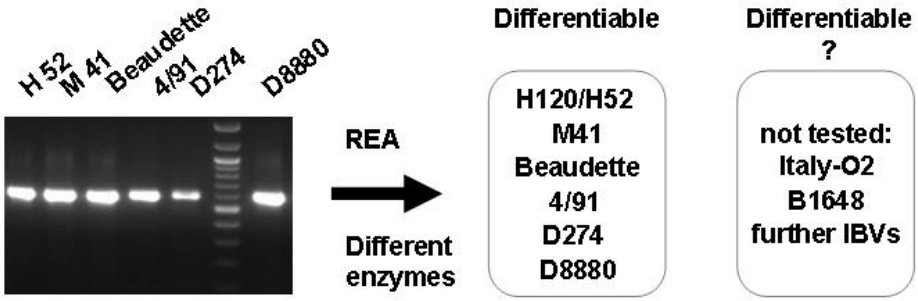


Fig. 2 Differentiation of IBV reference strains by REA of PCR products

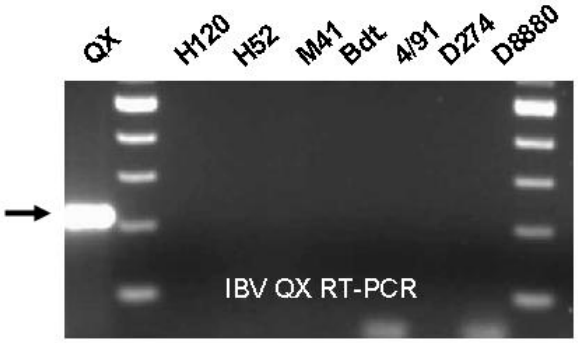


Fig. 3 Specificity of IBV QX RT-PCR

NEW APPROACHES TO POULTRY DISEASE CONTROL

David A. Halvorson

University of Minnesota, 1971 Commonwealth Avenue, Saint Paul, Minnesota 55108
Halvo002@umn.edu

INTRODUCTION

In 1992 exotic Newcastle disease (END) struck a flock of range turkeys on a farm in North Dakota. Authorities quickly moved in to quarantine the affected flock. They were killed with CO_2, pushed into a pit and burned on the farm. At that point it was discovered that there was a second flock on the farm. This flock had been vaccinated for Newcastle disease and had experienced no clinical signs. It was sent to market. Later the flock service person wryly commented that both approaches, mass animal destruction (MAD) and controlled marketing (CM) had been successful. It was the comment about two successful control methods used in this outbreak that stimulated the thinking behind this presentation.

HISTORY OF SOME POULTRY DISEASE OUTBREAKS

In 1924 the fowl plague epidemic became the first and most widespread serious poultry disease in the U.S. It began in New York City live poultry markets and ultimately involved poultry in 10 states, spreading all the way to Saint Louis, Missouri. The disease was recognized as a serious outbreak in the fall of 1924 and was eradicated by the spring of 1925. The disease was largely controlled and eradicated by enforcement of an embargo of incoming poultry into the live poultry markets and sanitation measures directed at conveyances of poultry. This was the last time in the U.S. that a foreign animal disease caused a large multi-state poultry outbreak.

In 1970 END affected several hundred thousand laying hens on four commercial farms, many back yard flocks and hundreds of wild birds in the El Paso, Texas, and Chamberino, New Mexico, area. By the time the disease was recognized it had apparently killed or immunized all the wild and domestic birds in the area and it quietly disappeared.

In 1971-74 a large END outbreak affected millions of chickens in southern California and was successfully controlled and eradicated by application of movement control, quarantine, sanitation and MAD. Over 12 million chickens were killed.

In 1978 turkey growers in Minnesota experienced a large outbreak of low pathogenicity avian influenza (LPAI) due largely to H6N1, but H4N8, H6N2, H6N8 and H9N2 infections were also detected. One hundred thirty market turkey flocks, 11 breeder flocks and three egg laying chicken flocks were involved. This outbreak was successfully controlled by isolation of affected flocks, traffic control, sanitation and CM.

In 1983-84 Pennsylvania poultry producers in the Lancaster area experienced a LPAI H5N2 outbreak from which a highly pathogenic avian influenza (HPAI) virus emerged. This HPAI outbreak was controlled and eradicated by application of quarantine, movement controls, sanitation and MAD. Seventeen million birds were destroyed.

In 1988 and again in 1991 Minnesota turkey growers experienced large LPAI outbreaks involving 258 turkey flocks and one broiler breeder flock in 1988 and 110 flocks of turkeys in 1991. Subtypes H1, H2, H4, H5, H6, H7, H8, and H9 were detected. Even though LPAI H5 and H7 had been detected, turkey growers, seemingly unaware of the potential danger of HPAI emerging, utilized isolation of affected flocks, traffic control, sanitation and CM.

More recent large poultry disease outbreaks include the following:

- 1995 LPAI in Minnesota successfully controlled - CM
- 1995 LPAI in Utah successfully controlled - vaccination and CM
- 2000-3 LPAI in California successfully controlled - vaccination and CM
- 2002-3 END in California successfully controlled – MAD
- 2002 LPAI in Virginia successfully controlled - MAD
- 2003 LPAI in Connecticut successfully controlled - vaccination and CM
- 2004 HPAI in British Columbia successfully controlled - MAD.

Lessons learned from historical outbreaks. The lesson seems to be that both MAD and CM are associated with successful disease control.

If we look at more recent small outbreaks of H5 or H7 AI, there is a similar trend:

- 2007 LPAI in West Virginia successfully controlled – MAD
- 2007 LPAI in Minnesota successfully controlled – CM
- 2007 LPAI in South Dakota successfully controlled – MAD

- 2007 LPAI in Nebraska successfully controlled – CM
- 2007 LPAI in Virginia successfully controlled - MAD

All these small outbreaks were controlled on the index farm.

The origin of MAD has its roots in the beginning of the 18th century when Pope Clement XI decreed that clubbing and deep burial of affected cattle would be used to eradicate what we now know as Rinderpest. This divine procedure was soon followed by other European countries and later by the United States as well (2). Mass animal destruction of animals in a disease outbreak has been called "stamping out" by O.I.E.

A lot has happened since 1711. Microbiology was discovered; our arsenal of disease control tools has expanded; agriculture has evolved; dramatic sights on television shock consumers; and the ethics of culling healthy animals is being questioned.

According to the National Highly Pathogenic Avian Influenza Response Plan: "When HPAI outbreaks occur in poultry, the preferred eradication and control methods are quarantine, enforcement of movement restrictions, and depopulation (culling) of all infected, *exposed, or potentially infected birds*, with proper disposal of carcasses and rigorous cleaning and disinfection of farms and surveillance around affected flocks." (emphasis mine)

With the advent of LPAI control plans, similar goals and language appear in some state control plans as well. So if both approaches are associated with success, the natural question is: "What do MAD and CM have to do with control?"

It appears that after the disease is under control either approach can be used successfully. There are cases when neither strategy has been particularly successful, and those cases involve actively infected birds being transported to market or being depopulated. So when birds are actively infected either strategy can fail; when the infection is under control either strategy can be successful. It would appear that MAD and CM are disposal strategies rather than control strategies.

Even though the public finds it distasteful, there is support in the veterinary community for stamping out when applied to emergency diseases. This support is strong but not universal, but what happens if healthy flocks are involved? It is often said that stamping out is the most cost effective strategy; however, recent stamping out programs involving poultry disease all eclipsed the $100 million mark in their total costs (Virginia, California, Italy, the Netherlands and British Columbia). It was recently pointed out that stamping out programs for low path AI may cost 10 to 100 times more than controlled marketing (1).

A PROPOSAL

It is questionable whether the modern poultry industry can tolerate the expense and drama of MAD in the control of LPAI, and it is questionable whether some developing countries can tolerate this method in the control of HPAI. The question of whether an alternative strategy would have been more effective has not been asked. In the absence of research trials to document the advantage of this archaic approach, regulatory officials should examine and document instances where emergency diseases were satisfactorily brought under control with a different approach. Low pathogenic avian influenza outbreaks have been effectively controlled by vaccination and CM as well as MAD, but for substantially less money.

Because industry-driven CM programs as well as government-driven MAD programs have been successful, a thoughtful examination of MAD programs leads to the idea that their success is related, not to the destruction of infected, susceptible and convalescent poultry, but to the enforced downtime, designation of infected zones, imposition of quarantines, and intensive monitoring. There is nothing special about killing and burying or burning poultry because disease outbreaks have been stopped by alternative means. Thus we can infer that it is the government's authority to quarantine, order cleaning and disinfecting, monitor and permit repopulation that accounts for its success in controlling disease. These strengths in government programs match up well with the major weakness of industry programs.

The modern poultry industry is driven by the companies' needs for meat and eggs. The weakness of industry-driven disease control is that this need for a continuous supply of meat and eggs may cause companies to act in ways that do not contribute to disease control and may actually contribute to disease spread.

A new hybrid disease control program is proposed that encompasses the best that industry and government programs have to offer. Industry, State and APHIS veterinarians, in a cooperative arrangement, could initiate well-thought out measures when a disease outbreak occurs. These measures would include biosecurity, flock scheduling, processing, vaccination, and area repopulation.

CONCLUSION

CM and MAD are disposal strategies. Successful disease control strategies have more to do with the universally accepted isolation or quarantine, movement control and sanitation rather than to one disposal technique or another. There is widespread agreement on the importance of isolation, traffic control and sanitation even if agreement is lacking on what to do with infected, exposed, potentially infected, and convalescent birds.

REFERENCES

1. Halvorson, D.A., *et al*. The economics of avian influenza control. Proceedings of the 52nd Western Poultry Disease Conference. Pp 5-7, March 8-11, 2003. Sacramento, California. 2003.

2. Kaleta, Erhard. A critical time for poultry diseases. Proceedings of the XIIIth Congress of the World Veterinary Poultry Association. July 19-23, 2003. Denver, Colorado. 2003.

CONTROL OF AVIAN PATHOGENIC *E. COLI* IN COMMERCIAL POULTRY

D. Karunakaran, C. Kromm, G. Siragusa, and T. Rehberger

Agtech Products, Inc. Waukesha, WI

INTRODUCTION

Avian pathogenic *Escherichia coli* (APEC) comprise a specific subset of pathogenic *E. coli* that cause extraintestinal diseases of poultry (4). APEC consists mainly of enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) serovars (5). APEC is found in the intestinal microflora of healthy birds and infections are enhanced or initiated by secondary environmental and host predisposing factors. Colibacillosis is a common systemic infection caused by APEC, and occurs most commonly as acute septicemia or subacute airsacculitis and polyserositis in chickens, turkeys, and other avian species (6).

Recently, virulence factors associated with avian colibacillosis have been identified. These genes include the aerobactin iron sequestering system (*iuc*C), temperature-sensitive hemagglutinin (*tsh*), increased serum survival (*iss*), and the structural genes of the colicin ColV operon (*cva*C). The aerobactin iron sequestering system allows bacteria to grow in low levels of iron (3) while the Tsh protein is an autotransporter that may contribute to the development of lesions within the air sacs of birds (4). The increased serum survival gene has been found to contribute to complement resistance of a human *E. coli* isolate and its presence is strongly correlated with *E. coli* isolated from birds with colibacillosis (7). The Col V plasmid codes for colicin V which causes membrane leakage in target cells (2).

The presence of these four genes identified through multiplex PCR can facilitate the identification of avian pathogenic *E. coli* isolates and when combined with randomly amplified polymorphic DNA (RAPD) PCR will be useful for examining the diversity of the avian pathogenic *E. coli*. This study reports the prevalence, distribution and diversity of APEC in commercial poultry. Understanding the distribution and diversity of APEC is the first step toward developing methods for controlling colibacillosis in poultry production.

MATERIALS AND METHODS

Three birds are collected from several commercial broilers and turkey flocks ranging from 17 to 77 days of age were collected. Whole intestinal tracts were removed, tied off at the esophageal and cecal ends, and immediately placed in whirl-pak bags containing enough sterile saline to cover the entire tract. Tracts were shipped overnight to Agtech Products, Inc. for analysis of pathogenic *E. coli*.

Three sections (upper, mid, and lower) were aseptically removed from each intestinal sample and pooled together for a composite sample for each bird. All three sections were rinsed with sterile phosphate buffer until all contents were washed out and the liquid ran clear. The sections were cut lengthwise to expose the epithelial lining and the sterile rinse was repeated. The three sections were weighed and combined in a sterile whirl-pak bag. Sterile 0.1% peptone water (99 mL) was added to each bag and the contents masticated for 60 seconds. All samples were plated on CHROMagar (CHROMagar Paris, France) for the enumeration of *E. coli*. Spiral plating techniques were used at 10^{-2} and 10^{-4} dilutions on the Autoplate 4000 (Spiral Biotech, Inc., Norwalk, MA). Plates were incubated at 37°C for 24 h before counting on the Q-Count system (Spiral Biotech). The counts were recorded and the plates with *E. coli* colonies were saved for further analysis.

Five colonies were picked from each sample unless there were less than five present. Colonies were grown in Trypticase Soy Broth (TSB) at 37°C for 24 h. DNA was isolated using a High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany). A multiplex PCR procedure (8) was used to determine the virulence genes present in each isolate. The PCR mixture contained: 5µL 10X buffer without $MgCl_2$, 2 µL 10mM dNTP mix, 0.3 µL 5U/µL Platinum Taq Polymerase (Invitrogen, Carlsbad, CA), 4 µL 50mM $MgCl_2$, 0.5 µL *iss* upper and lower primer, 0.3µL *tsh*, *iuc*C, *cva*C upper and lower primers, 31 µL sterile dH_2O, and 5 µL genomic DNA to give a final volume of 50 µL. Amplification for the multiplex analysis was performed in a GeneAmp PCR System Thermal Cycler (Applied Biosystems, Foster City, CA). The PCR program began with a pre-incubation of 95°C for 5 min.; then nine cycles of: 95°C for 59 s, 55°C for 30 s, 72°C for 59 s; 28 cycles of: 94°C for 30 s, 55°C for 30 s, 72°C for 30 s; and finished with a seven min. incubation at 72°C. PCR products were identified by electrophoresis on a 3% Nu-Sieve agarose gel (BioWhittaker, Rockland, ME) and visualized by UV transillumination after staining in ethidium

bromide solution. Gel images were captured using the Syngene BioImaging System.

Images from the RAPD PCR gels were incorporated into a dendrogram using the Bionumerics software program (Applied Maths, Belgium). The molecular weights of the banding patterns were analyzed and both primers used band matching at 2.00% tolerance. Dice comparisons were created for each primer separately before composing a composite data set. Both primers were weighted equally and by combining the Dice comparisons a composite dendrogram was created. *E. coli* was enumerated using a spiral plating technique on CHROMagar and counted on the Q-Count system.

A multiplex PCR procedure was used to determine the pathogenicity of the *E. coli* isolates. In this procedure, four virulence genes were identified as markers for pathogenicity. A positive control was used to indicate where the four genes migrated on the gel. The gels were analyzed visually for bands at the appropriate molecular weight.

As stated by Skyberg, *et al*. (8), the possession of two or more of these genes is a strong predictor of virulence. While the use of a chick embryo lethality assay may be the best way to determine virulence of avian *E. coli*, a multiplex procedure could allow for rapid screening of possible pathogenic *E. coli*.

RAPD PCR is performed to examine the diversity between the *E. coli* isolates. The dendrogram constructed from the RAPD PCR analysis shows the relative relatedness of the isolates to one another. The virulence genes present for each isolate along with the ages of the birds are included on the dendrogram to show the distribution of the pathogenic *E. coli*. A line is drawn at an 80% similarity coefficient results in clusters or family groups.

CONCLUSIONS AND APPLICATION

- All commercial poultry farms show varying prevalence levels of *E. coli* within sites.
- Subclinical levels of APEC compromise bird health and performance.
- The molecular tools discussed help understand the diversity of APEC and the distribution of virulence factors.
- *E. coli* isolates with two or more virulence factors indicating a possibility of being pathogenic isolates.
- Application of Direct Fed Microbial products in reducing APEC levels will be discussed.

REFERENCES

1. Altekruse, S.F., F. Elvinger, C. DebRoy, F.W. Pierson, J.D. Eifert, and N. Sriranganathan. Pathogenic and fecal *Escherichia coli* strains from turkeys in a commercial operation. Avian Diseases 46:562-569. 2002.
2. Brock, T.D., M.T. Madigan, J.M. Martinko, J. Palmer. Biology of microorganisms. Seventh Edition. pp 264-265. 1994.
3. Dho-Moulin, M., and J.M. Fairbrother. Avian Pathogenic *Escherchia coli* (APEC). Vet. Res. 30:299-316. 1999.
4. Dozois, C.M., M. Dho-Moulin, A. Bree, J.M. Fairbrother, C. Desautels, and R. Curtiss III. Relationship between the Tsh autotransporter and pathogenicity of avian *Escherichia coli* and localization and analysis of the *tsh* genetic region. Infection and Immunity. 68:4145-4154. 2000.
5. La Ragione, R.M., A.R. Sayers, and M.J. Woodward. The role of fimbriae and flagella in the colonization, invasion and persistence of *Escherchia coli* 078:K80 in the day-old-chick model. Epidemiol. Infect. 124:352-363. 2000.
6. Lee, M.D. and L.H. Arp. Colibacillosis. A laboratory manual for the isolation and identification of avian pathogens. Fourth Edition. pp 14-16. 1998.
7. Pfaff-McDonough, S.J., S.M. Horne, C.W. Giddings, J.O. Ebert, C. Doetkott, M.H. Smith, and L.K. Nolan. Complement resistance-related traits among *Escherchia coli* isolates from apparently healthy birds and birds with colibacillosis. Avian Diseases. 44:23-33. 2000.
8. Skyberg, J.A., S.M. Horne, C.W. Giddings, C. Doetkott, R.E. Wooley, P.S. Gibbs, and L.K. Nolan. Development of a multiplex PCR protocol to discern virulent from avirulent avian *Escherchia coli*. American Association of Avian Pathologists and American Veterinary Medical Association Meeting, Nashville, Tennessee. Poster 14, p 40. 2002.

A NOVEL HATCHING METHOD ON APPLICATION TO AN EXISTING COMMERCIAL HATCHERY SYSTEM RESULTS IN A MARKED DECREASE IN LATE EMBRYO LOSS

R.W. Keirs[A], E.D. Peebles[A], and M.A. Dekich[B]

[A]Mississippi State University, Mississippi State, Mississippi, USA
[B]AviTech, LLC, Salisbury, Maryland, USA

ABSTRACT

Under typical field conditions is a common multistage incubation system, hatching eggs from a single 28 week broiler breeder flock was compared to itself on being transferred into two adjacent hatchers following inovo-injection on their 18th day of incubation. Novel hatching inserts were placed in all 90 trays of the test hatcher prior to transfer placing all eggs in the vertical position for the remaining incubation period. The first three vertical columns A, B, and C consisting of 45 trays in the test hatcher were compared to their respective negative controls located in the adjacent hatcher, vertical columns A, B, and C and whose 18 day transfer was conventional, whereby all eggs were removed from their setter flats and placed horizontally in a loose array in hatchery trays. Comparisons were made utilizing hatching eggs residues as outlined in the Hatching Efficiency Analysis System (HEAS), Keirs, *et al*. Results depicted a 44.8% reduction in late embryonic loss with a 2.74% increase in estimated hatching efficiency.

Total embryonic loss of broiler hatching eggs is approximately 7.00% divided between early embryonic loss (EEL), three through 12 days (D) and late embryonic loss (LEL), 16 through 21 D at 3.80%, Hatching Efficiency Analysis System (HEAS), Keirs *et al*. Estimated hatch of fertilized eggs or Estimated Hatching Efficiency (Est HE) was 92.50%. This information comes from a base line HEAS composite of over 5000/machine/flock records which included over 500,000 hatching egg residue tabulations. EEL was primarily due to bacterial contamination of 1.82%. HEAS doesn't note but does include the mid embryonic loss 13, 14 and 15 D which would account for no more than 0.20% of embryonic loss. LEL had the more erratic loss between hatch trays. With many dead 18 and 19 D appearing normal with the majority of the 21 D being alive.

A recent study utilizing a Latin Square experimental design, abstract #30258, International Poultry Scientific Forum, Keirs *et al*, resulted in nonconventional transfer of hatching eggs having a significant reduction in 18 D embryonic loss of 54% ($P = \leq 0.01\%$) and a 19 D numerical decrease of 60%.

The objective of that study was to discern variables other than ovo-injection impacting on 18 D embryonic loss. After ovo-injection at 18 D incubation eggs conventionally transferred from setter flats to a horizontal position on hatcher trays were compared to a nonconventional transfer whereby eggs in the vertical position remained in their respective setter flats and were transferred in total to hatcher trays to complete the hatching process.

The objective of this field study was to compare the impact of a novel 168 egg capacity hatching insert on hatching efficiency and specifically on 18 D embryonic loss in eggs from a young broiler breeder flock. Such an insert could obstensibly be used from set through completion of hatch with the eggs remaining equally spaced in the vertical position, air cell up. Eggs from a single 28 week old Cobb x Cobb breeder flock were hatched simultaneously in adjacent Jamesway Super J hatchers after a 18 D ovo-injection by AviTech's Intelliject. The entire 90 trays of one hatcher were the recipient of all 15,120 eggs from setter flats during a conventional transfer. The test hatcher had all trays receive the novel hatching tray inserts whereby after the ovo-injection, the eggs were deposited on the inserts, remaining in the vertical position throughout hatch. The HEAS method for tabulating hatch residue was conducted on each tray with emphasis on LEL and Est. H.E. The first three vertical columns (A, B, C) (15 trays/column) of the conventional transfer were compared to their respective columns of the test hatcher with inserts. This allowed for 15 trays of hatch residue per replicate and three replicates per treatment for a total of 45 trays of residue per each treatment.

Compared to the conventional method the novel insert transfer resulted in a reduction for 18 D, 19 D and LEL of 45%, 54% and 47% respectively. EST HE rose to 93.87% compared to 91.28% for the conventional method.

From a pragmatic perspective such changes would be economically dramatic, for a 1% increase of chicks produced in the annual US 9 billion broiler chicks would equate too nearly 25 million dollars in chick cost.

Further studies should be actively pursued.

Table 1. Conventional LEL Embryonic Loss vs. Novel Insert

Transfer Method		16D	18D	19D	21D	LEL	EST HE
Conventional 45 Trays	A	0.44	0.56	3.68	0.20	4.88	91.49
	B	0.28	0.48	3.65	0.32	4.72	91.33
	C	0.16	0.75	3.69	0.28	4.88	91.03
A B C		0.29	0.60	3.68	0.26	4.83	91.28
Novel Insert 45 Trays	A	0.04	0.20	1.87	0.63	2.74	93.44
	B	0.04	0.36	1.35	0.48	2.22	94.02
	C	0.08	0.44	1.83	0.40	2.74	94.16
A B C		0.05	0.33	1.68	0.50	2.57	93.87
Reduction %			45	54		47	2.84*

Transfer % *increase

TIME-SCALED PHYLOGENIES OF BOTH GENOME SEGMENTS OF INFECTIOUS BURSAL DISEASE VIRUS SUGGEST MULTIPLE RE-ASSORTMENT EVENTS IN ITS EVOLUTIONARY HISTORY

Chi-Wai Yip, Chung-Chau Hon, Tsan-Yuk Lam, Meng Shi,
Fanya Zeng, and Frederick Chi-Ching Leung

School of Biological Sciences, The University of Hong Kong, Hong Kong SAR, China

ABSTRACT

Infectious bursal disease virus (IBDV) causes an immunosuppressive disease in chickens, resulting in serious economic impacts in the worldwide poultry industries. The evolutionary history of IBDV was puzzled by the phylogenetic discordance between the two genome segments for years. In this study, phylogenies of segments A and B were reconstructed to estimate the earlier divergence events in the evolutionary histories of both segments. The evolutionary rates and the time of most recent common ancestor (tMRCA) results of individual lineage in each segment were estimated independently. These evolutionary rates and tMRCA results were implemented as prior timestamps for the reconstruction of the phylogenies of the two segments. The earlier divergence events of various genotypes from the two phylogenies jointly suggest multiple re-assortment events in the evolutionary history of IBDV. This study is expected to provide a better understanding on the evolutionary history of IBDV and highlight the multiple occurrences of reassortment events which shaped the phylogeny of IBDV nowadays.

IDENTIFICATION OF *CAMPYLOBACTER JEJUNI* IN A LOCAL POULTRY FARM WITH STUDY OF TRANSMISSION ROUTE

Ning Jia, Yip Chi Wai, and Frederick Chi-Ching Leung

School of Biological Sciences, The University of Hong Kong, Hong Kong SAR, China

ABSTRACT

Campylobacter jejuni is the leading cause of food-borne human gastroenteritis in developed countries but its occurrence in Hong Kong still remains unclear. In this study, 24 chickens and certain amount of environmental samples were collected from a local poultry farm in Hong Kong for the detection of *Campylobacter jejuni*. Chicken samples including skins, toes, intestines, and feces, and environmental samples including feed and water were tested respectively. We cultured for Campylobacter isolates from each sample and examined them by polymerase chain reaction assay and DNA sequencing analysis of flaA gene. Seven out of the 24 chickens were confirmed as *Campylobacter jejuni* positive, indicating the existence of *Campylobacter jejuni* in Hong Kong. A phylogenetic tree was eventually constructed according to the sequence of flaA. This study revealed the potential threat of our isolated *Campylobacter jejuni* to the public because the phylogenetic tree shows its close relationship with some human-infecting strains discovered in USA.

AVIAN H5N1 - AN ANIMAL VIRUS!

Frederick C. Leung

University of Hong Kong
fcleung@hkucc.hku.hk

As of November 12, 2007, the cumulative number of confirmed human cases of avian influenza A/(H5N1) reported to World Health Organization (WHO) was 335, with 206 deaths. Avian influenza including H5N1 refers to a large group of different influenza viruses for which the primary host is birds and only on rare occasions will these cross over and infect other species including pigs and humans. Pandemic influenza disease occurs when a new subtype emerges that has not previously circulated in humans. Since H5N1 is a strain which posses high potential for causing serious disease in humans, WHO and other health experts have been priming the world to prepare for this threat along with OIE and FAO calling for culling million of poultry world-wide.

During my presentation, I will present a model based on the most recent event of an animal virus "crossing over" to become a human virus. I will argue that the scale of the warnings appears to outstrip the magnitude of the real threat. Further, that the culling of millions of chicken may not have actually lowered the actual risk for human health. Rather that efforts and resources should be directed to re search in understanding the molecular and genetic mechanisms which are underlying the virus crossing between species. Only then can effective barriers can then be put in place: specifically to limit the direct contact of susceptible species; to lower the transmission rate; and to avoid establishing/adaptation to a new host. In addition, I shall review recent scientific findings that avian H5N1 has remained an animal virus for the past ten years since the first scientific evidence was obtained that avian H5N1 can infect human without an intermediate host.

The probability and feasibility that the avian H5N1 will successfully adapt to human as a new host is therefore assessable in my opinion as remaining low at this particular moment.

(This paper was presented at the Fifty-Seventh Western Poultry Disease Conference, April 9-12, 2008.)

MYCOPLASMA IOWAE ASSOCIATED WITH SKELETAL LESIONS IN COMMERCIAL TURKEYS

David H. Ley, Rosemary A. Marusak, Eduardo J. Vivas, Oscar J. Fletcher, and H. John Barnes

Department of Population Health and Pathobiology
College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606
E-mail to: david_ley@ncsu.edu

Husbandry practices, metabolic and infectious diseases, and rapid growth may all play a role in the development of skeletal diseases in poultry (3). Skeletal lesions and diseases in poultry include: tibial dyschondroplasia, spondylolisthesis, chondrodystrophy, osteomyelitis, synovitis, mycoplasmosis, and others (2,4). *Mycoplasma iowae* (MI) infection in turkey breeders causes reduced hatchability (2 to 5%), embryo mortality (in later stages of incubation), and leg deformities (1,5). Trampel (5) described 17-day-old turkey poults with leg weakness, dehydration, chondrodystrophy of the hock joints, clear fluid in hock joint spaces, valgus deformities and shortening of the tarsometatarsal bones, and curled toes associated with MI. Experimentally in chickens and turkeys, MI has also induced airsacculitis, tenosynovitis, and arthritis, rupture of digital flexor tendons, rotated tibia and cartilage erosion (1,5).

Swollen hock joints (arthritis) and skeletal lesions occurred at very low incidences in commercial turkey flocks from two primary breeders. MI was identified by culture/immunofluorescence and MI-specific polymerase chain reaction (PCR) from some of the vertebral (back and neck) lesions, but not from swollen joints. Mycoplasma culture of vertebral lesion samples was a more sensitive diagnostic method than MI PCR. Gross skeletal lesions were consistent with chondrodystrophy. Histologic lesions were consistent with osteochondrosis, with dyschondroplasia and osteomyelitis also present. This is the first report of MI associated with vertebral chondrodystrophy and osteochondrosis in turkeys, and should now be considered in the differential diagnosis of turkeys with these lesions.

(The full-length article will be published in *Avian Pathology*.)

REFERENCES

1. Bradbury, J.M., and S.H. Kleven. Mycoplasma iowae Infection. p. 856-862. *In* Y. M. Saif (ed.), Diseases of Poultry, 12th ed. Blackwell Publishing, Ames, Iowa. 2008.

2. Crespo, R., and H.L. Shivaprasad. Diseases of the Skeleton, p.1154-1162. *In* Y.M. Saif (ed.), Diseases of Poultry, 12th ed. Blackwell Publishing, Ames, Iowa. 2008.

3. Morrow, C.J., J.M. Bradbury, M.J. Gentle, and B. H. Thorp. The development of lameness and bone deformity in the broiler following experimental infection with *Mycoplasma gallisepticum* or *Mycoplasma synoviae*. Avian Path. 26:169-187. 1997.

4. Sullivan, T.W. Skeletal problems in poultry: estimated annual cost and descriptions. Poult. Sci. 73:879-882. 1994.

5. Trampel, D.W. and F. Goll Jr. Outbreak of *Mycoplasma iowae* infection in commercial turkey poults. Avian Dis. 38:905-909. 1994.

ANTIBODY RESPONSE TO H5 POX-VECTORED VACCINE IN UNCONVENTIONAL POULTRY

Benjamín Lucio-Martínez

Avian Diagnostic Laboratory, Animal Health Diagnostic Center
College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

SUMMARY

Inactivated water-in-oil (W/O) vaccines have been widely used to prevent economic losses in chickens and turkeys exposed to low-pathogenicity avian influenza (LPAI) in Mexico, Pakistan, Guatemala, El Salvador, Italy, USA, and Hong Kong (1). These vaccines are well tolerated by chickens and turkeys, but may not be acceptable for use in other poultry species, frequently raised outdoors and at an even a higher risk of exposure to avian influenza.

Objections to the use of O/W vaccines in non-conventional poultry may include behavior modification due to pain, and formation of granulomatous lesions in the muscle as is the case in cattle (5). In addition, vaccines in game birds are more likely to be applied by untrained personnel, increasing the risk of accidental auto-injection of O/W vaccines, resulting in severe lesions in humans (2,4). Fowl pox-vectored avian influenza H5 vaccines have also been used extensively in chickens (1), and reported to induce immunity in cats (3). This study was done to determine the antibody response of ring-neck pheasant, chuckar partridge, turkey, white Peking duck, peafowl, and Guinea fowl after one or two subcutaneous injections of a fowl pox-vectored avian influenza H5 vaccine (Trovac-AIV H5, Merial Select, Inc., Athens GA).

MATERIALS AND METHODS

Birds. Ring-neck pheasants, chuckar partridge, turkey, peafowl, and Guinea fowl were obtained from commercial sources. White Peking ducks were obtained from Cornell's specific-pathogen-free flocks. The birds were raised in isolation from one day of age at Cornell University.

Experimental procedure. At experimental day 0 (21 days of age) the birds were bled and randomly assigned to one of four groups. Each group was made of five birds. The first group was used as negative control and was given no treatment. The remaining 15 birds were vaccinated subcutaneously with a calculated dose of 1 million infectious doses in 0.5 mL. At experimental day 21, negative control and vaccinated birds were bled. The vaccinated groups were injected again subcutaneously with a calculated dose of 1 million infectious doses in 0.5 mL. The birds were bled on experimental day 42, and humanely euthanized using CO2.

RESULTS

Hemagglutination-inhibition (HI). HI tests were performed using 4 HA units of inactivated A/tky/Ire/83 H5N8. Negative control groups had titers of 1.2 log2 or less in all cases. The vaccine induced antibodies in all vaccinated birds as shown in Table 1.

DISCUSSION

These results indicate that fowl pox-vectored H5 avian influenza vaccines induced a good antibody response in turkey, chuckar partridge, white Peking duck, ring-neck pheasant, and peafowl. In Guinea fowl the response was considerably lower. Whether these levels of antibodies will protect against disease is an open question, and it will likely depend on the pathogenicity of the challenging virus.

ACKNOWLEDGMENTS

Our deep appreciation to Dr. Kemal Karaca who provided the vaccine used in these studies, and to Dr. David Swayne for the inactivated H5 antigen.

REFERENCES

1. Capua, Ilaria and D.J. Alexander. Avian influenza: recent developments. Avian Pathology 33:393-404. 2004.

2. Fukumi, H. Effectiveness and untoward reactions of oil adjuvant influenza vaccines. Symp. Series Immunobiol. Stand. 6:237. 1967.

3. Karaca, K., D.E. Swayne, D. Grosenbaugh, M. Bublot, A. Robles, E. Spackman, and R. Nordgren. Immunogenicity of Fowlpox Virus Expressing the Avian Influenza Virus H5 Gene (TROVAC AIV-H5) in Cats. Clin. Diagn. Lab. Immunol. 12(11): 1340–1342, 2005.

4. Ogonuki,H., S. Hashima, and H. Abe. Histopathological tests of tissues in the sites of local reactions caused by injection of oil adjuvant cholera vaccine. Symp. Series Immunobiol. Stand. 6:125. 1967.

5. O'Toole, D., L. Steadman, M. Raisbeck and R. Torpy. Myositis, lameness, and recumbency after use of water-in-oil adjuvanted vaccines in near-term beef cattle. J. Vet. Diag. Inv. 17:23-31. 2005.

Table 1. Hemagglutination inhibition response in several unconventional poultry species vaccinated with fowl pox-vectored H5 avian influenza.

	Experimental Day		
Species	**0**	**21**	**42**
Turkey	0	4.0[A]	3.3
Chuckar	0	4.8	2.9
Duck	0	3.1	5.0
Pheasant	0	4.0	4.9
Peafowl	0	4.3	5.2
Guinea fowl	0	2.2	2.9

[A] Geometric mean of the reciprocal $\log_2$ of the highest dilution to inhibit 4 hemagglutination units.

THE EFFICACY OF BIOSEALED IN PREVENTING *SALMONELLA* SPP. FROM COLONIZING CONCRETE SURFACES

K.S. Macklin, D.M. Paiva, and M. Singh

Dept. of Poultry Science, Auburn University, Auburn, AL 36849-5416

SUMMARY

One of the challenges facing the poultry industry is controlling *Salmonella*. Biosealed™ is a cement sealer that claims to seal pores inside cement and to have antimicrobial activity. Using this product in the hatchery should help minimize bacterial contamination. A series of experiments were performed to determine the efficacy of this product against *Salmonella.* Cement blocks were divided into one of four treatments: Bacterial inoculation (CON), Biosealed applied before inoculation (BA), Biosealed applied after inoculation (BI) Biosealed applied before and after inoculation (BAI). Sampling was performed on the interior and exterior of the cement blocks. The exterior swabbing results showed that BI and BAI had significantly ($P < 0.05$) lower *Salmonella* levels compared to CON and BA. *Salmonella* was not isolated from the interior of blocks in BA, BI and BAI; however it was isolated from CON. It is concluded that Biosealed is an effective cement sealer that appears to have anti-microbial activity.

(The full-length article will be published in *Poultry Science*.)

COMPARISON OF TURKEY GROWER MANAGEMENT PRACTICES ON FLOCK HEALTH AND PERFORMANCE

Rosemary A. Marusak, Michael P. Martin, David Rives, David Ley,
J. Michael Day, and H. John Barnes

INTRODUCTION

When animals are reared in a confined environment with limitations on space and activity, additional management practices are necessary to ensure optimal health, growth, and well-being (1). Genetic selection for larger birds and rapid growth as well as economic and management factors lead to high bird density, creating potential for high pathogen loads and behavioral problems such as persecution. Although gold standard practices for bird management in the hatchery and grow-out facility are available (2), changes in bird genetics, microbial evolution, and altered resources and technology continually challenge current practices.

Herein, we attempt to identify some key management and welfare factors necessary for optimal turkey health and growth by comparing two sister flocks (SF) of Hybrid Converter (HC) turkeys reared in different settings: the Teaching Animal Unit (TAU) at North Carolina State University and a standard commercial grower (SCG) operation.

MANAGEMENT METHODS

Two thousand (2000) turkey poults were placed at the TAU in a single 5600 sq ft curtain-sided house that is used to grow one turkey and one chicken flock per year. The house is located next to a swine barn as well as nearby cattle, horse and small ruminant pastures, that are all part of the TAU. Five research animal units are in close proximity to the poultry TAU and may contain poultry intermittently but are run with separate staff and equipment. No commercial poultry are located near the TAU. Complete poultry house cleaning with a three month down time and fresh litter replacement is done between flocks. The house has three wall fans, two wall heaters, six air vents per N/S side and three ceiling fans for ventilation. The anteroom is equipped with a disinfectant footbath and sink. When birds are present, the house is evaluated four times per day for temperature, ventilation, and equipment function and birds are assessed for mortality, overall health, and comfort. Plasson drinkers were cleaned daily and litter was turned under drinkers and feeders as needed, but at least once per week. Caked litter was removed and replaced with dry or fresh litter in select areas as needed. Behavior and medical issues were addressed during each walk-through. Birds were culled as needed.

Because of increased persecutions at eight weeks of age management practices were implemented to minimize bird losses. Damaged wing and tail blood feathers were removed and blood stains quickly cleaned with water. When additional hemostasis was indicated, a small amount of Clotisol® was applied. Birds with head and neck persecution were placed on ladder rungs approximately four ft off the ground and provided feed and water until recovery. All persecuted birds were left in the flock and no isolation pens or additional space was required. Enrichment items were added to the house to break cycles of persecution.

Seven thousand (7000) turkey poults were placed on the SCG operation; the birds were housed in one 16,000 sq ft curtain-sided barn. Grounds around the house were in good condition. The farm included five other adjacent barns (two for brooding and grow-out and three for grow-out only) with a total of 48,000 birds on the farm. The SCG birds shared the house with another flock until 5.5 wks at which time the other flock was moved to their own grow-out barn. SCG birds were under the management and supervision of an integrated turkey company and contract grower, and no attempts were made to influence those management practices. A daily walk through the house by the grower involved picking up dead and adjusting ventilation according to the recommended industry practice. Culling of birds by the grower was not carried out until three wks prior to processing. Due to the large number of birds on the farm and only one farm-hand, routine litter, feeder, and drinker maintenance was difficult. Water sanitation relied solely on chlorine additive. Standard barn clean-out practices with four wk downtime between flocks (three flocks/year) were followed. Greater than 30 large commercial poultry and swine houses are located within a two mile radius from the farm. Cattle graze in a lot adjacent to the barns.

For both the TAU and SCG operations, one half the poults were placed day of hatch (d0) and the other half placed day after hatch (d1). Birds were commingled on ~ day three and opened to full house (TAU) and ½-house (SCG) on day nine. Full house access for SCG birds was on ~ day 38. D0 and d1 birds were identified by unique toe trimming patterns. Both operations were delivered the same feed (turkey starter 1 with nitarsone, turkey starter 2 with nitarsone, turkey

grower 1 with lasalocid, and turkey grower 2 with virginiamycin). SCG birds were given five additional medications during weeks 1-3, 5, and 7 to treat enteric disease.

Bird weights and fecal samples for culture and parasitology were collected at regular intervals until week 10 after which time TAU birds were processed. All TAU and select SCG mortalities were necropsied; intestines and viscera with gross lesions were submitted for bacterial analysis. Limited resources prevented *Salmonella* spp. identification. Select samples were submitted for histopathology. *Mycoplasma* testing was carried out on select abnormal joints. Darkling beetles were also collected from the SCG house and submitted for virus analysis. Uniformity index was calculated as (S.D.*100)/mean weight.

RESULTS

***TAU* birds.** Cumulative mortality (10 wks) was 3.75%. Gross pathology diagnoses included neonatal/early brooding mortality associated with *E. coli* infection (21.3%), non-infectious neonatal/early brooding mortality (4%), *Staphylococcus* associated arthritis/musculoskeletal (9.3%), *M. Iowae* associated chondrodystrophy (17.3%), and cardiovascular (18.7%) diseases, pendulous crop (10.7%), trauma (14.7%), and persecution (9.3%). Uniformity index was generally <10% throughout the lifetime of the flock. *Salmonella* was not detected in feces of d0 birds on arrival, but was detected in the feces of d1 birds. D0 birds became *Salmonella* positive on day one. *Salmonella* was shed by both groups intermittently throughout the grow-out period. Overall mortality of d1 birds was 1.2 times that of d0 birds. At processing (10 wks), TAU birds averaged 5.73 kg, 0.42 kg heavier than the Hybrid Converter standard (5.69 kg); d0 birds were 0.177 kg heavier than d1 birds. Daily gain was 0.1827 and adjusted feed conversion for TAU birds was 2.00.

***SCG* birds.** Cumulative mortality was 2.9% (10 wks) and 6.3% (13 wks). *Salmonella* shedding in feces of both d0 and d1 birds was detected in the earliest sampling (day three) and intermittently throughout the grow-out period. Birds showed flushing and decreased growth from wk one, and uniformity index was generally >10% throughout the life of the flock. A diagnosis of poult enteritis complex was made wk three with isolation of *C. perfringens* and *campylobacter* from pooled feces. *Cochlosoma* was identified from intestines. SCG birds also had *M. Iowae*-associated chondrodystrophy. Aggressive culling of "small birds" and an increase in mortality due to persecutions occurred in wks 10-13 accounting for 53% of the total mortalities. Mortality of d1 birds was 1.5 times that of d0 birds. SCG birds averaged 4.47 kg at 10 wk and d0 birds were 0.31 kg heavier than d1 birds. At processing (wk 13), feed conversion was 2.44 (farm average).

DISCUSSION

TAU and SCG birds differed markedly in their morbidity/mortality profiles and rates of growth. TAU birds had higher mortality from neonatal/early brooding diseases. In contrast to the TAU birds, SCG birds experienced gastrointestinal (GI) disease beginning in the first week. This resulted in a marked decreased growth rate and increased feed conversion. Birds placed day after hatch were affected to a greater extent than birds placed day of hatch.

The interaction between feed, gut health, immunity, and muscle growth is well-established (3). Immediate post-hatch feed accelerates GI and immune system maturation, establishes gut microflora, and positively influences muscle development (4,5). When a poult's GI tract is compromised at a very early age, immune system development is also affected. Rectal exposure to pathogens is met with decreased systemic and intestinal antibody responses and decreased lymphocyte populations in the bursa, ceca, and colon (3).

GI disease in SCG birds was most likely due to environmental challenges that promoted a higher load of pathogens combined with management issues. Lack of resources may have contributed to the management problems. The SCG house temperature and ventilation were not adjusted for optimal conditions throughout the growth of the birds, increasing potential stress that could lead to health and growth problems. As an example, during the first week of growth the in-house temperature reached 110^{o}F. Relatively high temperatures were sustained for at least the first two weeks of the birds' life, which could promote decreased appetite, jejunal atrophy, and decreased feed absorption (6). Litter quickly became caked, Plasson drinkers were not routinely cleaned, and darkling beetle infestation was not controlled. Further, sick birds were not regularly culled. The flock experienced continuous morbidity likely due to the environmental pathogen load. Medications were provided to the flock but it is unclear if they were efficacious as the causative factors for the morbidity were not diagnosed. It is also unclear if the medications may have altered the normal gut flora, thereby contributing to sustained illness and decreased growth. Once growth is inhibited, it is not possible for the birds to recover and regain weight up to the level and efficiency of an unaffected bird (4,5). This was observed in the study as the SCG flock uniformity index was >10% for most of the flock's life and TAU birds were on average 1.26 kg heavier than SCG birds at 10 wks.

Compounding the differences between the TAU and SCG flocks was the contribution of persecution to the morbidity and mortality. Simple and fast yet aggressive management of birds in the TAU flock decreased persecution mortalities over that seen in previous years. With no intervention, persecution remained an important cause of death in the SCG flock.

In conclusion, several factors contributed to major performance differences between the TAU and SCG sister flocks. Firstly, environmental conditions (e.g. area poultry density, number of houses on farm and number of flocks per year) led to increased pathogen exposure for the SCG flock. Secondly, the greater number of birds and houses on the SCG farm overwhelmed the resources available so that certain management practices were not performed. Thirdly, oversight of the TAU flock emphasized rapid responses to medical/behavioral issues such as bird persecution.

REFERENCES

1. Tabler, G.T. The Challenges Facing Turkey Growers. Avian Advice. 6 (1): Spring 2004. http://www.thepoultrysite.com/articles/264/the-challenges-facing-turkey-growers last accessed 1/15/09; Gross, W. B., P.B. Siegel. Why Some Get Sick. J. Applied Poultry Science pp. 453-460. 1997.

2. Schwartz, D.L. General Information and Fundamental Factors in Disease Prevention. In Poultry Health Handbook 4th ed. The Pennsylvania State University, University Park, PA. pp. 3-39. 1994.

3. Sklan, D. Early gut development: the interaction between feed, gut health and immunity in Interfacing Immunity, Gut Health and Performance. Tucker, LA and Taylor-Pickard, JA eds. Nottingham University Press. pp. 9-31. 2004.

4. Moore, D.T., P.R. Ferket, and P.E. Mozdziak. The effect of early nutrition on satellite cell dynamics in the young turkey. Poultry Science 84: 748-756. 2005.

5. Halevy, O., Y.Nadel, M. Barak, I. Rozenboim, and D. Sklan. Early posthatch feeding stimulates satellite cell proliferation and skeletal muscle growth in Turkey Poults. Halevy, O.J. Nutrition. pp. 1376-1382. 2003.

6. Azevedo, L.F. and J.J. Dibner. Maximizing gut health for peak performance. Inter. Poultry Production. 14: 17-20. 2006.

COMPARISON OF TURKEY GROWER MANAGEMENT PRACTICES ON FLOCK HEALTH AND PERFORMANCE

Rosemary A. Marusak, Michael P. Martin, David Rives, David Ley,
J. Michael Day, and H. John Barnes

INTRODUCTION

When animals are reared in a confined environment with limitations on space and activity, additional management practices are necessary to ensure optimal health, growth, and well-being (1). Genetic selection for larger birds and rapid growth as well as economic and management factors lead to high bird density, creating potential for high pathogen loads and behavioral problems such as persecution. Although gold standard practices for bird management in the hatchery and grow-out facility are available (2), changes in bird genetics, microbial evolution, and altered resources and technology continually challenge current practices.

Herein, we attempt to identify some key management and welfare factors necessary for optimal turkey health and growth by comparing two sister flocks (SF) of Hybrid Converter (HC) turkeys reared in different settings: the Teaching Animal Unit (TAU) at North Carolina State University and a standard commercial grower (SCG) operation.

MANAGEMENT METHODS

Two thousand (2000) turkey poults were placed at the TAU in a single 5600 sq ft curtain-sided house that is used to grow one turkey and one chicken flock per year. The house is located next to a swine barn as well as nearby cattle, horse and small ruminant pastures, that are all part of the TAU. Five research animal units are in close proximity to the poultry TAU and may contain poultry intermittently but are run with separate staff and equipment. No commercial poultry are located near the TAU. Complete poultry house cleaning with a three month down time and fresh litter replacement is done between flocks. The house has three wall fans, two wall heaters, six air vents per N/S side and three ceiling fans for ventilation. The anteroom is equipped with a disinfectant footbath and sink. When birds are present, the house is evaluated four times per day for temperature, ventilation, and equipment function and birds are assessed for mortality, overall health, and comfort. Plasson drinkers were cleaned daily and litter was turned under drinkers and feeders as needed, but at least once per week. Caked litter was removed and replaced with dry or fresh litter in select areas as needed. Behavior and medical issues were addressed during each walk-through. Birds were culled as needed.

Because of increased persecutions at eight weeks of age management practices were implemented to minimize bird losses. Damaged wing and tail blood feathers were removed and blood stains quickly cleaned with water. When additional hemostasis was indicated, a small amount of Clotisol® was applied. Birds with head and neck persecution were placed on ladder rungs approximately four ft off the ground and provided feed and water until recovery. All persecuted birds were left in the flock and no isolation pens or additional space was required. Enrichment items were added to the house to break cycles of persecution.

Seven thousand (7000) turkey poults were placed on the SCG operation; the birds were housed in one 16,000 sq ft curtain-sided barn. Grounds around the house were in good condition. The farm included five other adjacent barns (two for brooding and grow-out and three for grow-out only) with a total of 48,000 birds on the farm. The SCG birds shared the house with another flock until 5.5 wks at which time the other flock was moved to their own grow-out barn. SCG birds were under the management and supervision of an integrated turkey company and contract grower, and no attempts were made to influence those management practices. A daily walk through the house by the grower involved picking up dead and adjusting ventilation according to the recommended industry practice. Culling of birds by the grower was not carried out until three wks prior to processing. Due to the large number of birds on the farm and only one farm-hand, routine litter, feeder, and drinker maintenance was difficult. Water sanitation relied solely on chlorine additive. Standard barn clean-out practices with four wk downtime between flocks (three flocks/year) were followed. Greater than 30 large commercial poultry and swine houses are located within a two mile radius from the farm. Cattle graze in a lot adjacent to the barns.

For both the TAU and SCG operations, one half the poults were placed day of hatch (d0) and the other half placed day after hatch (d1). Birds were commingled on about day three and opened to full house (TAU) and ½-house (SCG) on day nine. Full house access for SCG birds was on about day 38. D0 and d1 birds were identified by unique toe trimming patterns. Both operations were delivered the same feed (turkey starter 1 with nitarsone, turkey starter 2 with

nitarsone, turkey grower 1 with lasalocid, and turkey grower 2 with virginiamycin). SCG birds were given five additional medications during weeks 1-3, 5, and 7 to treat enteric disease.

Bird weights and fecal samples for culture and parasitology were collected at regular intervals until week 10 after which time TAU birds were processed. All TAU and select SCG mortalities were necropsied; intestines and viscera with gross lesions were submitted for bacterial analysis. Limited resources prevented *Salmonella* spp. identification. Select samples were submitted for histopathology. *Mycoplasma* testing was carried out on select abnormal joints. Darkling beetles were also collected from the SCG house and submitted for virus analysis. Uniformity index was calculated as (S.D.*100)/mean weight.

RESULTS

TAU birds. Cumulative mortality (10 wks) was 3.75%. Gross pathology diagnoses included neonatal/early brooding mortality associated with *E. coli* infection (21.3%), non-infectious neonatal/early brooding mortality (4%), *Staphylococcus* associated arthritis/musculoskeletal (9.3%), *M. Iowae* associated chondrodystrophy (17.3%), and cardiovascular (18.7%) diseases, pendulous crop (10.7%), trauma (14.7%), and persecution (9.3%). Uniformity index was generally <10% throughout the lifetime of the flock. *Salmonella* was not detected in feces of d0 birds on arrival, but was detected in the feces of d1 birds. D0 birds became *Salmonella* positive on day one. *Salmonella* was shed by both groups intermittently throughout the grow-out period. Overall mortality of d1 birds was 1.2 times that of d0 birds. At processing (10 wks), TAU birds averaged 5.73 kg, 0.42 kg heavier than the Hybrid Converter standard (5.69 kg); d0 birds were 0.177 kg heavier than d1 birds. Daily gain was 0.1827 and adjusted feed conversion for TAU birds was 2.00.

SCG birds. Cumulative mortality was 2.9% (10 wks) and 6.3% (13 wks). *Salmonella* shedding in feces of both d0 and d1 birds was detected in the earliest sampling (day three) and intermittently throughout the grow-out period. Birds showed flushing and decreased growth from wk one, and uniformity index was generally >10% throughout the life of the flock. A diagnosis of poult enteritis complex was made wk three with isolation of *C. perfringens* and *Campylobacter* from pooled feces. *Cochlosoma* was identified from intestines. SCG birds also had *M. iowae*-associated chondrodystrophy. Aggressive culling of "small birds" and an increase in mortality due to persecutions occurred in wks 10-13 accounting for 53% of the total mortalities. Mortality of d1 birds was 1.5 times that of d0 birds. SCG birds averaged 4.47 kg at 10 wk and d0 birds were 0.31 kg heavier than d1 birds. At processing (wk 13), feed conversion was 2.44 (farm average).

DISCUSSION

TAU and SCG birds differed markedly in their morbidity/mortality profiles and rates of growth. TAU birds had higher mortality from neonatal/early brooding diseases. In contrast to the TAU birds, SCG birds experienced gastrointestinal (GI) disease beginning in the first week. This resulted in a marked decreased growth rate and increased feed conversion. Birds placed day after hatch were affected to a greater extent than birds placed day of hatch.

The interaction between feed, gut health, immunity, and muscle growth is well-established (3). Immediate post-hatch feed accelerates GI and immune system maturation, establishes gut microflora, and positively influences muscle development (4,5). When a poult's GI tract is compromised at a very early age, immune system development is also affected. Rectal exposure to pathogens is met with decreased systemic and intestinal antibody responses and decreased lymphocyte populations in the bursa, ceca, and colon (3).

GI disease in SCG birds was most likely due to environmental challenges that promoted a higher load of pathogens combined with management issues. Lack of resources may have contributed to the management problems. The SCG house temperature and ventilation were not adjusted for optimal conditions throughout the growth of the birds, increasing potential stress that could lead to health and growth problems. As an example, during the first week of growth the in-house temperature reached 110^{o}F. Relatively high temperatures were sustained for at least the first two weeks of the birds' life, which could promote decreased appetite, jejunal atrophy, and decreased feed absorption (6). Litter quickly became caked, Plasson drinkers were not routinely cleaned, and darkling beetle infestation was not controlled. Further, sick birds were not regularly culled. The flock experienced continuous morbidity likely due to the environmental pathogen load. Medications were provided to the flock but it is unclear if they were efficacious as the causative factors for the morbidity were not diagnosed. It is also unclear if the medications may have altered the normal gut flora, thereby contributing to sustained illness and decreased growth. Once growth is inhibited, it is not possible for the birds to recover and regain weight up to the level and efficiency of an unaffected bird (4,5). This was observed in the study as the SCG flock uniformity index was >10% for most of the flock's life and TAU birds were on average 1.26 kg heavier than SCG birds at 10 wks.

Compounding the differences between the TAU and SCG flocks was the contribution of persecution to the morbidity and mortality. Simple and fast yet aggressive management of birds in the TAU flock decreased persecution mortalities over that seen in previous years. With no intervention, persecution remained an important cause of death in the SCG flock.

In conclusion, several factors contributed to major performance differences between the TAU and SCG sister flocks. Firstly, environmental conditions (e.g. area poultry density, number of houses on farm and number of flocks per year) led to increased pathogen exposure for the SCG flock. Secondly, the greater number of birds and houses on the SCG farm overwhelmed the resources available so that certain management practices were not performed. Thirdly, oversight of the TAU flock emphasized rapid responses to medical/behavioral issues such as bird persecution.

REFERENCES

1. Tabler, G.T. The Challenges Facing Turkey Growers. Avian Advice. 6 (1): Spring 2004. http://www.thepoultrysite.com/articles/264/the-challenges-facing-turkey-growers last accessed 1/15/09; Gross, W. B., P.B. Siegel. Why Some Get Sick. J. Applied Poultry Science pp. 453-460. 1997.

2. Schwartz, D.L. General Information and Fundamental Factors in Disease Prevention. In Poultry Health Handbook 4th ed. The Pennsylvania State University, University Park, PA. pp. 3-39. 1994.

3. Sklan, D. Early gut development: the interaction between feed, gut health and immunity in Interfacing Immunity, Gut Health and Performance. Tucker, LA and Taylor-Pickard, JA eds. Nottingham University Press. pp. 9-31. 2004.

4. Moore, D.T., P.R. Ferket, and P.E. Mozdziak. The effect of early nutrition on satellite cell dynamics in the young turkey. Poultry Science 84: 748-756. 2005.

5. Halevy, O., Y.Nadel, M. Barak, I. Rozenboim, and D. Sklan. Early posthatch feeding stimulates satellite cell proliferation and skeletal muscle growth in Turkey Poults. Halevy, O.J. Nutrition. pp. 1376-1382. 2003.

6. Azevedo, L.F. and J.J. Dibner. Maximizing gut health for peak performance. Inter. Poultry Production. 14: 17-20. 2006.

SEROLOGIC RESPONSE AGAINST H5N2 OIL EMULSION AI VACCINE IN DOMESTIC DUCKS

Andres Morales Garzon, Donaji Garcia Lopez, Raul Montalvo Ramirez, Ruth Larios, and Eduardo Lucio Decanini

Investigación Aplicada S. A. DE C.V., 7 Norte 416 Tehuacan Puebla, Mexico, 75700

SUMMARY

Thirty-five two-week-old commercial ducks were divided into five groups and maintained on wire cages. Groups 1 and 3 received 1.0 and 2.0 mL respectively of a water killed LAI vaccine (H5N2, strain Chicken/Mexico/232/94/CPA) identified as Optimune AIV by subcutaneous route (SC). Groups 2 and 4 received 0.4 and 0.8 mL, respectively, a concentrated water killed LAI vaccine (H5N2) identified as Optimune C-AIV by SC. Group 5 was the control group without treatment. All birds were bled before vaccination and at 1, 2, 3, 4, and 5 wks post vaccination to quantify antibodies level against AI by Inhibition Hemagglutination (IH) test. All groups were negative to AI by IH before vaccination. Groups 2 and 4 (concentrated vaccine) showed higher titer than Groups 1 and 3 (normal vaccine) after five wks with a geometrical mean (GM) of 296 (8.2 log 2) and 485 (8.9 log 2) respectively, Group 5 showed a GM of 5 (2.3 log 2) during all experiment period. We conclude that oil emulsion vaccine containing subtype H5H2 could be an alternative to protect commercial ducks and avoid transmission to other species, mainly poultry.

INTRODUCTION

There was a theory that wild aquatic birds are believed to be the primordial reservoir of type A influenza viruses. Avian influenza causes asymptomatic infection in natural host; however, in aberrant hosts including poultry, swine, and humans, clinical disease could be observed. Avian influenza virus is classified in 15 hemagglutinin subtypes (HA) but high pathogenicity has been associated with some strains of the H5 or H7 HA subtypes. Since 1994 H5N2 subtype AI has been present in Mexico, and an extensive vaccination program has been established to avoid high pathogenicity virus in farms. However, commercial and backyard ducks could be affected by H5N1 subtype and infect poultry or humans. The objective of this work was to investigate if two commercial vaccines to H5N2 could be an alternative to protect against H5N1.

MATERIALS AND METHODS

Birds. Thirty-five two-week-old commercial ducks were maintained in wire cages.

Vaccines. *Normal vaccine*: Killed oil emulsion Avian Influenza Vaccine Optimune AIV®; *concentrated vaccine*: Optimune C-AIV® (Investigacion Aplicada S.A. de C.V. Tehuacan, Puebla, Mexico). The schedule of vaccination was according to Table 1.

Serology. A sample of serum was collected from each group at 0, 1, 2, 3, 4, and 5 wks post vaccination. IH test was run and geometric mean was calculated.

RESULTS AND DISCUSION

The aim of this paper was to show seroprevalence in commercial ducks using two commercial vaccines against H5N2 LPAI killed vaccine. All birds had not antibodies against avian influenza virus before vaccination, and negative control remained with very low titers until they were slaughtered. The first one is a normal vaccine containing AIV strain Chicken/Mexico/ 232/94/CPA and the other is a concentrated vaccine containing same strain. Both met minimal requirements established by Mexican authorities. In the case of poultry, normal vaccine is recommended to a dose of 0.5 mL per bird and concentrated vaccine is recommended to be administered at 0.2 mL. So, in ducks manufacturer recommended use double doses for each vaccine. Serologic results are showed in Table 2. As we can see, the best serologic response was obtained with concentrated vaccine reached a GM of 296 (8.2 log2) and 485 (8.9 log2) with a single or double doses at end of trial. Nevertheless, normal vaccine could be used.

In 2006, Swayne *et al*. (1) showing that H5N2 vaccines protect chickens from Asian H5N1 high pathogenicity avian influenza virus. So, we believe that H5N2 vaccines could be used to protect commercial ducks not only in Mexico but in other countries to reduce environmental contamination by H5N1 HPAI. Also, we recommend that challenge trials be carried out with H5N1 AI strains.

REFERENCES

1. Swayne, D.E., Chang-Won Lee, and E. Spackman. Inactivated North American and European H5N2 avian influenza virus vaccines protect chickens from Asian H5N1 high pathogenicity avian influenza virus. Avian Pathol. 35(2): 141-146. 2006.

Table 1. Vaccination schedule.

Group	Treatment	Doses (mL)	Administration	Number of birds
1	Normal	1.0	Subcutaneous	10
2	Concentrated	0.4	Subcutaneous	10
3	Normal	2.0	Subcutaneous	5
4	Concentrated	0.8	Subcutaneous	5
5	Control	-	None	5

Table 2. Serologic results with (H2N2) AI vaccines in commercial ducks.

W.P.	Group 1		Group 2		Group 3		Group 4		Group 5	
	GM	Log 2	GM	Log 2	GM	Log 2	GM	Log 2	GM	Log 2
1	5	2.3	5	2.3	5	2.3	5	2.3	5	2.3
2	20	4.3	83	6.3	27	4.7	98	6.6	5	2.3
3	74	6.7	109	6.7	80	6.3	320	8.3	5	2.3
4	109	5.5	148	7.2	121	6.9	422	8.7	5	2.3
5	127	7.0	296	8.2	160	7.3	485	8.9	5	2.3

W.P. Wks post vaccination
GM. Geometric mean

INFECTIOUS LARYNGOTRACHEITIS IN ITALY: CLINICAL CASES, DIAGNOSIS, AND CHARACTERIZATION BY PCR-RFLP

A. Piccirillo[A], E. Morandini[B], L. Gavazzi[C], R. Ceruti, P. Pesente[B], D. Giovanardi[B], A. Mondin[A], and A. Moreno[D]

[A]Department of Public Health, Comparative Pathology and Veterinary Hygiene, Veterinary Medicine Faculty, University of Padua - Viale dell'Università, 16, 35020 Legnaro (PD), Italy
[B]Veronesi Verona Spa., Via Valpantena, 18, 37034 Verona, Italy
[C]Gesco Consorcio Operativo, Via del Rio, 400, 47020 San Vittore, Cesena (FC), Italy
[D]Virology Department - Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Via A. Bianchi, 9, 25124 Brescia, Italy

INTRODUCTION

Infectious laryngotracheitis (ILT) is an acute respiratory disease of chickens caused by *Gallid herpesvirus 1*, a member of the subfamily *Alphaherpesvirinae*, family *Herpesviridae*. Although infectious laryngotracheitis virus (ILTV) has been the first poultry pathogen controlled by vaccination, ILT is still one of the most important diseases in the poultry industry, especially in areas where high density of poultry populations are reared (3). Mild and severe forms of the disease were commonly described in layers worldwide, but in the last years numerous cases of mild disease have been described in broilers in many countries (7,8).

During the period 2007 to 2008, several cases of mild ILT were observed in broilers in North Italy. The causative agent was ILTV in all outbreaks. The present paper reports the diagnostic findings from 36 cases and the molecular characterization of 17 field ILTV strains and four ILTV CEO vaccines by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis. In addition, a preliminary genomic characterization based on the sequencing of gE, gG, TK, ICP18.5 and ORFB-TK genes was carried out on three field strains and one CEO vaccine in order to better understand the eventual correlation between ILTV field and vaccine strains.

MATERIALS AND METHODS

Clinical cases. From May 2007 to October 2008, 36 cases exhibiting upper respiratory disease were observed in broiler flocks located in North Italy, divided in the following provinces: 18 in Brescia; five in Cremona; three in Teramo; two in each of these sites: Bergamo, Forlì, Verona, and Padova; and one in each of these sites: Mantova and Treviso. Out of these, 32 occurred in birds between 40 to 50 days of age and only four in younger animals. Thirty outbreaks occurred in industrial farms whereas six from dealer farms. No ILTV vaccination was applied in any industrial farm. In dealer farms, where layers and broilers, although in separate units, were reared in close proximity, only layers were vaccinated against ILTV.

Viruses. Seventeen ILTV field isolates and four live attenuated ILT vaccines were analyzed in this study. Only four vaccines are authorized in Italy and they are all of chicken embryo origin (CEO): Nobilis® Laringovac (Intervet), Poulvac ILT (Forte-Dodge Animal Health), Bio Laringo PV (Merial), and LAR-VAC (Fatro).

Virus isolation and identification. Tracheal tissue homogenates from 17 outbreaks were inoculated into nine to eleven day-old SPF chicken embryonated eggs (CEE) by chorionallantoic membrane (CAM) route. The CAMs were observed for the presence of pocks lesions after several days and passages and the presence of ILTV was evaluated by polymerase chain reaction (PCR). The presence of other respiratory pathogens, like infectious bronchitis virus (IBV), Newcastle disease virus (NDV) and avian influenza virus (AIV) were evaluated on all homogenates by previously described procedures (1,4,9).

DNA extraction. Viral DNA was extracted using the High Pure Viral Nucleic Acid Kit (Roche Diagnostics Corporation) from 200 µL of a suspension of the infected CAMs and reconstituted vaccines according to the manufacturer's instructions.

Real-time PCR. Tracheal tissue homogenates from all the outbreaks were analyzed by real-time Taqman® PCR assay for the detection of ILTV as previously described (2).

PCR-RFLP. PCR-RFLPs were performed as described by Kirkpatrick *et al.* (5). Except for ICP4 gene, our study analyzed gE, gG, TK, ICP18,5 and ORFBTK genes. The ORFB-TK region consists of several unique genes, including ORFB, ORFC, ORFD and ORFE. PCR products were digested with the

specific restriction endonuclease enzymes (New England Biolabs) i.e. EaeI for gE, MspI for gG and TK, HaeIII for ICP18.5 and FokI for ORFB-TK. After SybrSafeTM DNA Gel Stain (Invitrogen) staining, RE DNA fragments were visualized by Gel DocTM XR (Bio-Rad). Molecular weights of both PCR products and RE fragments were calculated by using Quantity One® 1-D Analysis Software (Bio-Rad).

Sequence analysis. Amplicons from three field isolates and one vaccine strain were purified using High Pure PCR Purification kit (Roche Diagnostics Corporation) and submitted to BMR Genomics (Padua, Italy) for sequencing. Analysis of the chromatograms, assembly of nucleotide sequences and the prediction of amino acidic sequences were performed using the Chromas Pro Software v. 1.42 (Technelysium Pty Ltd., Australia). Alignment of both nucleotide and amino acidic sequences was performed with CLUSTALW Software version 2.0 (6).

RESULTS

Clinical signs and pathology. Clinical sings included reduction of feed and water consumption, gasping with expectoration of blood-stained mucus, evident swelling of the infra-orbital sinuses, conjunctivitis, closed and watery eyes, and persistent nasal discharge. Low mortality (>10%) was reported. Gross lesions observed in tracheal and laryngeal tissues were variable from mild tracheitis with excess of mucus to severe with hemorrhagic or diphtheritic changes. Other lesions such as edema and congestion of conjunctiva and infraorbital sinuses were observed.

Real-time PCR and virus isolation. Tracheal homogenates from all 36 outbreaks resulted positive by ILTV real-time PCR. Of these, 17 field samples and four vaccine strains were propagated on CAMs of CEE and the presence of ILTV was demonstrated in all samples by real-time PCR. All cases were negative for NDV, AIV, and IBV.

PCR-RFLP. For all ILTV field isolates and vaccine strains, specific PCR products were generated for each individual gene (gE, gG, TK, ICP18.5 and ORFB-TK). All the field isolates and the vaccine strains under study produced PCR products of the same molecular weights and of the expected sizes as indicated by Kirkpatrick et al. (5), except for ICP18.5 which was about 6,400 bp instead of 5,890 bp. Restriction endonuclease digestion of all the genes produced identical patterns in all the field isolates and the vaccine strains. These patterns consisted of three bands of 1.10, 0.49, and 0.26 kb for gE PCR products, eleven bands of 0.28, 0.26, 0.23, 0.20, 0.18, 0.08, 0.07, 0.05, 0.04, 0.03, and 0.02 kb for gG PCR products, six bands of 0.89, 0.56, 0.24, 0.18, 0.13, and 0.12 kb for TK PCR products, ten bands of 2.00, 1.00, 0.80, 0.70, 0.45, 0.42, 0.35, 0.32, 0.24, and 0.22 kb for ICP18.5 PCR products and seven bands of 2.40, 0.90, 0.54, 0.35, 0.23, 0.13, and 0.06 kb for ORFB-TK PCR products. In some cases, the analysis of RFLP patterns was difficult for the presence of additional bands which sizes suggested that they could be due to partially digested products.

Sequencing and sequence analysis. The comparison of the nucleotide sequences and successive analysis of restriction sites of the field isolates and vaccine strain confirmed the patterns obtained by PCR-RFLPs of each individual gene. ClustalW2 analysis demonstrated 100% homology between the three field isolates for all the genes sequenced. Homologies among field and vaccine ILTV strains were 100% for gE and TK, 99.9% for gG, 99.9% for ICP18.5 and 99.8% for ORFB-TK genes. Deduced amino acid (aa) sequences revealed one aa substitution in ORFE (A135 to G) and ICP18.5 (H715 to R), and one additional aspartic acid residue at position 67 in ORFC in the field strains.

DISCUSSION

In 2007-2008 thirty six cases of ILTV were reported in broilers in North Italy in an area characterized by a high density of industrial poultry (broilers and layers) farms and dealer farms. These are the first cases observed in broiler population in this area of the country. ILTV was demonstrated by real-time PCR in all 36 tracheal homogenates submitted to the laboratory whereas other respiratory viruses AIV, NDV and IBV were excluded as the cause of the disease. The clinical signs were classified into mild forms of ILT in all cases. Most of the outbreaks (89%) occurred in broilers of 40 to 50 days of age. These flocks were “mixed sex” and in many cases it has been observed that clinical ILTV appeared one to two wks after the partial depopulation of female broilers and could be related to contact with contaminated fomites, trucks or personnel. Also the close proximity between farms and the spreading of contaminated litter neighboring the susceptible farms could likely have spread ILTV.

The characterization by PCR-RFLP of gE, gG, TK, ICP18.5 and ORFB-TK revealed the same RFLP patterns for all the ILTV strains under investigation. These results suggest a close relationship between the field isolates and the vaccine strains. However, the comparison of full sequences of these five genes for three field and one vaccine strains revealed some interesting results. The sequences of the five genes of the three field isolates showed 100% of homology at nucleotide level. The nucleotide sequences of gE and TK genes were identical among field isolates and vaccine strain whereas some differences either at

nucleotide and amino acidic level of the other examined genes/genomic regions were evidenced between them. On the basis of these preliminary results it could be hypothesized that field isolates are closely related to CEO vaccine strains but not identical.

A further genomic characterization of the other field isolates described in this study should be undertaken in order to verify if the same differences are presented in all field strains. In addition, further sequence analysis will be carried out to provide a better comprehension if these amino acid mutations could be involved in the virulence of ILTV strains and in the rapid spread of the disease during this epidemic. Since the PCR-RFLP analysis used in this study could not discriminate between field isolates and vaccine strains, Authors emphasize the necessity to characterize the ILTV strains by comparing RFLP patterns with gene sequencing.

REFERENCES

1. Adzhar A., R.E. Gough, D. Haydon, K. Shaw, P. Britton, and D. Cavanagh. Av Pathol, 26:625-640. 1997.
2. Callison S.A., S.M. Riblet, I. Oldoni, S. Sun, G. Zavala, S. Williams, R.S. Resurreccion, E. Spackman, and M. Garcia. J Virol Meth, 139:31-38. 2007.
3. Guy, J.S. and M. Garcia. Diseases of Poultry, Iowa State Press, Ames, pp.137-152. 2008.
4. Kho, C.L., M.L. Mohd-Azmi, S.S. Arshad , and K. Yusolff. J Virol Meth, 86: 71-83. 2000.
5. Kirkpatrick N.C., A. Mahmoudian, D. O'Rouke, and A.H. Noormohammadia. Avian Dis., 50:28-34. 2006.
6. Larkin, M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson, and D.G. Higgins. Bioinformatics, 23: 2947-8. 2007.
7. Ojkic, D., J. Swinton, M. Vallieres, E. Martin, J. Shapiro, B. Sanei, and B. Binnington. Av Pathol, 35: 286-292. 2006.
8. Sellers, H.S., M. Garcia, J.R. Glisson, T.P. Brown, J.S. Sander, J.S. Guy J.S. Av Dis, 48: 430-436. 2004.
9. Spackman, E., D.A. Senne, T.J. Myers, L.L. Bulaga, L.P. Garber, M.L. Perdue, K. Lohman, L.T. Daum, and D.L. Suarez. J Clin Microbiol, 40:3256-60. 2002.

APPLICATION OF *MYCOPLASMA SYNOVIAE* VACCINE (MS-H) IN LAYERS

Teruaki Ouchi[A], Yasuhisa Munakata[B], and Hiroshi Sakamoto[A]

[A]Nippon Biologicals, Inc., Kita Aoyama 1-5-12/5F, Minato-ku, Tokyo 107-0061 Japan
[B]Nippon Zenyaku Kogyo Co., Ltd., 1-1 Tairanoue, Sasagawa, Asaka-machi, Koriyama, Fukushima 963-0196 Japan

Significant advances in the control of mycoplasmosis have been realized since the introduction of attenuated live MG vaccines (e.g., ts-11). Until recently, the disease induced by *Mycoplasma synoviae* (MS) has remained problematic due to the lack of an effective vaccine. Additionally, MS may be contributory to other disease syndromes. Recent studies have shown that MS, aside from causing production losses in its own right, it can also be a significant factor in initiating *E. coli* peritonitis, a major cause of mortality in layers. At the 2004 American Association of Avian Pathologists meeting, Dr. Kenton Kreager reported that field evidence suggested synergy between the two pathogens to be a major problem for US layer flocks (1). Furthermore, an experimental study has supported the postulation that a virulent MS strain to be a primary factor in this syndrome (2).

NBI Technology Committee conducted two field studies in large multiple-age commercial layer farms to determine whether MS live, MS-H strain vaccination could cost-effectively increase production and decrease mortality (3).

Layer flocks vaccinated with MS-H showed significant improvements compared to non-vaccinated flocks previously placed on the same farm. In the field studies, reduced eggshell top cone abnormalities from 2 to 4% to 0% was seen in flocks vaccinated with MS-H. This is an important observation since MS is suspected to cause eggshell top cone abnormalities (4). In the first study, the cumulative egg weight per hen was 795 g greater at 57 wks. In the second study, the cumulative egg weight average was 787 g greater at 50 wks. Additionally, there was a reduction of mortality from *E. coli* peritonitis. From these results, NBI Technology Committee concluded MS-H vaccination to be effective and economic.

FIELD STUDY 1

MS-vaccinated group. 82,000 Hy-Line Gray layers (four subgroups placed March-October 2006)

Control group. 184,000 Hy-Line Gray layers (Nine subgroups placed 2000 to 2005)

***Vaccination programs**. Identical for both groups, except the MS-H live vaccine administration on day 31 via eye drop to layers in MS-H vaccinated group.

1 (d)	Marek's
7	IB (H120) + ND (VG/GA)
15 & 26	IBD
31	MG (ts 11) + IB (H120) + ND (VG/GA) **+[MS-H]***
50	Pox +ILT
63	ND (VG/GA) + IB (C-78)
70	AE
83	ND/IB2/Coryza (A&C) + SE

Historically, pullets on this farm were grown MS free for 120 days before being transferred to the laying house. By 150 days of age almost 100% of birds tested MS positive by serum agglutination testing. However, almost 100% of pullets vaccinated with MS-H tested MS positive by six wks post vaccination. This is an expected result from the MS-H vaccination.

RESULTS

Comparison 1. Table 1 compares the **MS-H vaccinated group,** consisting of four subgroups placed into production March - Oct. 2006 to the Control group (all nine subgroups placed 2000 to 2005).

MS-H vaccinated group: Significant Improvements.

1) Age at 50% egg production was 4.7 days earlier.
2) Peak egg production rate was 1.5% higher.
3) HD egg production rate was 6.4, 2.8 and 2.2% greater for Stage 1, 2 and 3, respectively.
4) HH egg production rate was 6.5, 3.7 and 2.9% greater for Stage 1, 2 and 3, respectively.
5) Mortality (compared with the control group):
Stage 1) 0.042%/wk lower (0.68%/15 wks),
Stage 2) 0.029% lower (0.44%/15 wks),

Stage 3) no significant difference.

6) Cumulative egg wt. at 57 wks was 795 g greater

7) Overall FCR was 0.13% better.

8) Feed intake: Stage 1) 2.3 g greater,
Stage 2) 3.3 g less,
Stage 3) 3.6 g less.

Comparison 2. Table 2 compares MS-H vaccinated group to the Control group consisting only the most recently placed subgroups (the four subgroups placed 2004 to 2005) to minimize impact of differences of time and conditions.

MS-H vaccinated group: Significant Positive Results.

1) The time to 50% egg production was 9 days earlier.

2) Peak egg production rate was 1.6% higher.

3) HD egg production rate was 9.9, 2.1 and 1.4% greater for Stage 1, 2 and 3, respectively.

4) HH egg production rate was 9.8, 2.7 and 1.8% greater for Stage 1, 2 and 3, respectively.

5) Mortality (compared with the control group):

Stage 1) 0.036%/wk lower (0.57%/15 wks),
Stage 2) 0.013% lower (0.2%/15 wks),
Stage 3) 0.033% lower (0.5%/7 wks).

Data analysis suggests the lower mortality is attributable to fewer cases of E. coli peritonitis.

6) Cumulative egg wt at 57 wks was 787 g greater

7) FCR was 0.1% lower during Stage 1.
The overall difference was 0.07% (not significant).

8) Feed intake: Stage 1) 5.6 g greater, Stage 2) no difference, and Stage 3) 3.5 g less.

Comparison 3. Table 3 provides data related to forced molting**.** The mean time for forced molting in the MS-H vaccinated group was 5.2 wks later than that in the Control group (all subgroups combined 2000 to 2005) and 3.8 wks later than that in the most recently placed Control group (four subgroups 2004 to 2005). Even though forced molting in the MS-H vaccinated group was four wks later, the egg production was similar at the time of molting for both groups. The MS-H vaccinated group sustained a longer period of good egg production.

SUMMARY

The MS-H vaccinated layers tended to increase egg weight rapidly during the early egg production stage, while reducing it during the late egg production stage due to improved laying persistency. The benefits of the vaccine are as follows:

1) Prevents delay of egg production
2) Decreases mortality
3) Improves egg production rate
4) Improves laying persistency
5) Reduces under-grade eggs in the late production
6) Increases cumulative egg production
7) Improves FCR

ECONOMIC BENEFITS

HH cumulative egg production is considered the best indicator of the economic benefit gained from use of the vaccine. The HH cumulative egg production out to 57 wks was approximately 800 g (13 eggs) greater in the vaccine group, which is estimated to represent a net profit of about 100 yen ($0.94)*. This is a conservative estimate. This extra income comfortably covers the cost of the MS-H vaccinations. Feed conversion ratio was better in the MS-H vaccinated group by approximately 7%, compared to all nine flocks of Control group. This savings amounts to a 120-yen ($1.13)* reduction in the cost of feed per hen (assuming that the hens are fed up to 78 wks).

FIELD STUDY 2

MS-H vaccinated group. 315,000 Lohmann (Julia-LSL)

(nine subgroups) placed April 2006 to Feb. 2007.

Control group. 245,000 Lohmann (Julia-LSL) layers

(seven subgroups) placed prior to the above period.

Vaccination programs. are identical to Field Study 1.

The study was conducted at a farm that historically maintains an egg production rate greater than 90% for approximately 20 wks, followed by a period of relatively good and stable egg production. Pullets are raised MS-free but within 30 days after transfer to layer house, all hens became sero-positive for MS. The farmer suspected that MS was negatively influencing the duration of the 90% egg-production rate and increasing the mortality.

RESULTS

MS-H vaccinated layers showed significant improvements compared to MS non-vaccinated layers. When performance comparisons were made, the flocks vaccinated with MS-H laid ≥ 90% rate for approximately 10 wks longer. By 50 wks, this resulted in a 5% increase in egg production (additional seven eggs per hen) compared with the non-vaccinated flocks. (Data available on request.) The other benefits gained from administration of MS-H vaccine were similar to results of the Field Study 1.

CONCLUSION

In these two large-scale field studies, flocks vaccinated with the MS-H consistently performed better than previously placed, non-vaccinated flocks. The actual benefits of using MS-H vaccine in other commercial settings in the future might vary from farm to farm, depending upon such factors as management practices, concurrent disease, and severity of wild MS field challenge. However, these current studies provide strong evidence that MS-H vaccine will easily prove to be economically justified in today's competitive layer industry.

REFERENCES

1. Kenton Kreager. Symposium on Emerging & Re-emerging Diseases. AAAP meeting, July 25, 2004, Philadelphia, PA. 2004.
2. Ziv Raviv, N. Ferguson-Noel, V. Laidinis, R Wooten, and S.H. Kleven. Role of *Mycoplasma synoviae* in commercial layer *Escherichia coli* peritonitis syndrome. Avian Diseases 51:685-690, 2007.
3. MS-H: Vaxsafe® MS (strain MS-H)*, Bioproperties, Australia
4. Ferberwee A., J. de Wit, and W. Landman. *Mycoplasma synoviae* associated eggshell apex abnormalities. The 15th Congress & Exhibition of the World Veterinary Poultry Association, Sept. 10-15, 2007: Beijing China, pg. 234. 2007.

* Exchange rate: 2005: 1 USD=106 Yen

** Registered in Australia, Mexico, Japan, S. Africa, Columbia, Brazil, and currently pending registration USA.

Table 1. MS-H vaccinated group compared to control group (all nine subgroups 2000-2005).

Difference (I–N)			**+2.2**	**+2.9**	**–**	**+2.6%**	**+0.795 kg**	**–0.2 g**	**–3.6 g**	**–0.11**

Table 2. MS-H vaccinated group compared control group (four subgroups most recently placed).

(Nov. 29, 2007, T. Ouchi)	Age at 50% Egg Prod.	Peak Egg Prod. (%)	HD Egg Prod. (%)	HH Egg Prod. (%)	Mortality%/wk	Rate of Normal Eggs (%)	Cumulative Egg Weight (kg/hen)	Mean Egg Weight (g)	Feed Intake (g/hen/day)	FCR
Stage 1 (21-35 wks)										
MS-H vaccinated	148.8	94.7	85.5	84.8	0.098	97.3	5.225	57.7	102.1	1.91
Control group (N)	157.8	93.1	75.6	74.9	0.134	97.8	4.650	57.3	96.4	2.01
Difference (I–N)	**–9.0**	**+1.6**	**+9.9**	**+9.8**	**–**	**–0.5%**	**+.575 kg**	**+0.5 g**	**+5.6 g**	**–0.10**
Stage 2 (36-50 wks)										
MS-H vaccinated			87.5	85.4	0.163	97.7	10.913	63.4	104.4	1.88
Control group (N)			85.4	82.7	0.175	97.6	10.150	63.7	104.5	1.93
Difference (I–N)			**+2.1**	**+2.7**	**–**	**+0.1%**	**+.763 kg**	**–0.3 g**	**–0.2 g**	**–0.04**
Stage 3 (51-57 wks)										
MS-H vaccinated			80.8	76.7	0.256	98.5	13.362	64.3	105.9	2.04
Control group (N)			79.4	74.8	0.289	95.2	12.575	64.8	109.4	2.12
Difference (I–N)			**+1.4**	**+1.8**	**–**	**+3.3%**	**+0.787 kg**	**–0.5 g**	**–3.5 g**	**–0.08**

Table 3: Forced Molting

(Dec 2, 2007, T. Ouchi)	Subgroup No.	Age at Forced Molting (wks)	Egg Production Rate at Forced molting (%)	Mean Egg Prod. Rate Before Forced molting (%) **	Substandard Eggs (%)*
Control group No vaccine	00531	65	78.7	86.1	0.9
	20907	56	79.6	83.4	1.5
	30405	57	76.6	81.8	1.9
	30619	62	77.4	82.5	3.7
	30827	60	74.2	83.3	2.6
	40610	58	75.5	82.5	2.7
	41030	66	78.7	84.7	6.0
	50323	62	75.3	82.5	10.0
	50604	64	78.3	85.0	12.9
Mean (2000-2005)		**61.1**	**77.1**	**83.5**	**4.7**
Mean (2004-2005)		**62.5**	**77.0**	**83.7**	**7.9**
MS-H vaccinated Group	60321	64	72.3	83.0	2.4
	60531	67	79.4	86.7	2.3
	60812	68	79	86.3	2.7

	61021	(not molted)	Not included in molting data comparisons		
Mean (March-Oct 2006)		**66.3**	**76.9**	**85.3**	**2.5**

* % are taken before grade and packaging.

** Average egg production rate from 50% of flock producing eggs until just before molting.

FIELD TRIALS TO TEST SAFETY AND EFFICACY OF A LIVE ATTENUATED VACCINE OF AVIAN PATHOGENIC *ESCHERICHIA COLI* SEROVAR O78 IN BROILER CHICKENS

Tetsuji Nagano, Rie Kitahara, and Shinya Nagai

Nippon Institute for Biological Science, 9-2221-1, Shin-machi, Ome, Tokyo 198-0024, JAPAN

INTRODUCTION

In the 56th Western Poultry Disease Conference, we showed that a rational attenuated *crp* mutant of avian pathogenic *E. coli* (APEC) serovar O78 can be a candidate as a safe and effective live vaccine against avian colibacillosis. Administration of the mutant strain via various routes (fine spray, coarse spray, eye drop, and *in ovo*) evoked an effective immune response that could protect chickens from challenge with the virulent wild-type *E. coli* O78 strain under laboratory conditionS. In this presentation, we will describe the results of the field trials using the mutant strain and will further evaluate safety and efficacy of the mutant as a vaccine candidate.

MATERIALS AND METHODS

In four broiler chicken farms where colibacillosis had frequently occurred, a total of 63,208 birds were vaccinated with the mutant strain and 61,508 chickens were served as non-vaccinated control birds. In each farm, approximately 7,400 to 25,500 birds were housed separately. Both sexes of Ross308 chickens were used in Farms A and B, male Cobb500 chickens were used in Farm C and female Ross308 chickens were used in Farm D. The lyophilized trial vaccine (Lot.19, 1,000 doses per vial) was dissolved in 300 mL of sterilized physiological saline. Chickens of Farms A and B were first vaccinated via fine spray at one day of age and were secondarily vaccinated via coarse spray three weeks later. In Farms C and D, chickens were vaccinated twice via fine spray at one day and four-weeks of age. The efficacy and safety of the vaccine were assessed by comparatively monitoring the status of individually housed birds: general clinical signs, existence or nonexistence of colibacillosis, weight gain, and productivity until slaughter and experimental challenge exposure. When clinical colibacillosis was not observed in both the vaccinated and the non-vaccinated birds, ten randomly selected birds from each household were introduced to the authors' laboratory and experimentally challenge-exposed to the virulent wild-type *E. coli* O78 strain via intravenously injection. After the challenge exposure, all birds were monitored daily for signs of illness and deaths. One week later, the surviving chickens were euthanized, and gross lesions representing colibacillosis (pericarditis and perihepatitis) were recorded.

RESULTS

After each vaccination, no adverse symptoms were observed in any of the treated birds. In Farm A, where clinical colibacillosis was observed, mortality and condemnation rates of the vaccinated birds were significantly lowered compared to those of the control. In the other three farms (Farms B, C, and D), where clinical colibacillosis was not observed, no significant differences were detected between the vaccinated and control birds except that the mean body weight was higher in the vaccinated birds in Farm C. In the experimentally challenge-exposed birds, improved results were observed in vaccinated birds; in survival rate and clinical score in Farm B, in survival rate, clinical score and weight gain in Farm C and in weight gain and pericarditis score in Farm D.

DISCUSSION

In field trials using a total of 63,208 birds, the trial vaccine was shown safe for commercial broiler chickens. The efficacy of the vaccine was indicated by reduction of deleterious effects by the colibacillosis, *i.e.* reduced mortality rate, clinical scores and other increased productivity. Additionally the trial vaccine protected against the experimental challenge-exposure of APEC strain.

The results presented here demonstrate that this vaccine can confer protection against acute and chronic manifestation of colibacillosis caused by APEC infection.

ROLE OF *CLOSTRIDIUM PERFRINGENS* AND *CLOSTRIDIUM SEPTICUM* IN CELLULITIS IN TURKEYS

K. V. Nagaraja, A. J. Thachil, and D. A. Halvorson

Department of Veterinary and Biomedical Sciences, University of Minnesota,
1971 Commonwealth Ave, St. Paul, MN 55108

Cellulitis in turkeys has been causing a significant economic loss for turkey producers in USA. Its impact has caused significant concern for it to be currently considered as the high priority disease of turkeys in Minnesota and elsewhere. Cellulitis is associated with acute mortality and presence of inflammatory subcutaneous fluid and crepitus, most commonly in commercial male turkeys nearing market age. The mortality is reported to be as high as 2 to 3% per week in the affected flocks.

Cellulitis usually appears at the age of 13 to16 weeks and persists until the birds are marketed. But more recently, even 8-week old birds were found to be affected with cellulitis. The lesions have been seen in various areas of the body, including: the breast, abdomen, legs, thighs, groin, and the back of the bird. Interestingly, in most cases of cellulitis, there appears to be no trauma to the skin. Palpitation of the affected areas often reveals crepitation due to gas bubbles in the subcutis and musculature. At necropsy, there is accumulation of bubbly, serosanguinous fluid in the subcutis. For this reason, cellulitis condition in turkeys appears different from the gangrenous dermatitis (GD) reported in broilers. The underlying musculature may have a cooked appearance in severe cases. The liver and spleen are often enlarged and may contain large necrotic infarcts. The kidneys are usually swollen.

Diagnostic laboratories have consistently isolated clostridial organisms from turkey cellulitis lesions. Organisms isolated from cellulitis lesions in turkeys predominantly include *Clostridium septicum* and *C. perfringens*. Experimental induction of cellulitis lesions and mortality in turkeys is possible with *C. perfringens* and *C. septicum* indicating the significance of clostridia in causing cellulitis. The affected tissues contained large amounts of gelatinous exudates and gas bubbles, most of which are in the subcutaneous and intermuscular connective tissue.

In our study we found *C. perfringens* and *C. septicum* spore culture caused severe cellulitis and mortality in five-week-old turkeys under experimental infection

C. septicum was found to be more lethal in mice as well as in turkeys and are more capable of inducing cellulitis lesions and mortality than *C. perfringens* in poults. The results of this study will be presented.

CONSTRUCTION OF A RECOMBINANT FOWL ADENOVIRUS 9 EXPRESSING THE HEMAGGLUTININ GENE OF INFLUENZA A VIRUS

Dan Hui Yang[A], Juan C. Corredor[A], Peter J. Krell[B], and Éva Nagy[A]

[A]Departments of Pathobiology and [B]Molecular and Cellular Biology,
University of Guelph, Guelph, Ontario, N1G 2W1, Canada

SUMMARY

Fowl adenoviruses (FAdVs) are about 70-90 nm in diameter, non-enveloped viruses with icosahedral symmetry and they are in the family *Adenoviridae*, genus *Aviadenovirus*. FAdVs are classified into five species, each including one or more serotypes: *Fowl adenovirus A* (FAdV-1), *Fowl adenovirus B* (FAdV-5), *Fowl adenovirus C* (FAdV-4 and -10), *Fowl adenovirus D* (FAdV-2, -3, -9 and -11), and *Fowl adenovirus E* (FAdV-6, -7, -8a and 8b). FAdV genomes are about 10 kb longer than the 30-36 kb of the mastadenoviruses. Fowl adenoviruses have a worldwide distribution and appear to be ubiquitous in poultry farms. Certain FAdVs are more pathogenic than others, and diseases, such inclusion body hepatitis, are more often associated with isolates belonging to serotypes 2, 4, 6, 8 and 11. Low pathogenic FAdVs are good candidate as vector viruses for use in poultry.

We developed a system for construction of recombinant viruses based on fowl adenovirus 9 (FAdV-9). In our earlier work we showed that the tandem repeat region 2 (TR-2) which is on the right end of the genome can be deleted and replaced with a foreign gene. We also demonstrated that chickens can be immunized with the recombinant virus and the virus could be administered through the drinking water and feed. From these results we felt that FAdV-9 could be used as a vaccine against poultry diseases. To identify additional sites for insertion we conducted a deletion analysis and identified a 2.4 kb non-essential region. Here we report on the generation of a recFAdV-9 expressing the full-length hemagglutinin (HA) gene of an avian influenza A virus. Two recombinant viruses, FAdV-9ΔL-HA-R and FAdV-9ΔL-HA-L, carrying the HA coding sequence replacing this 2.4 kb region in two orientations were generated by homologous recombination and subsequent transfection of chicken hepatoma (CH-SAH) cells. Expression of the HA protein was detected by Western immunoblotting using anti-avian influenza A (H5N1) HA polyclonal antibody. The activity of the recombinant HA protein was also examined by hemadsorption and hemagglutination assays. Further characterization of the recombinant viruses, such as stability is currently under way.

(The full-length article will be published in *Vaccine*.)

ISOLATION AND IDENTIFICATION OF AN ADENOVIRUS FROM DUCKLINGS WITH A PROLIFERATIVE TRACHEITIS IN ONTARIO, CANADA

Marina L. Brash[A], Janet N. Swinton[A], Alexandru Weisz[B], and Davor Ojkić[A]

[A]Animal Health Laboratory, University of Guelph, Box 3612, Guelph, ON, Canada, N1H 6R8
[B]Smith and Weisz Poultry Veterinary Services, Guelph, ON, Canada, N1L 1G3

SUMMARY

Increased mortality was reported in two flocks of Muscovy ducklings from two consecutive hatches originating from the same breeder flock. Coughing, dyspnea and gasping were observed in some ducklings between six and 11 days of age. Opaque white plugs of exudate were seen in the tracheas with some ducklings having multiple tracheal plugs. Tracheal and bronchial epithelium was hyperplastic and superficial epithelial cells contained eosinophilic intranuclear viral inclusions. Virus particles compatible with adenovirus morphology were observed in tracheal epithelial cells by electron microscopy and in the supernatant from cell cultures inoculated with filtered tracheal homogenates. The isolated virus was genetically indistinguishable from duck adenovirus (DAdV-1). Our report confirms for the first time the presence of DAdV-1 in Canada and also reports for the first time adenovirus associated respiratory disease in ducklings and supports previous findings that some DAdV-1 can be pathogenic even in waterfowl.

(A full length manuscript will be submitted for publication to *Avian Diseases*.)

IT'S THE FEED! VITAMIN E DEFICIENCY IN TURKEYS

T. R. Olson

Moroni Feed Company, Moroni Utah

SUMMARY

Moroni Feed Company, a turkey cooperative located in the mountain valley of Sanpete County Utah, places approximately 5.8 million turkey poults annually. Throughout the 2007 production year, turkey performance was significantly less than expected. Most flocks had poor feed conversion and failed to reach market weights. Increased flock mortality was also considerable with mortalities reaching at best 6% and at worst 25% at five weeks of age. This mortality was attributed to poor starts, a sharp increase in poult enteritis, and chronic colibacillosis.

Unprecedented changes had occurred within the Moroni Feed Company production system just prior to the 2007 production year. Changes included: a switch from >90% Orlopp strain poults to all Nicholas/Hybrid strains, a company owned hatchery that moved from multiple stage to single stage incubation using new setters and hatchers, the purchasing of poults from an outside hatchery that required considerable shipping distances, and the addition of a new nutrition consulting firm with a concomitant change to a "hotter" diet with significant increased fat levels. To further complicate matters, a move was made by many member growers to upgrade their production facilities with new equipment. The most significant change was a move to radiant heat brooder stoves. Other changes included new ventilation equipment and a move to automatic nipple water systems.

Needless to say there were fingers pointed in just about every direction to place blame on the poor performance. Rule outs and areas of concern included poor poult quality, introduction of a new enteric and/or respiratory virus or viruses into production units, something "wrong" with the feed (bacterial contamination, mycotoxins or mixing errors). All this was complicated by the unfamiliarity of a new breed and the many changes in management strategies both good and bad.

All rule outs were examined thoroughly and some changes were made that resulted in some measure of success. However, it wasn't until late fall that a major break-through occurred. A submission was made to the Moroni Feed Company Veterinary Diagnostic Laboratory of some 21-day-old tom poults with the chief complaint of lateral recumbence and paddling. Lesions included a unilateral hyperemic wing and a cherry-red cerebellum, pathognomonic for nutritional encephalomalacia due to vitamin E deficiency. Tissue samples were submitted to the Fresno Branch of the California Animal Health & Food Safety Laboratory System where the diagnosis of encephalomalacia was confirmed.

During the next several months, great effort was made to find the cause of the vitamin E deficiency. It was later determined that the correct level of vitamin E, as part of a premix, was being added to all diets. However, fat and meat meal additions to the diet had not been protected with an antioxidant. Therefore as diets went through varying stages of rancidity, vitamin E acting as an antioxidant was oxidized rendering it inactive.

During the months that followed, as poults consumed feed with adequate levels of vitamin E, flock performance and mortality returned to normal levels.

AN INTRODUCTION TO AVIAN INFLUENZA VIRUS MOLECULAR EPIDEMIOLOGY

John Pasick

Canadian Food Inspection Agency, National Centre for Foreign Animal Disease, Winnipeg, Manitoba, Canada R3E 3M4

INTRODUCTION

Avian influenza (AI) outbreaks in gallinaceous poultry are not random; rather, they can usually be related to environmental and host factors that vary with time, place and population subgroup. Molecular epidemiology, which employs molecular techniques to type and subtype avian influenza viruses, is one useful way of providing insight into the potential source and distribution of the viruses responsible for these outbreaks.

The ultimate source of all avian influenza viruses is wild aquatic birds belonging to the orders Anseriformes and Charadriformes. These viruses are characterized by combinations of two surface antigens: the hemagglutinin (HA), of which there are 16 genetically and antigenically diverse subtypes and the neuraminidase (NA), of which there are nine genetically and antigenically diverse subtypes. In addition to these surface antigens, the segmented genome of AI viruses code for an additional eight proteins. Under some situations viruses from this natural reservoir compartment are transmitted to gallinaceous poultry where they may undergo adaptation. It is thought that the evolutionary dynamics of AI viruses within wild aquatic birds and gallinaceous poultry differ significantly. Viruses in wild aquatic birds are thought to be in a state of evolutionary stasis characterized by low rates of evolutionary change (1). By contrast, viruses that are introduced to abnormal hosts like gallinaceous poultry are thought to undergo higher rates of evolutionary change. Influenza viruses undergo genetic change by a number of different mechanisms. These include point mutations, insertions, deletions and, due to the segmented nature of the viral genome, segment reassortment.

BASIC CONCEPTS OF MOLECULAR EVOLUTION

AI viruses have RNA genomes and given the error prone nature of RNA polymerases, mistakes in nucleotide incorporation is a feature associated with the replication of their genes. Because of the degeneracy of the genetic code these nucleotide incorporation errors or substitutions can be either synonymous (i.e. result in no amino acid change) or non-synonymous (i.e. result in an amino acid change). Prior studies have shown that AI viruses from wild aquatic birds have relatively low numbers of nonsynonymous to synonymous substitutions per site (d_N/d_S) which may indicate that strong selective constraints are at work within this population (2). Nucleotide substitutions fall into two major groups. Those which involve a purine (adenosine and guanine) being replaced by another purine or a pyrimidine (cytosine and thymidine/uracil) being replaced by another pyrimidine are referred to as transitions. Those which involve a purine being replaced by a pyrimidine or visa-versa are referred to as transversions. For steric reasons, transitions are more easily made than transversions, a fact that is taken into consideration in many nucleotide substitution models. Other errors in viral gene duplication can result in the deletion or insertion of one or more nucleotides, referred to as *indels*. If the indel involves multiples of three nucleotides, the reading frame of the gene is preserved. However, when the indel involves one or two nucleotides, the reading frame of the gene is disturbed resulting in a truncated and often defective viral protein.

The above changes are random in nature. Their fixation within a population is termed the evolutionary rate. The evolutionary rate for AI viruses has been estimated to be quite high, ranging from 1.8 to 8.4 x 10^{-3} substitutions/site/year (3). In the presence of appropriate selective pressures, changes resulting in an adaptive advantage will increase in frequency and become fixed in a population after fewer generations than if the change were to have neutral effects. This is viewed as an adaptive evolutionary process on which natural selection is based. Alternatively, the neutral theory of evolution views that the majority of genetic changes are the result of the random fixation of neutral mutations. Positive selection still occurs but only a minority of mutations become fixed by this process. Hence, based on the neutral theory of evolution, the majority of mutations that become fixed within a population are a result of random genetic drift. Molecular phylogenetic analysis makes use of nucleotide and amino acid sequence data to infer phylogenetic relationships of AI viruses based on the concepts of natural selection and neutral evolution.

PHYLOGENETIC TREES AND METHODS FOR INFERRING THEM

Evolutionary relationships among AI viruses can be illustrated by a phylogenetic tree. A typical phylogenetic tree and its parts are illustrated in Figure 1.

Phylogenetic analysis is a multi-step process which can be summarized as follows:

1. Alignment of the nucleotide or amino acid sequences of the viral genes of interest.
2. Determine the presence of a phylogenetic signal i.e. the presence of conserved and random positions in an alignment that can be used in phylogenetic inference.
3. Determine the best tree building method for the data set.
4. Choose the strategy to find the best tree under the selected optimality criterion.
5. Scrutinize the tree obtained to determine the level of confidence of the results.

Multiple Sequence Alignment. The alignment of nucleotide or amino acid sequences is an essential first step for most phylogenetic analyses. Alignments can be done manually but are most often accomplished using computer programs like Clustal X. Sequences are aligned one on top of the other so that homologous (descending from a common ancestral residue) nucleotides or amino acids from different sequences line up in the same column. Sequences that are evolutionarily related begin as identical to one another and then diverge over time with the accumulation of substitutions and indels. Gaps are introduced to the alignment with the expectation that they will correspond to the indels, thus leaving the columns maximally aligned (Figure 2).

A commonly used measurement of sequence similarity is **sequence identity** which is defined as the number of identical residues in an alignment divided by the number of aligned positions. One can also count the number of nucleotide differences per homologous site to obtain a measure of sequence divergence. This measure known as the observed distance or **p-distance** in many cases underestimates the true genetic distance or **evolutionary distance** between sequences. This is because multiple hits or more than one mutation may have occurred at a particular site in a sequence. To compensate for this, various nucleotide substitution models are used to infer the evolutionary distance from the data.

Some Methods used for Constructing Phylogenetic Trees. A number of different methods exist for inferring phylogenetic trees. The resulting trees may or may not differ from the true phylogenetic tree. Methods can be grouped based on:

1. Whether they use discrete character states or a distance matrix of pairwise dissimilarities.
2. Whether the method employs stepwise clustering to construct a single tree or carries out an exhaustive search of all theoretically possible trees.
3. Whether or not an explicit model of evolution is employed.

Maximum parsimony (MP) and Maximum likelihood (ML) are both character-based methods that examine the theoretically possible tree topologies for a given number of virus isolates. ML typically utilizes an explicit model of evolution whereas MP does not. The Neighbor-joining (NJ) method is a non-character, distance matrix based method which also utilizes an explicit model of evolution.

In the **neighbor-joining** method (4) the aligned DNA sequences of interest are used to calculate the genetic distances according to the nucleotide substitution or evolutionary model being utilized. This results in a distance matrix in which the character states (A, T, C or G) of the original data matrix are lost. The NJ algorithm analyses this distance data by first grouping the two OTUs (virus isolates) with the smallest distance between them and then progressively adding more distant OTUs to the group or to new groups. The method assumes that the data are additive so that the observed distance between two OTUs in the resulting tree, is equal to the sum of the branch lengths connecting them.

The **maximum parsimony** method (5,6) analyses the character states (A, G, C, T) of the original data matrix using an optimality criterion. This method aims to find the tree topology for a set of aligned sequences that can be explained by the minimal number of character changes or fewest evolutionary steps. Minimization of the evolutionary change necessary to infer a phylogenetic tree reflects the philosophical arguments of "Ockham's Razor" which takes the position of simplicity over unnecessary complexity. The methods used for finding the optimal tree include an exhaustive search but for analyses that involve > 20 OTUs, heuristic methods are usually employed. MP is the method used most frequently by cladists, whose major interests are the ancestor-descendent relationships between organisms.

The **maximum likelihood** method (7) like MP, analyses the original data matrix using an optimality criterion. Phylogeny inference using this method sets out to determine the tree topology, branch lengths and the various parameters of the evolutionary model, such as the transition/transversion ratio, base frequencies and rate of variation among sites, which maximizes the probability of observing the sequences being analyzed. This method produces a large number of different trees, and estimates for each tree, the conditional probability

that it represents the true phylogeny given the data and evolutionary model being used. The "tree space" or number of bifurcated rooted trees for *x* taxa (viral gene sequences) is given by the equation:

$$\frac{(2x-3)!}{[2^{x-2}(x-2)]!}$$

A data set of just 10 influenza hemagglutin genes for instance, will generate 34,459,425 rooted trees that have to be examined if an exhaustive search is employed. This becomes computationally prohibitive for data sets > 10 necessitating the use of alternative strategies to search the "tree space" which may not guarantee that the best possible tree is examined.

The goal of any tree reconstruction method is to select the one tree that actually represents the historical branching order of the sequences that are being analyzed. One must understand that the trees obtained, regardless of the method that was used in their construction, are only estimates of the true tree and therefore the reliability of those estimates must somehow be measured. The most widely used method for doing this is the **bootstrap** method although other methods also exist. Bootstrapping involves generating artificial data sets by randomly choosing columns from the original alignment to create a new alignment. Each column in the original alignment can be selected more than once or not at all until the new set of sequences or bootstrap replicate is the same length as the original. A new tree is then constructed and the process repeated. The proportion of OTUs that cluster together among all bootstrap replicates is computed and this proportion gives an indication of the statistical significance of a cluster's monophyletic origin.

APPLICATIONS OF PHYLOGENETIC ANALYSIS OF AVIAN INFLUENZA VIRUSES

There are numerous examples of how phylogenetic analysis has enhanced our understanding of avian influenza outbreaks in poultry. It can provide clues as to the origin of an isolate – whether its ancestor was responsible for previous outbreaks in poultry or whether it was the result of a separate introduction from the wild bird reservoir. For instance, phylogenetic analysis carried out on H7 avian influenza viruses isolated between 1994-98 from live bird markets in the northeast USA (8), inferred that that the majority of the outbreaks were the result of a single virus introduction. These viruses continued to circulate within those markets where they underwent a progressive adaptive evolution to poultry. The LPAI H7N3 which was later responsible for the outbreak that occurred in the spring and summer of 2002 in Virginia, West Virginia and North Carolina was shown to be genetically related to the H7N2 viruses circulating in those live bird markets (9). A similar analysis of H5 subtype viruses isolated between 1998 and 2002 from US poultry (10), showed that they clearly differed from HPAI A/chicken/Pennsylvania/1370/83 (H5N2) forming a different clade that contained two distinct subgroups or clusters A and B. This clustering was based on analysis of the HA and NS genes of these isolates. Although these H5 viruses shared high sequence similarity in their HA genes, the genetic evidence suggested that they were likely the result of separate introductions from the wild bird reservoir rather than extended circulation within poultry. Epidemiologic, serologic and molecular phylogenetic methods (Figure 3) were used to implicate the wild bird reservoir as the most likely source of virus responsible for the HPAI H7N3 outbreak on a Saskatchewan broiler breeder farm in September 2007.

The LPAI H3N2 viruses identified as responsible for moderate to severe drops in egg production in turkey breeder layer flocks in the USA and Canada since 2004, were shown to be most closely related to triple reassortant H3N2 viruses that had circulated in the U.S. swine population since 1998 (11,12,13). In many cases the affected turkey flocks were in close proximity to swine farms supporting the conclusions of the inferred viral phylogeny. Phylogenetic analysis showed that genome of these viruses is comprised of human-like HA, NA and PB1 genes, swine-like M, NS and NP genes and avian-like PA and PB2 genes.

Finally, phylogenetic analyses have been essential to our understanding of the continued evolution and spread of Eurasian H5N1 HPAI viruses. These viruses, which have spread to three continents, have undergone numerous reassortments with other avian influenza viruses generating many different genotypes in the process. The HA protein, however, has not been replaced and forms the basis for comparing the different viral isolates (14). Based on phylogenetic analysis performed on all of the publicly available H5 HA sequences that have evolved from the 1996 goose/Guangdong H5N1 isolate, the currently existing Eurasian H5N1 isolates have been grouped into 19 clades. Clade designation is based of a hierarchical numbering system which is being advocated for universal acceptance by researches when referring to the currently circulating HPAI H5N1 viruses.

REFERENCES

1. Evolution and ecology of influenza A viruses. Microbiol. Rev. 56: 152-179. 1992.

2. Widjaja, L., S.L. Krauss, R.J. Webby, T. Xie, and R.G. Webster. Matrix gene of influenza A viruses isolated from wild aquatic birds: ecology and

emergence of influenza A viruses. J. Virol. 78: 8771-8779. 2004

3. Chen, R. and E.C. Holmes. Avian influenza virus exhibits rapid evolutionary dynamics. Mol. Biol. Evol. 23: 2336-2341. 2006.

4. Saitou, N. and M. Nei. The neighbour-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 4: 406-25. 1987.

5. Farris, J.S. Estimating phylogenetic trees from distance matrixes. Am. Nat. 106: 645-668. 1970.

6. Fitch, W.M. Toward defining the course of evolution: Minimum change for a specific tree topology. Syst. Zool. 20: 406-416. 1971.

7. Felsenstein, J. Evolutionary trees from DNA sequences: A maximum-likelihood approach. J. Mol. Evol. 17: 368-376. 1981.

8. Suarez, D.L., M. Garcia, J. Latimer, D. Senne, and M. Perdue. Phylogenetic analysis of H7 avian influenza viruses isolated from the live bird markets of the northeast United States. J. Virol. 73: 3567-3573. 1999.

9. Senne, D.A. Avian influenza in North and South America, 2002-2003. Avian Dis. 50:167-173. 2007.

10. Lee, C.-W., D.A. Senne, J.A. Linares, P.R. Woolcock, D.E. Stallknecht, E. Spackman, D.E. Swayne, and D.L. Suarez. Characterization of recent H5 subtype avian influenza viruses in US poultry. Avian Path. 33:288-297. 2004.

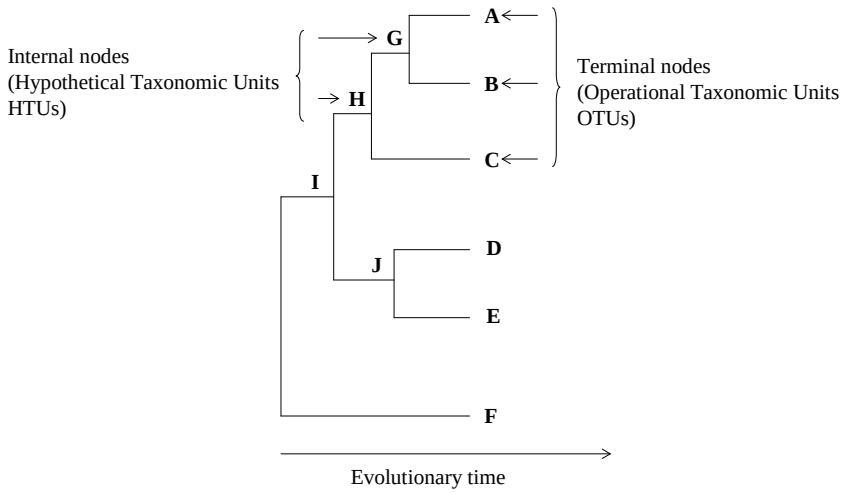
11. Tang, Y., C.W. Lee, Y. Zhang, D.A. Senne, R. Dearth, B. Byrum, D.R. Perez, D.L. Suarez, and Y. M. Saif. Isolation and characterization of H3N2 influenza A virus from turkeys. Avian Dis. 49:207-213. 2005.

12. Choi, Y.K., J.H. Lee, G. Erickson, S.M. Goyal, H.S. Joo, R.G. Webster, and R.J. Webby. H3N2 influenza virus transmission from swine to turkeys, United States. Emerg. Infect. Dis. 10:2156-2160. 2004.

13. Olsen C.W., A.I. Karasin, S. Carman, Y. Li, N. Bastien, D. Ojkic, D. Alves, G. Charbonneau, B.M. Henning, D.E. Low, L. Burton, and G. Broukhanski. Triple reassortant H3N2 influenza A viruses, Canada 2005. Emerg. Infect. Dis. 12:1132-1135.

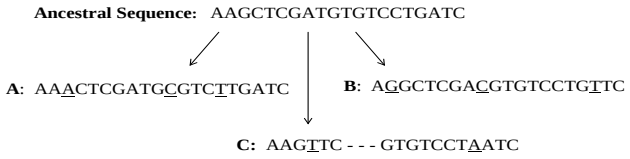
14. http://www.who.int/csr/disease/avian_influenza/guidelines/nomenclature/en/

Figure 1. Evolutionary relationships among AI viruses.



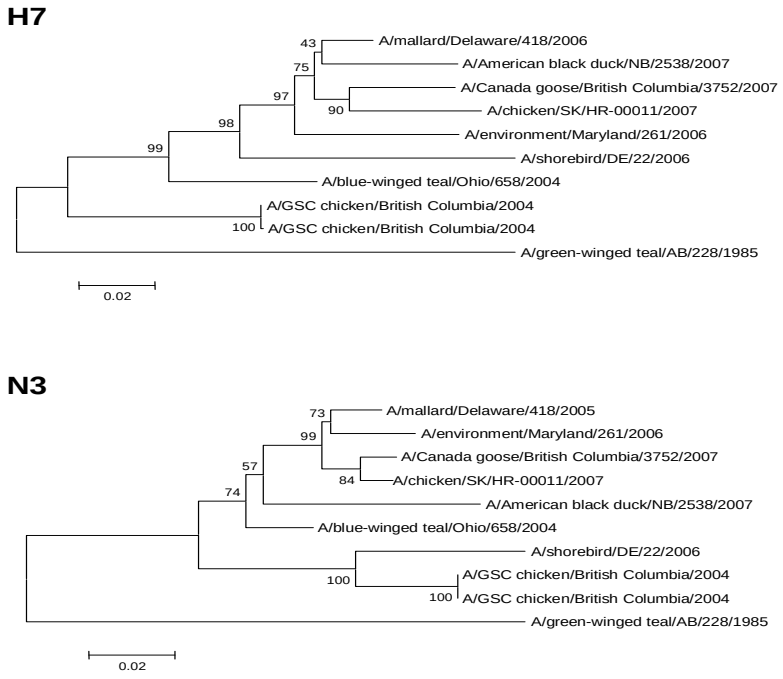
The OTUs A, B, C, D, E and F represent individual AI virus isolates. The HTU H represents a hypothetical AI virus from which A, B and C evolved. Since A, B and C cluster together they are said to have a monophyletic origin.

Figure 2. Three sequences which are derived from a common ancestral sequence mutate and diverge. Mutations include transitions, transversions and a three-nucleotide deletion.



A: AAACTCGATGCGTCTTGATC
B: AGGCTCGACGTGTCCTGTTC
C: AAGTTCGT - - - GTCCTAATC

Figure 3. Phylogenetic analysis of H7 and N3 genes of A/chicken/Saskatchewan/HR-00011/2007 and H7N3 viruses recently isolated from wild aquatic birds. The trees were generated using the neighbor-joining method. Evolutionary distances were computed using the method of Nei-Gojobori. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.



PROTECTION AGAINST VARIANT STRAINS CONFERRED BY THE RECOMBINANT HVT-IBDV VACCINE VAXXITEK®

Francisco Perozo[A], Pedro Villegas[A], Rafael Fernandez[B], and Julio Cruz[B]

[A]Poultry Diagnostic and Research Center, Athens, GA., USA
[B]Merial Select, Inc. Gainesville, GA, USA

SUMMARY

The use of viral vectors for transgenic expression of immunogenic proteins is a current trend in poultry disease control. The objective of this work was to assess the protection against variant E strain challenge conferred by day-one vaccination using VAXXITEK®, a recombinant herpesvirus of turkey expressing the immunogenic viral protein 2 from a classical IBDV. Specific pathogen free (SPF) and commercial one-day old birds were vaccinated with VAXXITEK by the subcutaneous route and challenged with the variant E strain at 18 or 28 days of age. The protection criteria included: bursa/bodyweight ratio and bursal histopathology scores. Adequate protection was demonstrated. Bursal indexes in vaccinated SPF and broiler groups were significantly higher than in the challenged controls. Vaccination protected against bursal damage as indicated by significantly lower bursal lesion scores in the vaccinated birds at both challenge points. These results indicate that single dose recombinant HVT-IBDV vaccination protects chickens against classical and variant strains.

EXPERIMENTAL DESIGN

One hundred twenty SPF birds and 120 broilers were divided in six groups. On day one and for each type of birds, two groups were vaccinated by the subcutaneous route with 0.1 mL containing 3000 plaque forming units of the recombinant vHVT013-19 (VAXXITEK). At 18 and 28 days, one vaccinated and one unvaccinated group was challenged by the intraocular route with $10^{3.2}$ EID_{50}/0.03 mL of variant E; a third group remained as unchallenged control. Bursa/bodyweight ratio and bursal histopathology were assessed as protection criteria.

RESULTS AND DISCUSSION

The results indicate an adequate protection in SPF and broilers: the relative bursa:body weight was significantly higher ($P < 0.05$) and the bursal lesion scores significantly lower in the vaccinate birds when compared with the unvaccinated-challenged controls regardless of the age at challenge (Table 1). The variant strains isolated and genotyped in the United States and in other countries exhibit antigenic drift affecting neutralizing epítopes in the capsid protein VP2 (1,2). The VP2 gene expressed by the recombinant HVT-IBDV comes from a classical strain (52/70 Faragher strain); hence variability in the protection against the neutralizing epitopes present in the variant strains VP2 is possible. The levels of protection observed in this trial suggest that under experimental conditions and against the variant E strain, VAXXITEK provides adequate protection. Similar levels of protection produced by another HVT-vectored IBDV vaccine have been previously reported (3).

REFERENCES

1. Letzel, T., F. Coulibaly, F.A. Rey, B. Delmas, E. Jagt, A.A. van Loon, and E. Mundt. Molecular and structural bases for the antigenicity of VP2 of infectious bursal disease virus. J Virol 81:12827-12835. 2007.

2. Snyder, D.B. Changes in the field status of infectious bursal disease virus. Avian Pathol 19:419-423. 1990.

3. Tsukamoto, K., S. Saito, S. Saeki, T. Sato, N. Tanimura, T. Isobe, M. Mase, T. Imada, N. Yuasa, and S. Yamaguchi. Complete, long-lasting protection against lethal infectious bursal disease virus challenge by a single vaccination with an avian herpesvirus vector expressing VP2 antigens. J. Virol. 76:5637-5645. 2002.

Table 1. Bursa:body weight ratio and the bursal lesion scores of SPF and broiler birds challenged with variant E strain.

		Early Challenge (18 days)*				**Late Challenge (28 days)**			
Treatment ***	**Challenge**	Bursa: Body** Weight		Busal Lesion Scores		Bursa: Body Weight		Busal Lesion Scores	
		SPF	Broiler	SPF	Broiler	SPF	Broiler	SPF	Broiler
VAXXITEK®	Variant E	4.68[a]	2.53[a]	2.43[b]	1.75[b]	5.49[a]	1.69[a]	2.1[b]	1.44[b]
Unvaccinated (+) control	Variant E	1.76[b]	0.85[b]	4.00[a]	3.95[a]	1.64[b]	0.80[b]	4[b]	4[a]
Unvaccinated (-) control	No challenge	6.03[a]	1.78[a]	2.00[b]	2.00[b]	5.46[a]	1.98[a]	1.7[b]	1.85[b]

* Each value represents the average of 20 birds per treatment.

** Bursa/body weight ratios calculated using the following formula: Bursa weight/body weight x 100.

*** Means with the same letter within column are not significantly different by the SNK test ($P < 0.05$).

A CRISIS COMMUNICATIONS/MEDIA RELATIONS PRIMER

Charlie Powell

Senior Public Information Officer, College of Veterinary Medicine,
Washington State University, Pullman, WA 99164-7010
Phone (509) 335-7073 (24 hrs.), Email: cpowell@vetmed.wsu.edu

Sound media relationships are a three-way business relationship between you, your stakeholders/ audience, and the media that will deliver your message. Your goal is to provide media with *saleable information* designed to go *through* them and *to* your audience. Anything less is destined to be ineffective at best (unheard message) and a failure (negative reaction) at worst.

What is a crisis? Any unscheduled event that can or will result in intense media scrutiny on an accelerated timeline and that could most likely negatively impact the primary concerns of a business or institution. Recall too, the lay public and media have a much more liberal definition of what they *feel* is a crisis and that evolves into a reality by broadcast. An emerging crisis can involve many conceivable things, especially one or more of the following:

- Crime;
- Negligence;
- Unethical conduct;
- Accidents;
- Public or personal health;
- Emergencies;
- Natural disasters;
- Catastrophes.

Successful crisis management requires:

- Prior planning;
- Pre-determined, trained, spokespeople;
- Open lines of communication established beforehand, including "new" media, i.e. blogs, webcasts, MySpace, YouTube, Facebook, etc.;
- Effective, purely honest media relations developed over a long period of time;
- Dedication;
- Attention to detail;
- An unwavering ethic to be reasonable, forthcoming, and truthful at all costs.

Important truths about media relations and crisis communications:

1. Even in the direst cases, each media contact is an extraordinary opportunity to reach your audience with your message. Rarely is it sound to pass up a media contact. If legal tells you otherwise, you need to build a better working relationship with them before a crisis. Welcome and work with media in a sound business manner.
2. Always, always, always confirm for yourself the details of a crisis as quickly as possible before speaking.
3. "Smoldering crises"—multiple studies have revealed that 80 percent of all "crises" involve issues or events known well in advance to those who must deal with them or who will be most negatively impacted by them.
4. "Perception is reality"—Well sort of. It's true for the most part, but perceptions are best fueled to realities by some element of fact also being present. That fact *may or may not be* related to the real issue. Nonetheless, you will have to address it and it will emotionally move your stakeholders.
5. "Truth and trust go hand in hand"—The only thing we all have in reality is credibility with others. If you lie, people don't trust you. Once people don't trust you, the truth no longer has any value to you. Once your character and credibility is called into question or worse yet exposed as fraudulent, you're done in the business.
6. "No comment" is not an appropriate answer nor will it help to resolve a crisis in your favor. Such a response is typically received by the public as, "they have something to hide." Empower those you prefer not talking to media by teaching them to say: "I'm not the person that has the information on that. Here is the contact information for the person who does. If you problems reaching them, call me back and I will help track them down."
7. Be willing to question any information you are given from any source before you convey it to the public as fact or advice. Have appropriate expertise speak to their areas of expertise.
8. The media is a necessary part of modern business—deal with them at each and every opportunity and develop sound business relationships early.

9. Develop a sound working relationship with your legal counsel in advance as this is growing in importance.

10. The best handling of crisis and risk communication occurs when all the players know each other BEFORE the crisis. There is no substitute for reaching out and getting to know all who might be involved as well as conducting training exercises.

11. Prepare for the long haul. Media interest will continue in a crisis as long as the story remains saleable to the public.

ARE YOU MEDIA READY?

Charlie Powell

Senior Public Information Officer, College of Veterinary Medicine,
Washington State University, Pullman, WA 99164-7010
phone 509-335-7073 (all hours), fax 509-335-2132
email: cpowell@vetmed.wsu.edu
website: www.vetmed.wsu.edu

Media relations are the most visible, successful, and frequently used public relations tool. This guide will help you develop skills to become media ready.

Tips on Speaking with Media Representatives:

- When a reporter calls, buy time. Ask when their deadline is and tell them you will call back in 20 or 30 minutes because you are speaking with someone right now.
- Use the time to develop your message. Three to five simple, declarative sentences, 20 words or less.
- Always provide a business card. If the subject is complicated, add a bulleted fact sheet.
- Be yourself.
- Be conversational, rather than formal in your approach. Speak in short, concise sentences, 20 words or less.
- Express passion for your topic.
- Be honest about your knowledge and skill levels.
- Never say anything that you don't want to see in print or hear on the air. There is no such thing as **off the record especially in today's world where people produce their own news**.
- Be prepared for the interview by having a comment ready for each of the topics and issues that touch your area of expertise.
- Each interview needs well thought out ideas. Take a second to think before speaking. If you don't understand the question, say so and it will be rephrased.
- Choose one or two messages that are important for the public to know. Emphasize those points through repetition and comments such as "This is very important."
- Offer to help the interviewer with as much information as you may have available. By feeding them information, you will have a good idea of what questions will be asked of you.
- Remember that people in the listening or viewing audience don't usually know industry jargon. Substitute common terms. Pretend you are talking to an aging friend.
- If a statement is made that is not true, refute it immediately and politely.
- This is not an oral examination or a court deposition. When you have delivered your message simply and clearly, stop talking. If asked again, make your statement again and then smile and stop talking.
- Get additional media relations training at each opportunity.

Television Tips:

- Choose an appropriate background.
- Direct your attention to the person conducting the interview—not the camera.
- Wear clean, conservative colors and clothing. Avoid harsh contrasts in color and patterns. Present a professional image.
- Make sure your hands and fingernails are clean.
- Try to keep movements to a minimum and underplay your gestures. Sit or stand still in a natural and relaxed manner.
- Visual aids are always helpful.
- Be yourself! Remember, TV is an intimate medium that seeks emotion above all else.

Radio Tips:

- Listen to the interview or show ahead of time to get to know your host and audience.
- Have facts and examples written on note cards for easy reference.
- Be relaxed; you have time to make your point.

- Be careful what you say during commercial breaks, as your microphone might still be on.

Newspaper Tips:

- Always ask for the reporter's deadline.
- Be flexible and prepared to change your schedule to accommodate the reporter.
- Make friends with the reporter—not enemies.
- You can ask if the reporter knows when the story will run; however, usually the reporter will not be able to give you an exact date.
- Reporters are simply a conduit for information; you are really talking to an audience.

Telephone Tips:

- Ask for the topic and the angle of the story.
- If you need time to prepare, ask if you can call the reporter back.
- Ask the reporter if responses are going to be taped directly off the phone.
- Answer the questions briefly and to the point. Remember nothing is **off the record**.

New media:

- This includes blogs, podcasts, RSS feeds, mobile feeds, and online video content **not just** your website
- Journalists today expect these other sources to be available
- People opposed to your enterprise will use these communication channels
- It can have profound influence
- In crisis and risk communication, these media are essential.

For Every Interview:

- Be personable
- Be accurate
- Be accessible
- Be careful
- Be exciting
- Be direct
- Be available

Most importantly…

Always remember you are talking THROUGH media to YOUR AUDIENCE. You are NOT talking TO media exclusively. All messages must be saleable for media's audience AND address your needs to your stakeholders.

IN VITRO BIO-MOS AGGLUTINATION OF *SALMONELLA* ISOLATES FROM CALIFORNIA POULTRY FARMS

Nancy Reimers, Kyle Newman, Francisco Uzal, and Gregg Cutler

SUMMARY

Twenty individual isolates of *Salmonella* spp. were selected from cases submitted to the California Animal Health and Food Safety Laboratory System. The cases represented 11 unique accessions from four companies in California. All companies routinely utilize a *Salmonella* vaccination program and occasionally include mannin-oligo-sacharride products in their rations.

All isolates were non-group D *Salmonellas*. Eighty-five percent of the submitted isolates were from routine chick paper screening and 15% were from mortality – one direct swab and two intestinal pools. BIO-MOS agglutinated 70% of the isolates. BIO-MOS agglutinated 100% of the mortality isolates and 65% of the chick paper isolates.

(The full-length article will be published in *Avian Diseases*.)

Sample Number	Agglutination by BIO-MOS	Source	Company
1A	Yes	Chick Papers	A
1B	Yes	Chick Papers	A
1C	Yes	Chick Papers	A
1D	Yes	Chick Papers	A
2A	No	Chick Papers	A
2B	No	Chick Papers	A
3A	Yes	Mortality - Direct Swab	B
4A	Yes	Chick Papers	C
5A	Yes	Chick Papers	C
6A	Yes	Chick Papers	C
6B	No	Chick Papers	C
7A	Yes	Chick Papers	C
7B	Yes	Chick Papers	C
8A	No	Chick Papers	C
9A	Yes	Chick Papers	C
9B	Yes	Chick Papers	C
10A	No	Chick Papers	D
10B	No	Chick Papers	D
11A	Yes	Mortality - Intestinal Pool	D
11B	Yes	Mortality - Intestinal Pool	D

PLATINUM BROODING

Stewart J. Ritchie

Canadian Poultry Consultants Ltd., Abbotsford, British Columbia

Managing poultry health in a constantly changing world is the theme of the 58th Western Poultry Disease Conference. The importance of optimum brood management cannot be overemphasized, not only in terms of allowing the bird to produce to its genetic potential, but also in terms of managing poultry health. For years poultry experts have provided information on getting chicks and poults off to a good start, however in the field this information is often overlooked.

In the field, problem solving exercises, often uncover deficiencies in brood management. In this constantly changing world, where enhanced automation, precision nutrition and precision genetic selection programs continue to change and where the specific requirements of neonates are numerous, the negative impact of a brooding deficiency is significant. Deficiencies in brood management can result in poor performance and may result in an increased incidence or severity of an infectious disease. As veterinarians, we have been very successful in providing our clients with an accurate and timely diagnosis. An accurate and timely diagnosis is important in order to initiate a cost effective treatment and to initiate and develop effective control and prevention strategies. More often than not, prevention strategies include recommendations for improvements to brood management.

In order to further emphasize and achieve a practical level of focus for commercial producers and service persons the Platinum Brooding Program is under development. The Platinum Brooding Program, at this time, consists of a one-day Platinum Brooding Class and a Platinum Brooding Service Field Call Protocol and Checklist. At this time, Platinum Brooding Distance Learning Modules are also being developed. This structured emphasis on brood management has developed, solely due to its current success in the field, where significant persistent performance and disease problems have been overcome by applying Platinum Brooding Protocols.

The Platinum Brooding Class takes place at a commercial broiler chicken farm and provides a thorough and comprehensive review of the critical brooding factors and an opportunity for hands-on experience. The Platinum Brooding Class begins with an emphasis and thorough review of biosecurity and biocontainment principles (1). Other agenda items include; an introduction and discussion of case reports, ready the barn section, chick delivery, barn equipment adjustments, completion of regulatory forms and chick necropsy and chick disease discussions (2,3). The Platinum Brooding Class notes consist of Integrated Poultry Health Management bulletins providing regionally accurate details on various topics or subsections, including for example; feed, light, litter, air, water, space, security and sanitation management.

The Platinum Brooding Service Checklist (attachment 1) is used to record important data. The Platinum Brooding Checklist is currently used as a guideline and provides the information necessary to identify critical deficiencies in brood management. The Platinum Brooding Checklist when accurately completed allows for an efficient, thorough and accurate review of current brood management practices. While Platinum Brooding Checklist targets vary on a regional basis, it has been accepted at this time for example; that **crop-fill** at 24 h post placement must be greater than 95%.

The identification of the **zone of comfort** has been considered one of the key components of the Platinum Brooding Program. Litter temperatures have been identified as a critical factor and have led to the development of litter temperature probes. Lighting intensity and distribution within the zone of comfort has also been identified as critical factors.

To date the Platinum Brooding Program has been used to provide critical information to farm managers and service-persons and used to solve various broiler performance and disease problems. The Platinum Brooding Program has also been used in the commercial layer industry, both in cages and on the floor brooding set-ups.

The Platinum Brooding Program simply provides a structured approach or system that is focused on brood management for use in the field, both for training and problem solving purposes. This presentation will discuss various field case reports where the Platinum Brooding Program has been used.

ACKNOWLEDGEMENTS

1. William Cox, British Columbia Ministry of Agriculture and Animal Health, Abbotsford, British Columbia.
2. Victoria Bowes, British Columbia Ministry of Agriculture and Animal Health, Abbotsford, British Columbia.
3. Mike McIlwee, Canadian Poultry Consultants Ltd., Abbotsford, British Columbia.

Figure 1. Platinum Brooding checklist.

Bulletin CPC-IPHM-102
Date: March 26, 2008

CANADIAN POULTRY CONSULTANTS LTD.

PLATINUM BROODING CHECKLIST
Integrated Poultry Health Management

Placement Date:		Premise Identification #:	
Farm Name:		# Birds Placed:	
Contact Name:		Date/Cycle:	
Farm Location:		CMS/PB#	
Feed Company:		Breed(s):	
Feed Representative:		Breeder ID:	
Hatchery:		Veterinarian Consulted:	
Hatchery Representitive:		Barn or Bin #:	
CHICKS		**SPACE**	
Rectal Temp. at Delivery	*Rectal Temperature Worksheet*	**Density in Brood Area**	
Weight @ Placement	*Placement Weight Worksheet*	**Density in Barn**	
Uniformity @ Placement	*Placement Weight Worksheet*		
Crop Fill @ 24 hours		**LITTER**	
Activity @ 24 hours		**Depth / area per unit**	
Distribution @ 24 hours		**Type of Litter**	
Navels	**Healed Unhealed**	**Brood Area**	
Hydration		**Temperature surface floor**	
FEED		**Moisture**	
Feeder Height		**Floor type**	Concrete Wood Other
Accessable	Yes No	**ZONE OF COMFORT**	
Distribution Comments		Infra-red Analysis	Yes No
Supplemental	Paper Trays #		
In Zone of Comfort	Yes No		
Feed Type	Mash Crumble		
Size of Crumble			
Feed Amount (grams/chick)			

Figure 2. Platinum Brooding checklist (cont.).

Bulletin CPC-IPHM-102
Date: March 26, 2008

CANADIAN POULTRY CONSULTANTS LTD.

PLATINUM BROODING CHECKLIST
Integrated Poultry Health Management

WATER		LIGHT	
Nipple Flow (ml/minute)		Intensity (Lux)	
Temperature		Light Type:	
pH		Brood Lights Zone of Comfort	Yes No
ORP		**AIR**	
Height (birds eye level) Uniform	Yes No	Temperature Sensor location	
Availability (#chicks/nipple in brood area)		Humidity (RH) Sensor location	
# chicks/nipple in barn		Static Pressure	
NVW		Ammonia	
Water Source	City Well Other	**SANITATION**	
Water Line Sanitation (describe program)		Litter Removed	Yes No
Product / concentration		Blown Out	Yes No
In Zone of Comfort	Yes No	Wash If yes:	Yes No Hot Water Cold Water
		Disinfectant / Insecticide Product/ concentration	
		Down Time (from manure out)	
		Mortality	Incinerate Compost Other
		Manure	Out Stored Distance

ASSESSING THE FINANCIAL IMPACT OF DISEASES OF EGG PRODUCING FLOCKS

Simon M. Shane

Adjunct Professor, North Carolina State University, Durham, NC, 27713
Phone: (919) 806-8695, sshane@nc.rr.com

INTRODUCTION

The Proceedings of the 2008 U.S. Animal Health Association incorporate a report on the current disease status of egg-producing flocks. Based on a survey among members of the Association of Veterinarians in Egg Production, avian pathogenic *E. coli* (APEC) complex was cited as the most common condition impacting profitability. A structured approach is necessary to develop appropriate and cost-effective strategies to minimize the impact of disease and to implement nutritional and management programs which support optimal egg production, feed conversion, livability and product quality. The emergence of the APEC complex and specifically peritonitis can be used as a model to evaluate specific diseases and to calculate the cost-effectiveness of preventive approaches incorporating biosecurity and immunization.

Manifestations of APEC infection. Primary APEC peritonitis emerged among large in-line complexes in the Midwest during the mid 1990s. The condition can cause up to 15% losses in a flock after peak production. In addition flocks may show APEC peritonitis during molting and at the onset of the second cycle of production. Erosive mortality of under 2% over a few weeks is generally not diagnosed specifically as APEC peritonitis unless routine post-mortem examinations are performed. The pathogenesis of APEC peritonitis has not been determined but it is assumed that inhalation of dust contaminated with pathogenic bacteria results in introduction of the organism into the abdominal air sacs. Local infection extends bacteria to the adjacent peritoneal surfaces and the serosal membranes surrounding the intestines, liver and reproductive tract. This contributes to extensive peritonitis within a short time. During the acute phase of infection, mortality may increase from a normal rate of 0.1% per week to over 1.5% per week. Affected hens are usually well-fleshed indicating the rapid onset of infection and the development of acute septicemia which precedes peritonitis.

Secondary APEC airsacculitis occurs when susceptible flocks are exposed to respiratory infections including MG, ND, IB, LT and coryza in some areas. The severity of the primary infection is influenced by the effectiveness of the vaccination program and administration, immune status of the flock, ventilation, climatic stress and nutrition. Both pullets and mature flocks may show up to 10% mortality following an outbreak of a primary respiratory infection followed by secondary APEC airsacculitis.

Generally treatment of mature flocks with an antibiotic is unproductive especially if FDA Prudent Use Principles are applied in medication. Some success has been achieved in reducing mortality in both pullets and young hens by administering a mannanoligosaccharide feed supplement. Management changes which may ameliorate the clinical course of APEC infection include chlorination of drinking water to a level of 2 ppm, cessation of dust and cobweb removal using air blowers and rectifying deficiencies in ventilation.

Financial impact of APEC peritonitis. The financial losses associated with an episode of APEC peritonitis can be projected using realistic assumptions related to standard production and the mortality characteristic of field infection. Table 1 quantifies the assumptions applied to calculating losses in a caged flock. These include projections of egg production during the first and second cycles, standard mortality, nest-run average revenue of $1.00 per dozen and an assumed production cost of $0.70 per dozen. In the specific example it is assumed that APEC mortality in the flock attains 5% by the 45th week of production. It is calculated that a flock of 100,000 hens would lose approximately five eggs per hen on average during the first cycle and seven eggs during the second cycle. Adjusting the contribution margin for feed not consumed by dead hens, the loss of one dozen eggs per hen spread over 100,000 hens started would amount to $27,000 over two cycles. If mortality due to APEC peritonitis occurred at the time of molting, the loss during a 30 week second cycle for 97,000 remaining hens would be $15,277 assuming mortality as shown in Table 2.

Infection of rearing pullets with APEC during the mid to second half of the growing cycle would result in cumulative mortality conservatively estimated at 2% due to airsacculitis. Assuming a pullet cost of $3.50, the loss for a flock of 100,000 chicks started would be $7,000. Generally flocks affected with respiratory disease and secondary APEC airsacculitis yields a proportion of pullets that are retarded in development.

This may result in a delay in onset of production by as much as four weeks for up to 5% of the flock. This will reduce the production of affected hens by 12 eggs or the equivalent of 0.6 eggs per hen spread over the flock. This loss is calculated to be $1,500. If pullets which die during rearing cannot be replaced with surplus birds or available hens, the 2% mortality will result in a decrease in contribution margin as a result of transferring a flock with 2,000 fewer hens. The flock operator would carry the same fixed costs for the flock but would not bear the depreciation or cost of feed consumed. The loss of these hens would depress average flock yield by eight eggs on average over the total life of the flock representing a value of $18,366.

Determining the cost-effectiveness of vaccination. The value of effective vaccination can be determined in relation to a specific disease or complex by considering cost, protective efficiency and the magnitude of losses due to infection. In the specific case of APEC, alternatives include autogenous inactivated vaccine or modified live *E. coli* products. Inactivated vaccines are generally expensive to prepare, require individual administration by injection and have variable effectiveness given the spectrum of APEC strains to which flocks may be exposed. A modified live *E. coli* vaccine based on O78 strain *E. coli* modified to delete the *aroA* gene, necessary for metabolism of cyclic amino acids has been licensed by the USDA. Over the past two years, vaccines administered by the coarse spray route during the first three weeks, and then subsequently at 12 to 14 weeks of age have been shown to suppress APEC peritonitis and airsacculitis. Given an approximate cost of $10 per 1,000 doses an egg producer would invest $2,000 in vaccinating a flock of 100,000 pullets. If successive outbreaks of APEC peritonitis generate losses of $30,000 per flock, vaccination will provide a potential benefit to cost ratio of 15:1. Suppressing airsacculitis mortality in pullets, resulting in losses during rearing and during the subsequent laying cycle would provide a benefit to cost ratio of 8:1.

The bottom line. Given a structured evaluation of the impact of disease, environmental or nutritional factor on productivity of a flock, it is possible to evaluate alternative preventive or therapeutic strategies. Since factors specific to the operation in addition to industry costs and returns and macroeconomic factors influence cost and revenue at different times, an approach such as gross marginal analysis can be applied to evaluate strategies for treatment or prevention alone or in combination. Frequently it is necessary for a veterinarian to project benefit to cost ratios in order to convince a profit-accountable manager that a recommended expenditure on prevention is justified in anticipation of a disease challenge, given a reasonable estimate of the probability, costs of prevention and consequences of an infection.

Table 1. Assumptions applied to calculating losses from *E. coli* infection in caged flocks.

Assumptions Relating to Standard Caged Flock:

Achievable Egg Production*	1st Cycle	260 eggs20-65 weeks
	2nd Cycle	140 eggs70-100 weeks
Mortality*	1st Cycle	3%
	2nd Cycle	2%

ǂ Nest run average revenue	100c/dozen
Assumed production cost (45¢ feed + 25¢ other) =	70c/dozen
Contribution margin	30c/dozen

*Based on Breeder's Management guides for Leghorn hybrids

ǂ Based on prevailing industry costs & revenue

Table 2. Calculation of losses attributable to *E. coli* peritonitis.

Cost of *E. coli* Peritonitis Resulting in Mortality in 100,000 Hen Flock
5% mortality 45 weeks to 50 weeks

Total eggs lost per hen over entire flock — 1st Cycle = 5 eggs; 2nd Cycle = 7 eggs

Decreased margin allowing for feed adjustment for dead hens:
$0.27/dozen x 12/12 eggs x 100,000 = $27,000

Loss with occurrence due to stress of molting (70-100 weeks)
Eggs lost 7 in 2nd cycle
$0.27/dozen x 7/12 x 97,000 = $15,277

EVALUATION OF THE CD8$^+$ T CELL MEMORY RESPONSE TO ADENOVIRUS EXPRESSING AIV HEMAGGLUTININ

Shailbala Singh[A], Worthie E. Briles[B], Haroldo Toro[C], De-chu C. Tang[D], Zongkai Shi[D], and Ellen W. Collisson[A,E]

[A]Texas A&M University, College Station, TX 77843
[B]Northern Illinois University, DeKalb, IL 60115
[C]Auburn University, Auburn, AL 36846
[D]Vaxin Inc., Birmingham, AL 35203,
[E]Western University of Health Sciences, Pomona, CA 91766

ABSTRACT

Non-replicating human adeno-virus vectored vaccine encoding the hemagglutinin from H5N9 avian influenza virus (AIV) (AdTW68.H5) has been shown to protect chickens from highly pathogenic viruses, with production of protective humoral immunity. The goal of the current study was to characterize the effective memory CD8$^+$ T lymphocyte response against the virus. Chickens were inoculated with AdTW68.H5 and the memory T cell responses were evaluated after ex-vivo re-stimulation with virus infected antigen presenting cells. MHC-I restricted effector T cell responses against the virus could be detected 10 days p.i. and memory T cell responses were detected at three weeks p.i. The peak response mostly mediated by CD8$^+$ T cells, was observed at five weeks p.i. and gradually decreased with time. The response was similar to that observed following inoculation of chickens with DNA plasmid expressing HA.

INTRODUCTION

Avian influenza virus (AIV) with its potential to mutate and evolve into highly pathogenic virus is a grave concern for the poultry industry. Additionally, its potential to evolve into a zoonotic pathogen also makes it a public health concern (1). Strategies to control the virus include vaccination in cases of low pathogenic viruses and culling of infected flocks in cases of a highly pathogenic virus infection. Currently poultry in affected areas is vaccinated either with inactivated whole virus vaccine (8,11) or with fowlpox vectored vaccines (10). Limitations to the efficacy of whole virus vaccine is the inability to distinguish infected from vaccinated birds (DIVA) and pre-existing immunity to the fowlpox virus (9). Viral vectored vaccines have an advantage over the use of whole virus vaccine since the risk of generation of new or virulent viruses is absent and DIVA is possible. Efficacy of vaccines is further enhanced if they are able to elicit cellular immune responses mediated by CD8$^+$ T lymphocytes which cause destruction of infected cells and thus additional viral clearance (5). Furthermore, cell mediated immune response has been shown to provide cross protection against heterologous viruses (7). A non-replicating human adenovirus vector encoding HA from H5N9 AIV (AdTW68.H5) has been developed and found to elicit an effective humoral immune response and protect chickens against highly pathogenic virus challenges (12,13). In mice recombinant adenovirus vaccine vectors have been shown to generate protective CD8$^+$ T cell immune response (2,3). In the current study CD8$^+$ T cell response to the AdTW68.H5 vaccine, shown to induce protective immunity, was demonstrated in chickens.

EXPERIMENTAL DESIGN

The AdTW68.H5 vectored vaccine was administered i.m. to B19/B19 MHC-I haplotype chickens at a dose of 1 x 10^8 ifu (12,13). PBMCs were collected at varying times post inoculation and T lymphocytes were purified as described by Seo and Collisson (6). Chicken kidney cells infected with the virus were used as antigen presenting cells. Purified T cells were restimulated *ex-vivo* by culturing with infected antigen presenting cells. The activation of T cells was determined by nitric oxide production as described by Karaca *et al.* (4).

RESULTS

The CD8$^+$ effector T cell response was detected at 10 days post-inoculation. This response was MHC-I restricted. Memory CD8$^+$ T cell response emerged at three weeks post-inoculation and were highest at five weeks post-inoculation. This T lymphoycte response was MHC-I restricted and specifically directed against AIV infected cells. The memory response did gradually decrease with time.

CONCLUSION

AdTW68.H5 vectored AIV vaccine induced CD8$^+$ T cell mediated immune response in chickens

when administered i.m. The non-replicating adenovirus expressing the HA provides a rational effective vaccine candidate since it induces both humoral as well as cell mediated immunity. Its practical application will require further studies.

REFERENCES

1. Fauci, A.S. Emerging and re-emerging infectious diseases: influenza as a prototype of the host-pathogen balancing act. Cell 124:665-670. 2006.

2. Gao W., A.C. Soloff, X. Lu, A. Montecalvo, D.C. Nguyen, Y. Matsuoka, P.D. Robbins, D.E. Swayne, R.O. Donis, J.M. Katz, S.M. Barratt-Boyes, and A. Gambotto. Protection of mice and poultry from lethal H5N1 avian influenza virus through adenovirus-based immunization. J Virol.80:1959-1964. 2006.

3. Hoelscher, M.A., Jayashankar, S. Garg, V. Veguilla, X. Lu, N. Singh, J.M. Katz, S.K. Mittal, and S. Sambhara. New pre-pandemic influenza vaccines: an egg- and adjuvant-independent human adenoviral vector strategy induces long-lasting protective immune Clin Pharmacol Ther. 82:665-671. 2007.

4. Karaca, K., I.J. Kim, S.K. Reddy, and J.M. Sharma. Nitric oxide inducing factor as a measure of antigen and mitogen-specific T cell responses in chickens. J Immunol Methods 192:97-103. 1996.

5. , G.F., R.A. , and A.D. . Influenza virus-specific cytotoxic T lymphocytes: a correlate of protection and a basis for vaccine development. Curr Opin Biotechnol. 18:529-36. 2007.

6. Seo, S.H. and E.W. Collisson. Specific cytotoxic T lymphocytes are involved in *in vivo* clearance of infectious bronchitis virus. J. Virol. 71:5173-5177. 1997.

7. Seo, S.H. and R.G. Webster. Cross-reactive, cell-mediated immunity and protection of chickens from lethal H5N1 influenza virus infection in Hong Kong poultry markets. J Virol.75:2516-2525. 2001.

8. Stone, H., B. Mitchell, and M. Brugh. *In ovo* vaccination of chicken embryos with experimental Newcastle disease and avian influenza oil-emulsion vaccines. Avian Dis. 41:856-863. 1997.

9. Swayne, D.E., J. R. Beck, and N. Kinney. Failure of a recombinant fowl poxvirus vaccine containing an avian influenza hemagglutinin gene to provide consistent protection against influenza in chickens pre-immunized with a fowl pox vaccine. Avian Dis. 44:132-137. 2000.

10. Swayne, D.E., M. Garcia, J.R. Beck, N. Kinney, and D.L. Suarez. Protection against diverse highly pathogenic H5 avian influenza viruses in chickens immunized with a recombinant fowlpox vaccine containing an H5 avian influenza hemagglutinin gene insert. Vaccine 18:1088-1095. 2000.

11. Tollis, M. and L. Di Trani. Recent developments in avian influenza research: epidemiology and immunoprophylaxis. Vet J. 164:202-215. 2002.

12. Toro, H., D.C. Tang, D.L. Suarez, J. Zhang, and Z. Shi. Protection of chickens against avian influenza with non-replicating adenovirus-vectored vaccine. Vaccine 26:2640-2646. 2008.

13. Toro, H., D.C. Tang, D.L. Suarez, M.J. Sylte, J. Pfeiffer, and K.R. Van Kampen. Protective avian influenza *in ovo* vaccination with non-replicating human adenovirus vector. Vaccine 25:2886-2891. 2007.

THE COMPATIBILITY OF HVT RECOMBINANTS WITH OTHER MAREK'S DISEASE VACCINES

G. Slacum, R. Hein, and P. Lynch

Intervet/Schering-Plough Animal Health, Millsboro, DE 19966

Recently, several recombinant turkey herpesvirus (rHVT) vaccines have been introduced in the US. These rHVT constructs are based on the insertion of immunogenic genes from Newcastle disease virus (NDV), infectious larygotracheitis virus (ILTV), or infectious bursal disease virus (IBDV). The HVT used in these recombinants belong to serotype 3 of Marek's disease virus (MDV). The common vaccines used to control MD are derived from three different serotypes of MDV, serotype 1, (eg. Rispens CVI 988), serotype 2 (eg. SB1) and serotype 3 (eg. HVT). In several cases, the poultry industry uses bivalent (HVT+SB1 or HVT+CVI988) or trivalent (HVT+SB1+CVI988) combinations based on the virulence of the MDV field challenge.

In situations where there is virulent MDV, producers will possibly combine the rHVT vaccines with other MD vaccine serotypes (Rispens CVI 988 and/or SB1), as is currently done with conventional HVT vaccines. Several trials have been carried out to investigate the potential interference that serotype 1 (Rispens CVI988) and serotype 2 (SB1) vaccines might have on the rHVT vaccines. A rHVT vaccine has been licensed in combination with SB1 (eg. INNOVAX-ND-SB). As expected, a synergistic affect was shown similar to that observed with the conventional HVT vaccine when combined with SB1. Data from our trials will be presented which show that Rispens CVI988 vaccine does not interfere with the rHVT vaccines, similar to the conventional HVT vaccine. In contrast, interference occurs when rHVT vaccines are simultaneous administrated with a conventional HVT vaccine. The interference is expressed as a decrease in the immunity against the virus from which the donor genes in the rHVT vaccine are derived.

INACTIVATION AND DEGRADATION OF INFLUENZA VIRUS AND NEWCASTLE DISEASE VIRUS DURING COMPOSTING

J. Guan, M. Chan, C. Grenier, D.C. Wilkie, B.W. Brooks, and J.L. Spencer

Canadian Food Inspection Agency, Ottawa Laboratory (Fallowfield), 3851 Fallowfield Road, P.O. Box 11300, Station H, Ottawa, Ontario, Canada, K2H 8P9

The fate of avian influenza (AI) and Newcastle disease (ND) viruses during composting was investigated by virus isolation and real-time RT PCR (1,2). Experiments included a comparison of survival of these viruses in cage layer manure and in litter taken from a floor pen of chickens. The latter was decomposed and resembled compost. These materials were contained within nylon mesh bags and were buried in compost or were held outside of compost at ambient temperatures. For control purposes, bacteria-free allantoic fluid that contained viruses was dispensed in vials that were sealed and held with other specimens. There were at least 5 $\log_{10}$ of virus per g of each specimen when the study began on day 0, but by day seven, the temperatures in compost had reached at least 50°C and the viruses had been inactivated. At ambient temperatures that ranged from 13° to 28°C, the viruses were inactivated by day 21. In sealed vials buried in compost the viruses survived to day 10, but at ambient temperatures they were viable to the termination of the experiment on day 21. At both the compost and ambient temperatures, the degradation of viral RNA was more rapid in used litter than in cage layer manure, suggesting that differences in microbial activity may have been a factor. In the absence of microbial activity, the viral RNA in sealed vials remained stable at ambient temperatures to day 21.

A role for microbial activity in the degradation of viral RNA was supported by other experiments, similar to the above, where the RNA of ND virus in embryonated eggs with intact shells, persisted during 21 days in compost. In comparison, the RNA of AI and ND viruses in muscle and liver specimens were fully degraded during this period. Likewise, the RNA of AI virus in eggs whose shells were crushed during composting was fully degraded. This suggested that the intact shells had prevented composting activity within the eggs that could have degraded the viral RNA.

In vitro studies compared the survival of viruses in water extracts containing microbes from compost with similar extracts from manure. At temperatures ranging from 35° to 55°C, survival of viruses was similar in the two extracts. However, at 25°C the killing of viruses was significantly more rapid in the suspension that contained microbes from compost than in the one that contained microbes from cage layer manure ($P < 0.05$). The findings suggest that microbes in manure, compost and water on poultry farms can influence the killing and degradation of AI and ND viruses and that this should be considered in formulating cleaning and disinfection programs following outbreaks of these diseases.

REFERENCES

1. Guan, J., M. Chan, B-L Ma, C. Grenier, D.C. Wilkie, J. Pasick, B.W. Brooks, and J.L. Spencer. Development of methods for detection and quantification of avian influenza virus and Newcastle disease viruses in compost by real time RT-PCR and virus isolation. Poultry Science 87: 838-843.

2. Guan, J., M. Chan, C. Grenier, D.C. Wilkie, B.W. Brooks, and J.L. Spencer. Survival of avian influenza and Newcastle disease viruses in compost and at ambient temperatures based on virus isolation real-time reverse transcriptase PCR. Avian Diseases, in press.

CASE REPORT DOCUMENTING AN OUTBREAK OF VERY VIRULENT INFECTIOUS BURSAL DISEASE IN NORTHERN CALIFORNIA PULLETS

Simone T. Stoute,[A,E] Peter R. Woolcock,[B] Daral J. Jackwood,[D] Susan E. Sommer-Wagner,[D] George L. Cooper,[A] Gabriel C. Senties-Cue,[A] Arthur A. Bickford, [A] Mark L. Anderson,[C] and Bruce R. Charlton[A]

[A]California Animal Health and Food Safety Laboratory System, Turlock Branch,
[B]Fresno Branch, [C]Davis Branch, University of California, Davis, 1550 N. Soderquist Road, Turlock, CA 95381
[D]Food Animal Health Research Program, Department of Veterinary Preventative Medicine, Ohio Agricultural Research and Development Center, The Ohio State University, 1680 Madison Avenue, Wooster, OH 44691

SUMMARY

Very virulent infectious bursal disease (vvIBDV) is antigenically similar to the classical strains of infectious bursal disease (Gumboro) but is characterized by high mortality and the ability to overcome previously protective levels of maternal immunity. In December 2008, 11 and 14 week old pullets from two ranches were submitted to the California Animal Health and Food Safety Laboratory (CAHFS), Turlock branch due to spiking mortality. Affected birds had been spray vaccinated with an intermediate infectious bursal disease vaccine at 10, 20, and 28 days of age. Post mortem lesions included enlarged edematous bursa of Fabricius, hemorrhages on the breast and thigh muscles, as well as hemorrhage at the junction of the gizzard and proventriculus. Histologically, there was severe lymphoid depletion, necrosis and inflammation of bursas. Necrotic lesions were also observed in the thymus, spleen, cecal tonsils, and livers. Diagnosis of vvIBDV was based on necropsy lesions, molecular detection of virus synonymous with vvIBDV strains and high mortality in SPF birds inoculated with virus. The mortality in affected, submitted flocks peaked at 26% and 34% before rapidly returning to normal. vvIBDV was first isolated from broilers in the 1980s and rapidly spread across Asia, Africa, and more recently, South America. This virus is relatively stable in the environment, highly contagious, and immunosuppressive. Establishment of vvIBDV in commercial flocks in the United States has the potential to cause substantial economical losses to the broiler and layer industry. This case is the first official documented report of very virulent infectious bursal disease in the United States. Details of the pathological findings will be presented at conference.

TRICHOMONIASIS PRIMARILY INVOLVING THE RESPIRATORY TRACT OF PIGEONS IN NORTHERN CALIFORNIA

S. T. Stoute, B. R. Charlton, A. A. Bickford, and M. C. Bland

California Animal Health and Food Safety Laboratory System- Turlock Branch
University of California, Davis
1550 N. Soderquist Road, Turlock, CA 95381

INTRODUCTION

Trichomonas gallinae is the etiological agent involved in pigeon trichomoniasis or pigeon canker. It is a flagellated protozoan parasite that primarily affects the upper digestive tract in avian species. The organism typically infects squabs during feeding and infected birds usually remain carriers for life. Almost all Columbidae are carriers of *T. gallinae* but manifestations of disease do not always occur. The typical form of the disease usually affects the upper digestive tract and organisms invade the mucosa of the oral cavity, sinuses, pharynx, esophagus, crop and occasionally the conjunctiva and proventriculus. Lesions can also extend into tissues of the head, neck, nasopharynx, orbits, and cervical soft tissue. Systemic trichomoniasis involving the liver, lung, mesentery, and heart has been documented. Degenerative lesions due to trichomoniasis have also been reported in the kidney and genitalia in pigeons infected with the virulent *T. gallinae*, Eiberg strain. The following report documents a novel manifestation of trichomoniasis with the primary tissue of involvement being the respiratory tract.

MATERIALS AND METHODS

Case history. The trichomoniasis outbreak occurred in a newly constructed squab facility in Northern California. The facility consisted of one building with 20 in-line lofts separated by wire fencing. The facility housed 1000 cross-bred white feathered breeder squab candidates with 25 breeding pairs in each loft. The affected breeder candidates were the first to be housed on the new facility and were purchased at four to five weeks of age from a single source. Increased morbidity and mortality was observed in these pigeons within two weeks of the birds being transferred to the new facility. Birds six weeks of age and older were primarily affected and exhibited signs of coughing and dyspnea. Subcutaneous emphysema in the region of the thoracic inlet was also noted in some birds and may have resulted from ruptured interclavicular air sacs, secondary to dyspnea. There was a slight increase in mortality from 5% to 7% within one month of the initial outbreak in January 2008. Mortality related to respiratory trichomoniasis continued for the next three months before gradually tapering off.

Necropsy findings. A total of 11 pigeons, four live and seven dead were submitted to the CAHFS-Turlock Branch. The predominant necropsy lesions were in the respiratory tract of dead pigeons with all seven dead birds exhibiting moderate to severe tracheal lesions. Hemorrhage was present throughout the tracheal mucosa (5/7), and a solid reddish tracheal plug was present in the lumen of the other two dead birds. Two dead birds also had a yellow, caseous exudate on the abdominal air sacs, with one of these birds also exhibiting areas of consolidation in the left lung. Only one of the four live submissions exhibited a moderate reddening of the tracheal mucosa but no tracheal plug was observed. Microscopic examination of wet mount preparations of crop fluid from the four live bird submissions revealed rare numbers of trichomonad organisms with no corresponding gross lesions in the oral cavity or upper digestive tract. There were no significant diagnostic findings based on serology, virology, parasitology, and bacteriology.

Histopathology. Hematoxylin and eosin (H&E). The most significant lesion was a severe necrotizing and hemorrhagic tracheitis (10/11) characterized by an infiltration of the tracheal epithelium with a mixed population of lymphoplasmacytic cells, heterophils, fibrin, denuded epithelium, and infiltration of large numbers of protozoan-like structures throughout the lamina propria of multiple tracheal sections. Lung sections had focal areas of fibrinopurulent pneumonia characterized by caseous necrotic debris, infiltration of coccoid bacterial organisms, heterophils, lymphoplasmacytic cells, and protozoan-like parasites especially around blood vessels (5/8). Most crop and esophageal sections were devoid of trichomonads or had only a few of the organisms. One esophageal section had a focal area of ulceration with trichomonads and bacterial colonies associated with it.

Immunohistochemistry. This stain detected trichomonads in multiple tracheal and lung tissue

sections. Multiple tracheal sections exhibited brownish red staining indicative of an extensive infiltration of the lamina propria and tracheal lumen with trichomonad organisms. The staining also correlated to regions where trichomonad-like organisms were observed on H&E. Extensive brownish red staining of trichomonads were also observed in the necrotic debris of lung parenchyma and around pulmonary blood vessels of lung sections.

DISCUSSION

This case, to the best of the authors' knowledge, is the first report of a natural infection of trichomoniasis that primarily involves the trachea and lung without significant involvement of other organs. The unique presentation and the severity of tracheal and lung lesions may be associated with the pathogenicity of the infecting strain of *T. gallinae*. It may also be possible that trichomonad organisms were aspirated from the oral and upper digestive tract and were able to invade and subsequently adapt to the tissues of the respiratory tract. Clinical disease may have also been triggered by the stress of relocation from one facility to another.

The primary pathological lesions in these submissions were a hemorrhagic, caseous tracheal exudate due to an infiltration of the tracheal mucosa and lung parenchyma with trichomonads, the associated tissue inflammatory response, and build up of necrotic debris. An interesting finding in this case was a lack of significant gross and microscopic trichomonad lesions in the oral mucosal cavity and upper digestive tract where the organism is usually identified. While the H&E staining of trachea and lung microscopic sections was highly suggestive of trichomoniasis, immunohistochemistry was a useful diagnostic tool in confirming this etiological agent. Immunohistochemistry testing was specific for the *Trichomonas* genus but not specific for the species *T. gallinae*. No morphological identification or molecular sequencing was undertaken to confirm that the species was *T. gallinae*; in spite of this, other species of *Trichomonas* have never been unequivocally demonstrated to be pathogenic for the avian host (4). Antibiotic therapy is not a standard regimen in the treatment of trichomoniasis as administration may exacerbate other subclinical conditions such as candidiasis. In the United States drugs active against avian trichomoniasis, such as dimetronidazole and ipronidazole are no longer available for use in food producing birds (1,2,5). Management of this condition depends on reducing stress in birds and removing severely affected birds from the flock. The establishment of a carrier state and production of humoral immunity have been observed to have a protective effect post exposure in adults (3). Pigeons appear to be immune to disease from virulent strains after recovery from sublethal trichomoniasis (4).

REFERENCES

1. Anonymous. Animal drugs, feeds, and related products; ipronidazole. (21 CFR parts 520, 556, and 558). Code of Federal Regulations No. 10. U.S. Government Printing Office, Washington, D.C. pp. 1685-1686. 1989.
2. Anonymous. Dimetronidazole; withdrawal of new animal drug applications. Code of Federal Regulations No. 128. U.S. Government Printing Office, Washington, D.C. p. 25312. 1987.
3. Kocan, R.M. A method for producing healthy carriers of the Jones' Barn strain of Trichomonas gallinae. J. Parasitology. 55:397. 1969.
4. McDougald, L.R. Other protozoan diseases of the intestinal tract- Histomoniasis (blackhead). In: Diseases of poultry, 11th ed. Y.M. Saif, H.J. Barnes, J.R. Glisson, A.M. Fadly, L.R. McDougald, and D.E. Swayne, eds. Iowa State University Press, Ames, IA. pp. 1001-1010. 2003.
5. McLoughlin, D.K. Observations on the treatment of Trichomonas gallinae in pigeons. Avian Dis. 10:288-290. 1966.

MAREK'S DISEASE VIRUS DETECTION IN THE FIELD BY PCR METHOD

Tamayo Maritza[A], Gallardo Jaime[A], Mireia Toldra[B], and Amanda Cook[C]

[A]Fort Dodge Animal Health, Mexico
[B]Fort Dodge Animal Health Olot, Spain
[C]Fort Dodge Animal Health Charles City, Iowa

SUMMARY

Marek's disease (MD) is a common lympho-proliferative disease of chickens, usually characterized by mononuclear cellular infiltrates in peripheral nerves and other organs and tissues including iris and skin. The disease is caused by a herpesvirus and has a major economic impact on the poultry industry because of cost of vaccination, mortality, condemnations, and loss of egg production.

There is no treatment is available, the main tool for prevention and control of the MD is the use of attenuated strains. Vaccine development for the control of MD was a highlight in avian medicine and basic cancer research, as this was the first example of a neoplasic disease preventable by immunization. For diagnostic purposes, we relied on histopathology and clinical signs, being also difficult to differentiate vaccinated from unvaccinated birds.

A PCR analysis has been specially designed to detect serotype 1 Marek's disease virus (MDV), and it consists in two different techniques:

-Real-Time PCR (quantitative PCR): permits the calculation of the number of MDV serotype 1 genomes present in 10.000 chicken cells.

-132 bp PCR (qualitative PCR): permits the ability to distinguish between the two different serotype 1 MDV strains (i.e. field and vaccine).

The qualitative PCR test detects the 132 bp repeat region of the MDV genome. This region is specific to serotype 1 and shows differences between Rispens vaccine strain and field strains. Since feather tips can be sampled in a non-invasive manner, and since they have higher virus levels than other tissues, they are selected as the tissue of choice for sampling. Non-invasive sampling also provides the opportunity to sample the same chick at several different time-points and to sample many birds in the field in order to get a statistic significance. Using FTA cards facilitates sampling and transportation.

CONCLUSION

This test allows the detection of serotype 1 MDV and to differentiate Rispens vaccine strain from field viruses. The use of this technique will be another tool that will help reduce losses due to vaccine failures and to detect susceptible birds prior to challenge.

REFERENCES

1. Baigent, S.J., L.J. Petherbridge, K. Howes, L.P. Smith, R.J.W. Currie,and V.K. Nair. Absolute quantitation of Marek's disease virus genome copy number in chicken feather and lymphocyte samples using real-time PCR. Journal of Virological Methods. 123, 53-64. 2005.

2. Baigent, S.J., K., L.P. Smith, R.J. W. Currie, and V.K. Nair. Replication Kinetics of Marek´s disease vaccine virus in feathers and lymphoid tissues using PCR and virus isolation. Journal of General Virology, 86,2989-2998. 2005.

3. Baigent, S.J., K., L.P. Smith, V.K. Nair, and R.J.W. Currie. Vaccinal Control of Marek´s disease: Current challenge and future strategies to maximize protection. Veterinaty Immunology and Immunopathology. 112,78-86. 2006.

4. Landman, W.J.M. and S.B.E. Verschuren. Titration of Marek's disease cell-associated vaccine virus (CVI988) of reconstituted vaccine and vaccine ampoles from dutch hatcheries. Avian Diseases. 47, 1458-1465. 2003.

5. Nair, V. Evolution of Marek's disease – a paradigma for incessant race between the pathogen and the host. The Veterinary Journal. 170, 175-183. 2005.

6. Venugopal, K. Marek's disease: an update on oncogenic mechanisms and control. Research in Veterinary Science. 69, 17-23. 2000.

7. Witter, R.L. and K.A. Schat. Neoplasic diseases: Marek's disease. In: Saif, Y.M., H.J. Barnes, A.M. Fadly, J.R. Clisson, L.R. Mcdougald, and D.E. Swayne. (2003). Diseases of poultry. 11th ed. (pp.407-465). Ames: Iowa State University. 2003.

EFFECTIVE CALORIC VALUE OF BIRD HEALTH AND MANAGEMENT

R. G. Teeter[A], A. Beker[A], C. Brown[A], C.Broussard[B], S.Fitz-Coy[B], J.Radu[B], and L. Newman[B]

[A]Department of Animal Science, Oklahoma State University, Stillwater, OK, USA
[B]Schering-Plough Animal Health, Summit, NJ
Phone: 405-880-1170; Fax: 405-744-7390; E-mail: teeterbob1@hotmail.com

ABSTRACT

Experiments were conducted to quantify the impact of coccidiosis upon dietary effective caloric value (ECV) and other energetic costs in broilers. Five 6 - day regions spaced throughout a 48 day growth curve were examined. Challenge consisted of an oral dose of sterile saline or a mixture of three *Eimeria* species administered as oocysts at 14, 21, 28, 35, and 42 days. Variables examined, six days post challenge, included gross and microscopic lesion scores, live weight gain and gain composition, FE and heat production (Kcal/h). Lesion scores for coccidiosis non-challenged (CNC) birds did not differ from zero ($P > 0.10$) throughout the testing period. Coccidiosis challenged (CC) scores were inversely correlated ($P < 0.01$) with live weight, weight gain, FE, energy consumption and retained energy. Effective caloric value declined markedly as lesion score increased and especially so as birds matured. Indeed the lesion score 1 ECV fell from a high of 3200 $\pm$ 4, matching the dietary MEn value with no lesion score, to just 3075, 2719 and 2520 at 20, 34 and 48 days, respectively. The ECV consequence of even low level lesion scores far exceeds husbandry considerations as lighting program and feed form. In summary results indicate that coccidiosis consequence in growing broilers is age dependent with deleterious impact being more pronounced in immunologically naïve birds late in the growth curve.

INTRODUCTION

Feeding equal quantities of MEn energy as carbohydrate, protein and lipid can result in marked differences in actual energy gain. This occurs as the heat increment associated with substrate utilization varies with the substrate type and metabolic fate. Numerous nonnutritive factors have also been documented to impact bird performance. For example, managerial issues as ventilation, stocking density, lighting program and feed processing have received considerable study for calorific impact upon broiler production. As a result, the metabolizable energy system may lead to unanticipated varying cellular energy/nutrient ratios. This is especially true when the aforementioned factors are coupled with coccidiosis. Coccidiosis is well known to adversely impact production and increase feed costs to achieve desired weights. The amount of dietary ME_n available to promote BWT and FCR has been defined as the effective caloric value (ECV; McKinney and Teeter, 2004) of dietary ME_n. Consequently, ECV transforms performance variability into dietary energy costs. The objective of the following study was to quantify the impact of coccidiosis upon ECV and measures of bird energy metabolism.

MATERIALS AND METHODS

Experiments using 1,200 Cobb X Cobb broilers were conducted to quantify coccidiosis impact upon bird energy balance. Five 6 - day regions, spaced throughout a 48 day growth curve, were examined. General bird management in floor pens and metabolic chambers as well as dietary ration specifications and ECV calculations have been previously described (1, 2). Challenge consisted of an oral dose of sterile saline or a mixture of three *Eimeria* species as E. *maxima*, *E. acervulina*, and *E. tenella* initially at 20,000; 50,000; and 30,000 oocysts per bird and increasing to 55,000; 105,000; and 50,000 oocysts per bird, respectively at 42 days to mimic production environments. Challenges were administered at 14, 21, 28, 35, and 42 days. Variables examined six days post challenge included gross and microscopic lesion scores live weight, FE, (upper small intestine: USI; mid small intestine: MSI; ceca: C), and microscopic lesion scores (*E. maxima*; *E. tenella*; and *E. acervulina*) with scores as 0 = none and 4 = high. Bird heat production (Kcal/h) was measured continuously by indirect calorimetry and body composition via x-ray analysis. Effective caloric value was estimated according to McKinney and Teeter, 2004.

RESULTS AND DISCUSSION

Study results are displayed in Table 1 for three of the five evaluation intervals. As the 21 and 35 day data were generally intermediate to the 14, 28 and 42 day results, they were not shown to conserve writing space. Coccidiosis challenge adversely impacted bird live weights, feed efficiency and MEn consumption. Reduced MEn consumption itself would be expected to worsen feed efficiency as maintenance energy costs become a higher percentage of energy consumption. However, with coccidiosis the maintenance energy expenditure itself increased significantly. In this study the determination of bird heat production and protein and lipid gain were determined enabling quantification of maintenance energy cost of all treatments as Kcal/day (2,7). Data indicated that bird maintenance cost increases with bird size, classically so for CNC birds, while the cost for CC birds increased linearly with lesion score. If CC birds exhibited higher body weights, then elevated maintenance would be expected. But, the live weight and FCR of CC birds was reduced ($P < .01$). Indeed, the elevated maintenance cost occurred with birds consuming less energy. Calories lost in excreta, measured by energy balance, further exacerbate coccidiosis consequence. In this study, CNC birds were within 95% of complete energy accounting throughout the growth curve. This deviation averaged 12% for score 2 birds on day 20 while exceeding 26% for the lesion score 2 birds at 48 days. As a result, with coccidiosis the combination of lowered appetite coupled with elevated maintenance and increased calories lost in excreta markedly impact bird performance.

Clearly, the combined actions of CC on bird appetite, maintenance and excreta energy loss impact bird performance. Effective caloric value expresses the combined impact of these variables as a dietary equivalence that would be needed to achieve the same action. Converting mash ration to 100% pellets has been valued at +187 Kcal/kg diet and lighting programs to +115 Kcal/kg ration. Conversely, since rations are not altered, the ECV provides an expression of ration caloric density loss due coccidiosis. In the reported study, the ECV of CNC birds differed little from the calculated energy value adding credence to the methodology. In contrast the ECV was sharply impacted by coccidiosis, falling as much as 71% (-2282 Kcal/kg ration) for lesion score 2 birds at 48 days while score 2 birds at 20 days only declined 18% (-601 Kcal/kg ration) 1 Lesion score 1 birds at 20 days declined just 4% (-121 Kcal/kg ration) while the score 1 bird ECV at 48 days declined over 21% (-685 Kcal/kg ration). The combination of lowered energy consumption coupled with elevated maintenance cost and excreta energy loss, makes the coccidiosis challenge critical to avoid. If some coccidiosis challenge is to occur, the data strongly suggests that early exposure will have less overall consequence to energy utilization.

ACKNOWLEDGEMENTS

The author wishes to thank Cobb-Vantress, Inc. Siloam Springs, AR, USA for research support and Schering Plough for their research and travel support.

This work has been approved for publication by the Director, Oklahoma Agricultural Experiment Station, Stillwater, OK 74087, USA.

REFERENCES

1. Belay, T., and R.G. Teeter. Virginiamycin effects on performance and salable carcass of broilers. J. Appl. Poult. Res. 3:111-116. 1994.

2. Brown, C. 2007. Evaluation of coccivac-B® and sacox 60® for control of 3 strains of *Eimeria* in broilers. MS. diss., Oklahoma State University, 2007.

3. McKinney, L.J., and R.G. Teeter. Predicting effective caloric value of nonnutritive factors: I. Pellet quality and II. Prediction of consequential formulation dead zones. Poult. Sci. 2004.

Table 1. Coccidiosis mediated lesion score effects upon production and energetic criteria at standardized weights[1].

Variable		Lesion Score		
Average Daily Gain		0	1	2
(Age, days)	Initial Live Wt. (g)			
14-20,	904	76.5	60.6	40.7
28-34	2096	92.6	54.3	27.3
42-48	3398	97.3	32.7	-7.0
Gain/Feed				
14-20,	904	0.64	0.60	0.38
28-34	2096	0.54	0.37	-0.04
42-48	3398	0.43	0.10	-0.49
ME_n Consumption/Day (Kcal)				
14-20,	904	386	342	300
28-34	2096	562	477	420
42-48	3398	701	570	482
Maintenance Cost (Kcal/Day)				
14-20,	904	124	151	281
28-34	2096	187	218	308
42-48	3398	281	308	315
Added Excreta (Kcal/Day)				
14-20,	904	16	22	35
28-34	2096	24	57	86
42-48	3398	38	94	130
Retained Energy (Kcal/Day)				
14-20,	904	188	149	100
28-34	2096	274	162	87
42-48	3398	305	110	-0.9
Effective Caloric Value (Kcal/kg feed)				
14-20,	904	3202	3075	2601
28-34	2096	3214	2719	2502
42-48	3398	3205	2520	923

[1]Values created using predictive models ($R^2 > 0.95$) and standardized initial weights.
[2]Homogenous arrays of lesion scores were applied.

A FIELD STUDY TO CONTROL CELLULITIS IN TURKEYS USING AN INACTIVATED TOXOID

Anil J. Thachil, David A. Halvorson, and Kakambi V. Nagaraja

Department of Veterinary and Biomedical Sciences,
University of Minnesota, 1971 Commonwealth Ave, St. Paul, MN 55108

Clostridium perfringens and *C. septicum* are currently considered as the organisms responsible for cellulitis in turkeys, a major cause of economic loss to turkey producers over the last few years. The involvement of other species of clostridia including *C. septicum* in the development of cellulitis in turkeys is increasingly reported recently. The objective of our study was to look at the effects of an experimental toxoid vaccine in controlling cellulitis in turkeys using *C. septicum* alone. *Clostridium septicum* was grown and allowed to sporulate producing toxins in a suitable media. The culture supernatants were used to make an experimental *C. septicum* toxoid. The experimental inactivated vaccine was found to be useful in reducing the antibiotic usage and mortality in cellulitis affected birds in the field.

HEMATOBIOCHEMICAL CHANGES OF LAYER CHICKENS IN EXPERIMENTAL AFLATOXICOSIS

N. K. Thapa[A], M. Muralimanohar[B], C. Balachandran[C], and G. Sarathchandra[D]

[A]Dy.cheif Veterinary officer, NCAH, Serbithang, Thimphu post box 155, Bhutan
Nkthapa08@hotmail.com
[B]Director, Centre for animal health studies, TANUVAS, Chennai-600 051
[C]Professor & Head, Department of veterinary Pathology, Madras Veterinary College, Chennai-600 007 India
[D]Associate professor and Head, CFFRAL(Central feed and food residue analytical laboratory), TANUVAS, Chennai-600 051, India

SUMMARY

Twenty seven-week old laying chicken were randomly distributed to three groups of 10 birds each and were fed with diets containing 0, 1 and 2 ppm AF for 42 days. The mean (±SE) PCV values were 27.60 ± 0.92, 25.60 ± 0.20 and 21.40 ± 1.16% for 0, 1, and 2 ppm respectively, indicating significant ($P < 0.05$) decrease in the PCV value in aflatoxin (AF) treated birds compared to the control. No significant differences were observed in hemoglobin (Hb) and total erythrocyte count (TEC) values. The means (±SE) of serum glucose values were 131.31 ± 11.11, 185.30 ± 1.11 and 177.71 ± 11.11 mg/dL. Significant increase ($P < 0.05$) in serum glucose values were observed in the AF fed layer chicken. However, no significant differences were observed in serum cholesterol, total protein, and albumin values. The means (±SE) of alkaline phosphatase (ALP) were 970.53 ± 128.04, 805.93 ± 128.04 and 1264.21 ± 128.04 U/L for 0, 1 and 2 ppm AF fed layer birds respectively indicating significant ($P < 0.05$) increase in the level of ALP in 2 ppm AF fed layer birds when compared to the 0 and 1 ppm AF fed birds. However, no significant differences were observed between the control and AF treated layer birds for alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Mean (±SE) values of blood urea nitrogen (BUN) were 12.90 ± 1.30, 10.33 ± 1.30 and 7.41 ± 1.30 mg/dL for 0, 1 and 2 ppm AF fed layer birds respectively which revealed significant ($P < 0.05$) decrease in the levels of BUN in AF treated birds and variable results were observed for serum creatinine when compared to the control. Mean (±SE) values of serum sodium were 165.19 ± 8.93, 166.99 ± 8.42 and 130.14 ± 8.42 mmol/L for 0, 1 and 2 ppm AF fed layer birds respectively. The values decreased significantly ($P < 0.05$) in 2 ppm AF fed layer chicken. Serum calcium, phosphorous and potassium values did not differ significantly between the control and AF treated layer chicken.

INTRODUCTION

Contamination of feed with mycotoxin is a global problem. Among mycotoxins, aflatoxins (AFs) are of the most important mycotoxins encountered in the feeds world wide (Williams *et al*., 2004). AFs are difurocoumarin derivatives produced by toxigenic fungi of *Aspergillus* species. Poultry industry suffers greater economic losses due to greater susceptibility of the species compared to other animals to the toxin and also due to continuing intermittent occurrences in feeds (Fraga *et al*., 2007).

Compared to the broilers the literature with regards to poultry layers are very limited, hence the study was conducted.

MATERIALS AND METHODS

Aflatoxin (AF) was produced on rice (7). Thirty numbers of 27 weeks old White Leghorn Forsgate layer chickens were randomly divided into three groups of 10 birds each and were fed with control and treatment diets containing 1 and 2 ppm AF for six weeks. Blood samples were collected after 42 days of trial by cardiac puncture in a vial containing Heller and Paul double oxalate mixture. Hb, PCV and TEC were estimated. Serum total protein, albumin, cholesterol, ALT, AST, ALP, BUN, creatinine gamma glutamyl transferase (GGT), calcium, phosphorous, sodium, potassium, and uric acid were analyzed using the reagents/kits (Agappe and Accurex).

Data were subjected to one/two way analysis of variance (ANOVA) using SPSS software version 10.00.

RESULTS AND DISCUSSION

The mean (±SE) PCV values were 27.60 ± 0.92, 25.60 ± 0.20 and 21.40 ± 1.16% Significant decrease in PCV was observed in 2 ppm AF fed layer chicken Compared to the compared to control. While Hb and TEC values did not differ from the control. Gounalan *et al*. (3) also reported significant reduction in PCV and

Hb values in layer chicken fed with 0.5 ppm of AF from 0 to 12 weeks age. However, Fernandez *et al*. (1) reported increase in TEC and hematocrit in layer chicken by feeding 2.5 and 5 mg/kg for 32 days.

Overall means (±SE) of serum glucose values were 131.31 ± 11.11, 185.30 ± 1.11 and 177.71 ± 11.11 mg/dL. The serum glucose levels showed significant ($P < 0.05$) increase in AF fed birds when compared to the control. No significant differences for cholesterol, total protein and albumin. Similarly, no significant changes were reported in serum protein in layers fed up to 5 ppm or more AF for 28 or 32 days (2,4). However, hypoproteinemia and hypoalbuminemia were reported in laying hens fed up to 5 ppm (3,8).

There was numerical decrease in GGT and ALP in 1 ppm AF treated birds. Earlier workers reported elevated serum ALT, AST and GGT in birds fed 500 ppb AF from two days onwards (3,5,6).

Mean (±SE) values of BUN were 12.90 ± 1.30, 10.33 ± 1.30 and 7.41 ± 1.30 mg/dL and serum creatinine were 0.42 ± 0.02, 0.47 ± 0.02 and 0.35 ± 0.02 mg/dL for 0, 1 and 2 ppm respectively. There was significant ($P < 0.05$) decrease in the level of BUN and creatinine in 2 ppm level when compared to the control. In AF fed birds, no significant changes were observed in serum uric acid. Increase in uric acid and creatinine were reported by feeding 0.5 ppm AF to layer chicken up to 12 weeks of age (3).

Feeding 1 and 2 ppm of AF to layers for 42 days resulted in significant decrease in serum sodium level in 2 ppm fed birds when compared to the control groups. Serum calcium, phosphorous and potassium levels did not show any differences between the control and toxin treated birds. However, significant reduction in serum calcium and phosphorus were reported by feeding AF up to 5 ppm (2,5,8,9).

Feeding of 1 and 2 ppm aflatoxin for 27 weeks in layer chicken caused significant ($P < 0.05$) decrease in the PCV value and significant increase ($P < 0.05$) in serum glucose values when compared to the control birds. No significant differences were observed between the control and AF treated layer birds for ALT and AST. There was significant ($P < 0.05$) increase in the level of ALP in 2 ppm AF fed layer birds when compared to the 0 and 1 ppm AF fed birds decrease in the levels of BUN. Variable results were observed for serum creatinine. Serum sodium values decreased significantly ($P < 0.05$) in 2 ppm AF fed layer chicken when compared to the control and 1 ppm AF fed layer birds. Serum calcium, phosphorous and potassium values did not differ significantly between the control and AF treated layer chicken.

REFERENCES

1. Fernandez, A., M.T. Verda, J. Gomez, M. Gascom, and J.J. Ramos. Research in Veterinary Science, 58 (2): 119-122. 1995.
2. Fernandez, A., M.T. Verda, M. Gascon, J. Ramos, J. Gomez, D.F. Luco, and G. Chavez. Avian Pathology, 23: 37-47. 1994.
3. Gounalan, S., C. Balachandran, and B. Murali Manohar. 2006. International conference on advance Veterinary practice in medicine and surgery augmenting health and production, Chennai, 21-25 June, 2006.
4. Iqbal, Q.K., P.V. Rao, and S.J. Reddy. Indian Journal of Animal Sciences, 53: 1277-1280.1983.
5. Kim J.G., L.W. Lee, P.G. Kim, W.S. Roh, and H. Shintani. Journal of Food Protection, 66 (5): 866–873. 2003.
6. Rao, V.N. and H.C. Joshi. Indian Veterinary Journal, 70: 344–347. 1993.
7. Shotwell, O.L., C.W. Hesseltine, R.D. Stubblefield, and W.G. Sorenson. Applied Microbiology, 111 (3): 425 – 428. 1966.
8. Stanley, V.G., M. Winsman, C. Dunkley, T. Ogunleye, M. Daley, W.F. Krueger, A.E. Sefton, and A. Hintom. Journal of Applied Poultry Research, 13: 533-539. 2004.
9. Umesh, D., V.N. Rao, and H.C. Joshi. Indian Journal of Veterinary Medicine, 15(1): 32-34. 1995.

Table 1. Hematobiochemical values of AF fed layer chicken.

Hematobiochemical parameters	Aflatoxin level (ppm)		
	0	1	2
Hematological values			
PCV (%)	27.60a±0.92	25.60ab±0.21	21.40b±1.16
Hb (g/dL)	10.60±0.06	11.32±0.07	10.56±0.04
TEC (millions/mm^3)	2.35±0.02	2.11±0.02	1.84±0.01
Serum Metabolites			
Glucose	131.31a ±11.11	185.30b±11.11	177.71b±11.11
Cholesterol	124.57± 11.81	111.83 ± 11.81	137.66± 11.81
Total protein	4.88±0.20	4.49±0.20	4.44±0.20
Albumin	1.94±0.10	2.00±0.10	2.06±0.10
Serum enzymes (U/L)			
ALT	4.74±0.74	5.26±0.81	3.56±0.70
AST	164.89 ±6.49	173.58 ±6.49	168.38±6.49
GGT	16.68ab±1.32	13.08a ±1.32	16.16b±1.32
ALP	970.53ab±128.04	805.93a±128.04	1264.21b±128.04
BUN, creatinine & uric acid values		(mg/dL)	
BUN	12.90a±1.30	10.33ab±1.30	7.41b±1.30
serum creatinine	0.42ab±0.02	0.47a±0.02	0.35b±0.02
uric acid values	4.50±0.52	4.46±0.52	4.95±0.52
Serum mineral and electrolytes			
Calcium (mg/dL)	10.63±0.21	11.02±0.21	11.18±0.21
Phosphorous (mg/dL)	5.70±0.57	7.43±0.57	6.87±0.57
Sodium (mmol/L)	165.191a±8.93	166.99a±8.42	130.14b±8.42
Potassium (mmol/L)	8.02±1.43	8.07±1.43	9.52±1.43

Means with same superscript within a column do not differ significantly ($P > 0.05$).

SAFETY OF FEEDING RACTOPAMINE TO FINISHING TURKEYS

D.J. Ivers, C.T. Herr, D.H. Mowrey, and J. D. Trites

Elanco Animal Health, Eli Lilly and Company, Greenfield 46140

Separate tom and hen turkey studies were conducted to evaluate the safety of ractopamine (RAC) when fed at various doses in the finisher phase. Each gender-specific study utilized 48 pens (eight birds per pen) with the pen being the experimental unit. Treatment design consisted of 0, 13, and 130 ppm of ractopamine added to finishing feed rations for 14 days. Health observations were observed twice daily and blood chemistries were taken on day 0 and day 14. Parameters measured included blood, coagulation, and hematology variables, as well as bone strength. Two birds per pen were necropsied on day 14 and selected tissues were evaluated microscopically. These studies demonstrate that feeding finishing tom and hen turkeys diets containing up to 130 ppm RAC for 14 days is safe to the birds.

PREVALENCE OF PARVOVIRUS INFECTION IN CHICKEN AND TURKEY FLOCKS IN THE UNITED STATES

Laszlo Zsak[A], Keith O. Strother, and J. Michael Day

Southeast Poultry Research Laboratory, USDA, Agricultural Research Service,
Athens, GA 30605
[A]Corresponding author, E-mail: laszlo.zsak@ars.usda.gov

SUMMARY

Enteric diseases of poultry cause significant economic losses because of decreased bird weight gain, increased morbidity and mortality and increased production costs from poor feed conversions and the cost of treatment. Pathogens involved in enteric disease include bacteria, protozoa and viruses. Currently, the role of these pathogens in the etiology of enteric disease of poultry is not completely understood.

Recently, we reported that using a random molecular screening method, novel parvoviruses were detected in intestinal samples of chickens and turkeys exhibiting characteristic signs of enteric disease (1). The technique is based on random amplification of particle-associated nucleic acids in clinical samples. Using this method, we successfully identified parvoviruses in intestinal homogenates from affected birds and demonstrated that the chicken and turkey parvoviruses were closely related to each other and representative of a novel member of the *Parvoviridae* family.

Here, we describe the development and application of a conventional PCR assay to detect parvoviruses in commercial poultry flocks. In a nationwide survey a total of 138 field enteric samples from poultry flocks were tested for parvovirus presence by PCR. Of the tested chicken samples that were collected in 54 farms, 77% showed the presence of parvovirus while 78% of the turkey samples that were received from 29 farms were parvovirus positive. For the first time, our data clearly demonstrate that parvoviruses are widely distributed in commercial poultry flocks in the US. The high prevalence of parvovirus infection in young birds suggests a potential role of these viruses in the etiology of enteric disease of poultry.

Phylogenetic analyses comparing NS gene segments revealed a strong similarity between the chicken and turkey parvoviruses. It was also evident that most of the chicken and turkey parvovirus isolates formed distinct phylogenetic groups, suggesting that these viruses might have diverged from a common ancestor and subsequently went through a host specific adaptation.

(The full-length article will be published in *Avian Diseases*.)

ACKNOWLEDGMENTS

The authors thank Fenglan Li for her excellent technical assistance and the sequencing unit at SEPRL for outstanding support.

REFERENCES

1. Zsak, L., K.O. Strother, and J. Kisary. Partial genome sequence analysis of parvoviruses associated with enteric disease in poultry. Avian Pathol. 37:435-441. 2008.