PROCEEDINGS OF THE FIFTIETH WESTERN POULTRY DISEASE CONFERENCE

March 24-26, 2001. University of California, Davis, California

WPDC SPECIAL RECOGNITION AWARD FOR DONALD D. BELL

Don Bell was born in Santa Ana, California, on 17th December 1933. He was raised on an egg ranch in Central California and attended Turlock High School. Don graduated from the University of California, Davis, with a BS degree in Poultry Science in 1955. He received an MS degree in Poultry Science from Colorado State University in 1972. Don was hired as a UC Poultry Farm Advisor for Orange County in 1958, and transferred to Riverside County in 1965. He was promoted to Poultry Specialist in 1983. Don, whose career is highlighted by numerous accomplishments and awards, retired in 2000.

Don completed over 150 applied research projects, and published 11 refereed journal articles, 16 abstracts and 57 research reports. In addition, he is author or co-author of 3 book chapters, 318 trade journal articles, 160 county publications, 70 slide sets, 12 computer programs and thousands of newsletter articles. His greatest contribution has been in the area of extension education, primarily on management and nutrition of egg production pullets and laying hens. Don is currently the past-President of the Poultry Science Association (PSA). He was also associate editor of the PSA journal and a PSA director. Don has received numerous awards including the PSA Pfizer Extension Award, USDA Award for Superior Service, UC Distinguished Service Award, and the Pacific Egg and Poultry Association Person of the Year and the Poultry Scientist of the Year awards.

Don enjoys golfing and fishing, and traveling with his wife, Lucy. They have two children, Sharon and Warren. Don plans to continue working part-time for the UC Extension on a few pet projects and to consult with United Egg Producers. The WPDC is honored to recognize Don Bell for his contributions to the poultry industry.

WPDC SPECIAL RECOGNITION AWARD FOR DR. ARTHUR BICKFORD

Dr. Art Bickford was born and raised on a dairy farm in Conventry, Vermont, in 1936. After 2 years of undergraduate studies at the University of Vermont, Dr. Bickford was accepted into the veterinary medicine program at the University of Pennsylvania, where he graduated with his VMD in 1960. Dr. Bickford then did a year of graduate work at both the University of Vermont and Colorado State University before heading to Purdue University. In 1964, he obtained his MS, and in 1966 his PhD, both from Purdue University.

Dr. Bickford has held numerous positions as a pathologist within universities and industries. He traveled back-and-forth across the country before accepting the position in 1975 as an extension veterinarian with the University of California, filling the position previously held by Dr. A.S. Rosenwald. In 1979, Dr. Bickford moved back to Missouri to become the head of the Department of Veterinary Pathology at the University of Missouri. However, after 4 years, Dr. Bickford realized his mistake and returned to sunny (and non-humid and tornado-less) California as the Branch Chief of the Turlock lab for the California Animal Health and Food Safety Laboratory System (CAHFS), of the School of Veterinary Medicine, University of California, Davis. In 1992, Dr. Bickford became the Associate Director for CAHFS, and continues in this position until his retirement in June 2001.

Dr. Bickford's contribution to the poultry industry has been tremendous. He is now recognized as one of the foremost avian pathologist in the country and as such was awarded the American Association of Avian Pathologists' C.A. Bottorff award in 1995. He served on numerous educational and scientific committees, and editorial boards. He is an author on 90 publications in refereed journals, 23 book chapters, and 141 abstracts or proceeding articles. In addition, Dr. Bickford is currently past-president of the Western Veterinary Conference, and for some reason truly enjoys his annual trips to the meetings in Las Vegas.

Dr. Bickford and his wife Margaret, have three daughters, Carolyn, Patricia and Andrea, all of whom live in the mid-West. They are blessed with three grandchildren, Alexandra, Matthew and Daniel, and look forward to getting in their first class RV and traveling across the country to visit them.

SPECIAL ACKNOWLEDGMENTS

The Western Poultry Disease Conference (WPDC) is honored to acknowledge the many contributions to the Conference. These contributions provide support for outstanding participants and to help pay for some of the costs of the Conference. Approximately 50 organizations, companies and individuals have given substantial financial support. Many companies and organizations, including some that also contribute, send speakers at no expense to the Conference. We thank all these people, and acknowledge their support and contribution.

We are extremely pleased to acknowledge three contributors at the Super Sponsor level. They are the American Association of Avian Pathologists, Merial, Inc. and Positive Action Publications. In addition, we would like to recognize the American Association of Avian Pathologists, IDEXX Laboratories, Inc., Lohmann Animal Health International and Nippon Biologicals, Inc. as Benefactors. Once again, our distinguished Patrons, Donors, Sustaining Members, and Friends of the Conference are listed on the following pages. We greatly appreciate their generosity and say thanks to them and their representatives.

Thanks and appreciations are due to Dr. Ken Takeshita, for taking over as the Program Chair at the last minute, especially before he realized what he was getting into! Ken has put together an excellent program, and with 50% more titles submitted this year than normally, he had a particularly tough time arranging the program.

Many have provided special services that contribute to the continued success of this conference. The WPDC would like to thank Ms. Nicole Gibson and the Center for Avian Biology for their secretarial support to Dr. "Rosy" Rosenwald; Gina Hardwood, Kim Buckner, Yvette Valesquez, Judy Brooks and the University of California, Veterinary Medicine Extension/Public Programs for administering the contributions and financial services; Dr. Rosenwald for once again garnering the outstanding contributions; and to all others who contributed to the program and conference.

A special thanks goes to Dr. Carol Cardona for local arrangements this year. As you know, the 50th WPDC is located on the campus of UC Davis. The last time we met in Davis was in 1988. Many things have changed since then and numerous arrangements were necessary to organize the location of the meeting rooms, especially with the change to double sessions, and the catering. We are especially grateful to Carol for her untold hours of work for WPDC.

We acknowledge and appreciate Ms. Lina Layiktez and her crew at the University of California, Davis, Conference and Event Services, who handle the registration and administrative duties of this conference.

We thank Dr. David Frame for accepting the position as Proceedings Editor and for editing and producing an outstanding Proceedings. We look forward to continued excellence with the WPDC Proceedings and hope Dave is now ready for the joint meeting with ANECA next year. A special appreciation is in order for Sherry Nielson, Senior Secretary of The Utah State University Turkey Research Center, for her untiring and seemingly endless work of proofreading and formatting the Proceedings for publication. We express our gratitude to all authors who submitted manuscripts – especially those who followed the instructions and submitted their papers on time! We again acknowledge and thank Ms. Rebecca Dodson and Ominpress, Madison, Wisconsin, for the handling and printing of this year's Proceedings. Once again, we thank Bruce Patrick (Graphic Communications, Brigham Young University) for the cover design.

2001 SUPER SPONSORS

American Association of Avian Pathologists
Kennett Square, PA

Merial, Inc.
Gainesville, GA

International Poultry Production
East Yorkshire, England

2001 BENEFACTORS

American Association of Avian Pathologists
Kennett Square, PA

EMBREX, Inc.
Research Triangle Park, NC

IDEXX Laboratories, Inc.
Westbrook, ME

Intervet, Inc.
Millsboro, DE

Lohmann Animal Health International – MBL, Vineland
Gainesville, GA

Nippon Biologicals, Inc.
Tokyo, Japan

2001 PATRONS

Jones-Hamilton Co. – PLT
Salisbury, MD

Productos Toledano, S.A.
Panama City, Panama

Sunrise Farms, Inc.
Catskill, NY

Synbiotics Corporation
Kansas, MO

Veterinary Service, Inc.
Salida, CA

2001 DONORS

Alltech Biotechnology Centre, Inc.
Guelph, Ontario, Canada

ANECA
Mexico City, DF, Mexico

Bachoco S.A. de C.V.
Celaya, GTO, Mexico

Bayer Intensive Production Unit
Shawnee Mission, KS

Biomune, Inc.
Lenexa, KS

Boehringer Ingelheim Vetmedica, S.A. de C.V.
Guadalajara, Jalisco, Mexico

Charles River, SPAFAS, Inc.
Storrs, CT

Gregg Cutler, DVM, MPVM
Moorpark, CA

Fort Dodge Animal Health
Overland Park, KS

Foster Poultry Farms, Inc.
Livingston, CA

Hubbard ISA LLC
Chattanooga, TN

Hy-Line International
West Des Moines, IA

Lasher Associates, Inc.
Millsboro, DE

Moroni Feed Company, Inc.
Moroni, UT

Perdue Farms, Inc.
Salisbury, MD

Pharmacia & Upjohn Co.
Staunton, VA

A.S. (Rosy) & Jo Rosenwald
(In memory of Paul DeLay)
Davis, CA

2001 SUSTAINING MEMBERS

Alternative Design Manufacturing
Siloam Springs, AR

Aviagen North America
Huntsville, AL

Creighton Brothers LLC
Santa Rosa, CA

Gemperle Enterprises, Inc.
Turlock, CA

Megan Health, Inc., Animal Health Division
St. Louis, MO

Merrill's Poultry Farms, Inc.
Paul, ID

Nicholas Turkey Breeding Farms
Sonoma, CA

Novus International, Inc.
St. Louis, MO

Orlopp Turkey Breeding Farms
Orosi, CA

Preserve International
Turlock, CA

Schering-Plough Animal Health/ASL
Union, NJ

Valley Fresh Foods
Turlock, CA

Vega Poultry Farms
Davis, CA

Vetech Laboratories, Inc.
Guelph, Ontario, Canada

Winkler Enterprises
State College, PA

Zacky Farms
Fresno, CA

2001 FRIENDS OF THE CONFERENCE

BioSentry
Stone Mountain, GA

California Poultry Federation, Inc.
Modesto, CA

M. A. Hammarlund, DVM
Riverside, CA

Pacific Egg and Poultry Association
Sacramento, CA

J.S. West Milling Company
Modesto, CA

50TH WESTERN POULTRY DISEASE CONFERENCE OFFICERS

Dr. Patricia S. Wakenell
Program Chair
SVM: PHR
University of California, Davis
One Shields Avenue
Davis, CA 95616

Dr. Ken Takeshita
Program Chair
Lohmann Animal Health International
4759 Ridgeview Lane
Vacaville, CA 95688

Dr. Barbara Daft
Program Chair-elect
California Animal Health & Food
Safety Laboratory System –
San Bernardino
105 W. Central Ave.
San Bernardino, CA 92408

Dr. David Frame
Proceedings Editor
Utah State University Cooperative Extension
USU Turkey Research Center
325 West 100 North
Ephraim, UT 84627

Dr. Richard P. Chin
Secretary-Treasurer
California Animal Health & Food Safety
Laboratory System - Fresno
2789 S. Orange Ave.
Fresno, CA 93725

Dr. Carol Cardona
Local Arrangements Chair
School of Veterinary Medicine
University of California, Davis
One Shields Avenue
Davis, CA 95616

Dr. A.S. Rosenwald
Contributions Chair
Veterinary Medicine Extension
University of California, Davis
One Shields Avenue
Davis, CA 95616

The **Proceedings** of the 50th Western Poultry Disease Conference are not 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 discussion and information presented.

Copies of these Proceedings are available from: Conference & Event Services
University of California, Davis
One Shields Avenue
Davis, CA 95616
(530) 757-3331

Price (includes shipping & handling): US$18.00 for USA.
US$15.00 for AAAP members and orders of 5 or more for USA.
US$19.00 for Canada and Mexico.
US$21.00 for all other countries.
Add an additional US$6.00 for airmail.

Make checks payable to: UC Regents

50th WPDC CD-ROM. Copies of the CD-ROM can be purchased from the AAAP. Phone: 610-444-4282, 610-444-5800-ext.2714. Fax: 610-925-8106. aaap@vet.upenn.edu. Web: http://cahpwww.nbc.upenn.edu/~aaap/index.html

WESTERN POULTRY DISEASE CONFERENCE (WPDC) HISTORY

	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	D. C. Young	W. J. Mathey		
4th Poultry Health Sym. (PHS)		**1st combined WPDC & PHS, 1st listing of distinguished members**		
20th WPDC – 1971	W. J. Mathey	Ramsay Burdett		
5th PHS				
21st WPDC – 1972	R. Burdett	Marion Hammarlund		
6th PHS				
22nd WPDC – 1973	M. Hammerlund	G. W. Peterson		
7th PHS				
23rd WPDC – 1974	G. W. Peterson	Craig Riddell		
8th PHS				
24th WPDC – 1975	C. Riddell	Ralph Cooper		
9th PHS				
25th WPDC – 1976	R. Cooper	Gabriel Galvan		
10th PHS				
26th WPDC – 1977	G. Galvan	Don H. Helfer	Hector Bravo	
11th PHS				
27th WPDC – 1978	D. H. Helfer	Art Bickford		
12th PHS				
28th WPDC – 1979	A. Bickford	J. W. Dunsing		
13th PHS				
29th WPDC – 1980	J. W. Dunsing (WPDC)	G. Yan Ghazikhanian	P. P. Levine	
14th PHS				
5th ANECA	Angel Mosqueda T. (ANECA)			
30th WPDC – 1981	G. Y. Ghazikhanian	Mahesh Kumar		
15th PHS				
31st WPDC – 1982	M. Kumar	Robert Schock		
16th PHS				
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
35th WPDC – 1986	D. W. Waldrip (WPDC)	Duncan A. McMartin(WPDC)	J. A. Allen	

	PRESIDENT	PROGRAM CHAIR	DEDICATION	RECOGNITION
11th ANECA	Jorge Basurto (ANECA)	Mario Padron (ANECA)	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	J. M. Smith (WPDC)	Richard P. Chin (WPDC)	A. S. Rosenwald	
16th ANECA	Martha Silva M.(ANECA)	David Sarfati M.(ANECA)	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	D. D. Frame (WPDC)	Mark Bland (WPDC)	Don Zander (WPDC)	Pedro Villegas
21st ANECA	R. Salado C. (ANECA)	G. Tellez I. (ANECA)	M. A. Marquez (ANECA)	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	K. Takeshita	Barbara Daft		

Minutes of the 49th WPDC Annual Business Meeting

President McMillan called the meeting to order on Monday, 6 March 2000, at 5:05 PM, at the Capitol Plaza Holiday Inn, Sacramento, California. There were approximately 40 people in attendance.

APPROVAL OF 48TH WPDC BUSINESS MEETING MINUTES

The minutes from the 48th WPDC business meeting were reviewed and a motion was carried to approve the minutes of the 48th business meeting as printed in the Proceedings of the 49th WPDC.

ANNOUNCEMENTS

President McMillan acknowledged all the contributors; in particular, those contributing at the Benefactor level. They included the American Association of Avian Pathologists, Elanco Animal Health, Intervet and Merial-Select, Inc. He also thanked all the contributors for their generous donations. President McMillan acknowledged the efforts of the current WPDC officers.

REPORT OF THE SECRETARY-TREASURER

Dr. R. Chin presented the Secretary-Treasurer report. There were 322 registrants for the 1999 WPDC. At the end of the 1998-99 fiscal year, contributions for the 1999 WPDC were $27,925 and income from the meeting was $43,170.66. There were expenses of $47,463.71 for the meeting with a net gain of $23,631.95. The current balance in the WPDC account is $47,815.99. It was asked if the money was being invested. The money is currently in an agency account at UC Davis and cannot be invested. Dr. Chin estimates a net gain of $3000 for the 1999 meeting.

REPORT OF THE PROCEEDINGS EDITOR

Dr. C. Riddell presented the Proceedings Editor report. In general, the production of the Proceedings went well with all papers being submitted by E-mail. There were 69 papers in the Proceedings, 53 oral presentations and 16 poster presentations. There are still problems with authors not following the directions. There is a possibility that the instructions can be shortened, so hopefully authors will read it. Dr. Riddell stated that he is not sure if he will be available to serve as Proceedings Editor next year due to his retirement. He will let the Conference know this spring.

REPORT OF THE LOCAL ARRANGEMENTS COORDINATOR

Dr. L. Woods reported that on the initial contract from Holiday Inn two years ago there were new charges of approximately $10,000 for meeting room rental. After negotiating with the Holiday Inn, we were able to receive complimentary room rental with $1600/day of food at breaks and an additional $5/guest room credit. Dr. Woods reported that we were having problems with the UCD Audio-Visual group. Finally, Dr. Woods noted that due to other obligations she has resigned as the Local Arrangements Coordinator and reported that Dr. Carol Cardona has agreed to accept the position. President McMillan thanked Dr. Woods for all her work and effort as Local Arrangements Coordinator.

OLD BUSINESS

Dr. R. Chin reported that since the WPDC was financially stable, he contacted members of the Executive Committee and received approval to reimburse Dr. Rosenwald for expenses accrued at the WPDC/ANECA meeting in Cancun. Dr. Rosenwald asked that he be reimbursed $4500 this year with the remaining $4272 reimbursed in 2001. A motion was carried to approve reimbursement to Dr. Rosenwald.

NEW BUSINESS

President McMillan reported that the WPDC Executive Committee would like to nominate Dr. Joan Jeffrey for Program Chair-elect of the 51st WPDC in 2002. There were no other nominations from the floor and nominations were closed. A vote was conducted and Dr. Jeffrey was unanimously elected as the Program Chair-elect for the 51ts

WPDC. President McMillan reported that the WPDC Executive Committee would like to nominate Dr. Carol Cardona as Local Arrangements Chair. There were no other nominations from the floor, nominations were closed, a vote was conducted and Dr. Cardona was unanimously elected as Local Arrangements Chair. Finally, President McMillan reported that the WPDC Executive Committee would like to nominate Dr. Ken Takeshita as Contributions Chair-elect. There were no other nominations from the floor, nominations were closed, a vote was conducted and Dr. Takeshita was unanimously elected as Contributions Chair-elect. President McMillan nominated the following officers for 2000-2001:

Program Chair: Dr. Duane Olsen
President: Dr. Pat Wakenell
Local Arrangement Coordinator: Dr. Carol Cardona
Contributions Chair: Dr. A.S. Rosenwald
Contributions Chair-elect: Dr. Ken Takeshita
Proceedings Editor: Dr. Craig Riddell
Secretary-Treasurer: Dr. Rich Chin
Program Chair-elect: Dr. Joan Jeffrey

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

The 50th WPDC, in the year 2001, will be at the University of California, Davis, California, 24-27 March 2000. Dr. Chin suggested that we produce a CD-ROM that contains the Proceedings from all previous meetings that will be presented to all registrants. One estimate came to about $45,000 for production of 800 CD's. A lengthy discussion followed regarding the cost. A motion was carried to produce the CD if financial support can be raised for half of the cost and that Dr. Chin investigate other vendors.

The 51st WPDC, in 2002, was discussed. Dr. Ernesto Soto, on behalf of ANECA, invited the WPDC to join ANECA in Mexico in 2002. There was a lengthy discussion regarding this invitation because of the serious financial problems that occurred with the last joint meeting in 1996. A motion was carried to have a joint meeting with ANECA if communication can be established that will assure financial responsibility from both organizations.

It was asked if the Executive Committee meeting on Sunday morning was an opened or closed meeting. It was agreed that all meetings are open to everyone.

President McMillan passed the presidency to Dr. Pat Wakenell. President Wakenell thanked outgoing President McMillan for his service to WPDC and adjourned the meeting at 6:20 PM.

TABLE OF CONTENTS

Note: Both the oral and poster presentations of the 50th WPDC are listed below in alphabetical arrangement by presenter. Authors and titles of all papers scheduled for presentation at the conference are listed.

PROCEEDINGS OF THE FIFTIETH WESTERN POULTRY DISEASE CONFERENCE

THE ONGOING THREAT OF AVIAN INFLUENZA AND NEWCASTLE DISEASE TO THE US POULTRY INDUSTRY

David L. Suarez, David E. Swayne, and Daniel J. King

Southeast Poultry Research Laboratory, 934 College Station Rd, Athens, GA 30605

Introduction to Avian Influenza. Type A influenza viruses are segmented, negative-sense RNA, enveloped viruses that can infect a wide variety of birds and mammals. However, the normal host range of the virus is considered to be in wild waterfowl, gulls, and shorebirds (11,18). Wild birds were first implicated in the ecology of influenza in 1972 with the first isolation from free living wild ducks (18). Since that first report, numerous surveillance studies of wild birds have shown that all 15 hemagglutinin and 9 neuraminidase subtypes of influenza can be found in wild birds (17, 19, 20, 24). However, the different HA and NA subtypes are not distributed equally within the sampled populations, and some important subtypes, including H5 and H7 subtypes, are rarely seen in wild birds. However, influenza virus from migratory waterfowl are still considered to be the primary source of infection for poultry (9). A review of the United States Animal Health Reports shows that H5 and H7 are regularly isolated from poultry in the United States (Table 1) (USAHA reports, various years).

Poultry, primarily chickens and turkeys, and mammalian species represent an abnormal host for influenza infection. This idea is supported by several different lines of evidence in poultry. First, little evidence of influenza infection in wild turkeys has been detected in three separate serologic studies (5, 6, 10). This would suggest that in the wild, turkeys do not play a role in propagating influenza viruses and are not a natural host species. Similar studies have not been performed with the ancestor to the domestic chicken, red jungle fowl, but it is likely that as another Gallinaceous bird, chickens would not play an important role in the ecology of influenza. For mammalian species, influenza viruses can infect on a regular basis a number of different species, but only rarely does it become endemic. Furthermore, different endemic influenza viruses often become extinct in the new host species. For example, the H2N2 subtype is no longer observed in humans, and in horses the H7N7 subtype is no longer isolated. In both cases the endemic viruses appeared to be supplanted by another, presumably more fit, influenza subtype. Some of the most convincing evidence that influenza does not normally infect these species of animals is the rapid rate of evolution of influenza observed in mammals and chickens and turkeys (8,15,21,22,23), which contrasts with the slow rate of evolution observed in influenza viruses from wild birds (21, 27).

Low pathogenic avian influenza outbreaks occur every year in the United States. During the 1980's and early 90's, infection of turkey flocks with many different subtypes of influenza were common, particularly in Minnesota where turkeys were often raised outdoors. The number of new infections of turkeys, however, have been decreasing, primarily because turkeys are now more commonly reared in confinement. The largest continuing threat to the U.S. poultry is the continued presence of low pathogenic H7N2 influenza viruses in the live bird markets in the Northeast U.S. and H5N2 influenza viruses in Mexico. The continued circulation of H5 and H7 influenza viruses in poultry provides the ideal conditions for the mutation of these low pathogenic avian influenza (LPAI) isolates to highly pathogenic avian influenza (HPAI) viruses which would result in high disease losses and loss of export markets.

H7 Influenza in the Live Bird Markets in the Northeastern United States. Since 1994, H7 avian influenza viruses (AIVs) have been isolated from different poultry species in the live bird markets (LBMs) of the Northeast United States. The presence of AIVs among birds in these markets is of considerable concern because of the potential for H7 LPAI viruses mutating into HPAI viruses. In 1997 and 1998, low pathogenic H7 influenza viruses spread from the live bird markets to several large commercial poultry operations in Pennsylvania causing serious economic losses(7), and the virus in the LBMs remains a threat to spread back into the large commercial poultry operations. As part of an ongoing surveillance of influenza viruses in the LBMs, selected H7 viruses have been sequenced for one or more influenza viral genes including the hemagglutinin, neuraminidase, nonstructural and matrix genes.

The hemagglutinin gene was compared from representative AIV isolates and most of the viruses were determined to be in the same lineage of virus, suggesting a single introduction of virus was responsible for a majority of infections (Figure 1). Two exceptions were observed. In 1998 a single H7N2 virus isolate was sequenced that was distinctly different from the principal lineage and this virus had an additional basic amino acid at the HA cleavage site at the –5 position. In 1999 several H7N3 viruses were

isolated, and sequence analysis showed a HA cleavage site similar to what is observed in wild birds.

Additional sequencing of the neuraminidase, nonstructural and matrix genes was also completed for selected H7 viruses. The majority of the H7 isolates from the LBMs were H7N2 viruses, but H7N3 viruses were isolated in 1994 and 1999. The hemagglutinin gene from the 1994 H7N3 viruses appeared to be similar to other viruses from the main lineage of virus and it was assumed that the N3 gene was the result of a reassortment event. All the N2 viral genes examined appeared to form a single lineage, and were likely the result of a single introduction of this viral gene. Four distinct nonstructural genes and three distinct matrix genes were observed from viruses that had H7 genes from the same lineage, demonstrating that reassortment with these two genes was common (22).

The H7 gene sequence was directly compared and analyzed by regression analysis to determine how rapidly the viruses were adapting and evolving among birds in the LBM system. The earliest isolate available was used as the index case to compare subsequent isolates for sequence changes. Evidence of rapid evolution was observed in the hemagglutinin gene with isolates accumulating nucleotide and amino acid substitutions. The rate of evolution was similar to what has been observed in influenza viruses in mammalian species. Two different changes were observed near the hemagglutinin cleavage site including a threonine to proline change at the –2 position and an asparagine to lysine change at the –5 position. Isolates with these changes became the predominant isolates in the LBMs. The additional basic amino acid at the –5 position causes greater concern about the possibility of the viruses becoming highly pathogenic, since multiple basic amino acids are required for a virus to be highly pathogenic. An additional unique change that has not been observed with other influenza viruses was a loss of eight amino acids in the HA1 portion of the protein that is believed to be near the receptor binding domain. Again, viruses with the deletion, first observed in 1995, became the predominant isolate observed in the LBMs.

The H7 influenza viruses are being maintained in the LBMs primarily as a single lineage, and not by new introductions of virus. It is unclear exactly what part of the LBM system that the viruses are persisting. It appears unlikely that the viruses are being perpetuated on individual poultry farms, since surveillance studies of poultry farms have found few birds positive for H7 infection. The viruses are likely being maintained in the distribution system or in the LBMs themselves, but no data is available to determine for sure where the viruses are being maintained. The sequence data also demonstrates that both viral reassortment and adaptation of the viruses to poultry are occurring. This rapid change of the virus makes it difficult to predict if and when these viruses could become highly pathogenic.

Efforts are increasing to eradicate the virus out of the live bird markets, and in March 1999 the Live Bird Market Working Group (LBMWG) was formed to help address the problem. The group was formed of state and federal regulatory officials, industry representatives, and other interested parties to coordinate efforts to eradicate influenza from the markets. The LBMWG has produced a Best Control Practices guideline to help standardize procedures related to the markets. It has also recommended that the best way to eradicate the virus in the LBMs is to have a three day “chicken holiday” where all live bird markets are depopulated, cleaned and disinfected at the same time. The chicken holiday will be funded as an emergency program by the Animal and Plant Health Inspection Service (APHIS). The proposed “chicken holiday” is planned in late spring or summer of 2001.

H5N2 viruses in the U.S., Mexico and Guatemala. The last widespread outbreak of H5N2 influenza virus in the U.S. was in 1993, primarily in the live bird market system. Additional isolates were observed in 1995, 1998 and 2000. However, three different isolates from Minnesota, New Jersey and New York were similar based on sequence analysis, and they are likely from a common source. No new isolations of H5N2 viruses were made in 1999, so this lineage was believed to have become extinct, but recent isolates from ducks in New York suggests it has been maintained at a low level. The cleavage site sequence for all these viruses are the same as the consensus for low pathogenic wild bird viruses, and there is no evidence that these viruses have a high potential for becoming highly pathogenic based on the 14 day embryonated chicken egg model.

The Mexican H5N2 outbreak that started in 1993 continues to circulate as LPAI in commercial and backyard poultry. The HPAI virus that occurred in 1994-95 was eradicated, and continued control efforts of surveillance, vaccination, and depopulation continue to try and completely eliminate influenza from poultry in Mexico. Sequence analysis of the hemagglutinin gene from isolates from 1993-1998 shows that all the isolates examined are in the same lineage of virus, although variants within the lineage are emerging.

In early 2000, as part of a surveillance program in Guatemala for different avian pathogens, serologic evidence of infection with avian influenza was found in both backyard and commercial poultry. A low pathogenic H5N2 avian influenza virus was isolated from commercial chickens in May of 2000. The hemagglutinin gene from five isolates were sequenced and compared to the HA database, and the viruses were highly similar to Mexican H5N2 viruses. The closest homology was from H5N2 viruses from the state of

Chiapas, which shares a border with Guatemala. This provides strong evidence that the Guatemala outbreak was an extension of the Mexican outbreak, although it is not known when this introduction first occurred.

Miscellaneous U.S. Outbreaks. In December 1999 a turkey breeder flock in Missouri developed a rapid drop in egg production. An H1N2 influenza virus was isolated and sequence analysis showed that the virus was a complex reassortant virus that had swine, human, and avian genes. A similar H1N2 virus had also recently been reported from swine in Indiana. Serologic evidence of other turkey flocks being infected with an H1N2 virus were also reported from Missouri, Illinois, and Ohio. It has been reported since the early 80's that classic swine H1N1 viruses could infect turkey flocks and cause egg production drops and respiratory infections, but this is the first case of a reassortant virus with swine genes infecting turkeys. Complex reassortant H3N2 influenza viruses have also recently been isolated from swine which could potentially cause infect turkeys, but the use of H1N1 vaccines will be ineffective in protecting these flocks.

In February 2000 a chicken layer flock in Southern California developed respiratory symptoms and a drop in egg production. A H6N2 was isolated and serologic evidence of H6N2 infections of other flocks was observed. Because of the size of the layer flock, vaccination of the flock was approved to try and control the outbreak. As of December, 2000 at least 300,000 doses of vaccine have been administered and no evidence of new infections have occurred in the last several months.

Hong Kong H5N1 and H9N2. In 1997 a H5N1 avian influenza virus infected at least 18 people in Hong Kong and resulted in six deaths. This virus was highly pathogenic for poultry and also caused severe disease in chicken flocks in Hong Kong. The virus appeared to spread directly from infected chickens to humans at the live bird markets that are common in Hong Kong. Fortunately, this virus spread poorly with little evidence of person to person transmission. The exact origins of this virus will probably never be known, however, by examining other viruses from Hong Kong and China, viruses with closely related gene segments have been found. A closely related H5 hemagglutinin gene was observed in Mainland China in 1996 in an outbreak of influenza in Geese, although the other seven genes were not closely related to the 1997 virus (28). A H6N1 virus also isolated in 1997 had a neuraminidase gene closely associated with the Hong Kong 97 virus, and it also had high homology with the six internal genes. A similar observation with high homology of the internal gene was also made with H9N2 viruses circulating in Hong Kong in 1997. It is likely that a reassortment occurred with two or more of these viruses that resulted in a HPAI virus with the ability to cross species. The H5N1 virus was eradicated from Hong Kong by total poultry depopulation. Since that time the Hong Kong Department of Agriculture and Fisheries has screened all live birds coming into Hong Kong, with Mainland China being the primary source. The birds are from monitored flocks and representative serologic samples are taken when they enter the province. In March of 1999 serologic evidence of an H5 infection in geese was detected, and although the birds had been marketed before the test results were complete, virus was isolated from the cages the birds had been in. This H5N1 virus was very similar to the Chinese goose isolate of 1996, where it had an H5 gene similar to the Hong Kong 97 virus, but the other seven genes were different. This virus was highly pathogenic in chickens, but it did not kill mice like the Hong Kong 97 viruses did. So although this virus did not directly appear to pose a human health risk, one of the precursor viruses from Hong Kong 97 virus still was circulating in Mainland China (4).

The H9N2 viruses that were first isolated in the mid 90's from China has spread widely in the countries of Asia and the Middle East. These viruses, although low pathogenic in standard pathotyping, have been associated with high mortality in some countries, although the mortality is probably the result of secondary pathogens. H9N2 viruses have also been associated with infections in humans and swine, although it has not been associated with severe disease (14). The H9N2 viruses remain a concern as both a poultry pathogen and as a potential human health threat.

Introduction to Newcastle Disease. On a global scale, virulent Newcastle disease (ND) outbreaks occur more frequently than outbreaks of HPAI. Those outbreaks of ND from infections with virulent ND viruses are officially called exotic ND in the U.S., but may also be referred to as velogenic ND (VND), viscerotropic velogenic ND, neurotropic velogenic ND, or simply ND in different countries. The clinical disease that is a consequence of infections with low virulence vaccine-like ND viruses is not a reportable disease and is not included here. In addition to the potentially devastating bird losses from a ND outbreak, a major negative impact of ND is on developed countries that rely on international exports of poultry and poultry products for healthy agricultural industries. Outbreaks of ND have occurred around the world during 1999 and 2000 including the continents of Africa, North America, South America, Asia, Europe and Australia (2). Below are the highlights of outbreaks in Australia, Brazil, Honduras, and Mexico. Information on the ND outbreak in Italy is provided separately in these proceedings. Other ND issues,

including the new OIE definition of ND, are reported after the summary of recent important outbreaks.

Australia. Velogenic ND outbreaks have occurred in several locations in the state of New South Wales in Australia between 1998 and 2000(13). Based on sequence data, the virulent ND viruses from all outbreaks are related and arose by mutation of an endemic low virulent ND virus and not the introduction of an exotic VND virus. This is a unique event in the history of ND because there may be only one other case of a low virulence virus mutating to a strain of high virulence. Low virulence strains have been indigenous in Australia since 1966. There had been no virulent ND outbreaks in Australia since 1932. However, the ND viruses underwent a series of accumulative mutations over time in the fusion (F) gene that changed the amino acid sequence in the fusion protein cleavage site and the virulence from low -RKQGRL- to high -RRQRRF-(16). Factors that precipitated the change in the virus at this time are unknown. The virulent ND virus was identified as Australian-origin rather than exotic to Australia based on an amino acid extension on the hemagglutinin-neuraminidase protein, a characteristic of Australian ND viruses. There was no evidence of wild bird involvement in the origin of the virulent ND virus. Extensive surveillance is ongoing to attempt to identify virus reservoirs. Some details of the Australian outbreaks follow.

The first outbreak occurred in September 1998 in a cluster of farms east of Sydney in New South Wales. The first case was in a multiage layer flock but later affected several broiler farms. The infection produced mostly neurological signs with mortality in some groups reaching 50%. In the layers, the disease spread slowly, had accompanying nervous signs, resulted in low mortality, and was initially misdiagnosed as Marek's disease. In broilers, the spread was more rapid and accompanied by higher mortality. The farms were depopulated of approximately 100,000 birds and disinfected. No ND virus was found on follow-up surveillance.

The second outbreak began in a pullet rearing farm in the Mangrove Mountain area, near Gosford in New South Wales, north of Sydney on 2 April 1999. ND spread to other farms in the vicinity. By 28 May 1999, 1,900,000 birds were depopulated within the Infected Zone, most were broilers but 2000 were aviary and other poultry on small non-commercial farms. An additional 2,000,000 broilers on farms in the surrounding Surveillance Zone were destocked under a special processing permit by 9 June 1999. Disinfection of all farms in the Infected and Surveillance zones was conducted twice, 14 days apart, before restocking.

The third outbreak was in layers on a farm in Schofield, West Sydney on 21 August 1999. A small number of birds had ataxia and other nervous signs. A mandatory vaccination program was implemented with Australian-origin V4 lentogenic vaccine strain in the Mangrove Mountain area of the second outbreak during December 1999 in response to isolation of low virulent ND viruses in flocks in the Surveillance zone. In December, ND viruses with a F protein cleavage site of a virulent ND virus were isolated in the Surveillance zone of Mangrove Mountain. The surveillance zone was extended to West Sydney, Cumberland Surveillance Zone. In January 2000, Virulent ND was identified in the new surveillance zone in West Sydney at farms in multiage layer farms at Orchard Hills and Llandillo. Virulent ND was also identified in three layer flocks near Tamworth in February. In these latter outbreaks, clinical signs was less frequent than in 1998 and 1999, and virus isolation more difficult. Quarantine zones were instituted.

Brazil. There were 3 outbreaks in poultry in June 2000 with 1400 deaths and 75,100 birds destroyed in chickens supplying the local market in the State of Rio de Janeiro. The poultry were reared on farms unconnected to commercial poultry production system. This is a similar situation the commercial poultry industry in the northeast U.S. faces in relation to LPAI (H7N2) in live-bird markets but not in commercial poultry.

Honduras. Six outbreaks were reported beginning in March 2000 in the departments of Francisco Morazan and Cortes involving 1,500 deaths and slaughter of 411,400 exposed and at risk birds. The NDV was confirmed by NVSL as a viscerotropic velogenic ND (VVND). NDV vaccination program was initiated in backyard flocks close to the outbreak using 43,000 doses of vaccine. Subsequently there were three additional outbreaks in July 2000 involving approximately 100,000 layers and backyard birds. Outbreaks were believed to be secondary with spread from the initially infected farms in the same area by movement of contaminated equipment.

Mexico. An outbreak of exotic ND began in the Comaraca Lagunera region within the states of Durango and Coahuila of Mexico on March 30, 2000. By July 2000, 13,087,787 chickens from 92 broiler flocks were infected with VVND virus. The flocks were depopulated under government supervision and buried on the farms. The farms were cleaned and disinfected. Replacement flocks have been placed and include some non-vaccinated sentinels. No indemnity was paid. The industry absorbed all monetary losses.

The affected flocks were under a lentogenic ND vaccination program similar to the one used in the U.S. broiler industry. Before the outbreak, 1/4 dose of ND virus (B1)-infectious bronchitis was given by spray in the hatchery and a boost at 10-14 days in the field by water or spray administration. The ND vaccination

program after the outbreak includes a live virus vaccination and injected, inactivated ND vaccine in the hatchery. Reportedly flocks not affected in the initial outbreak had been vaccinated in the hatchery with both live and inactivated virus vaccines.

A second issue was infection of backyard or village-type poultry in the vicinity of the commercial poultry with VVND virus. Forty VVND isolates were made from such poultry in 16 towns located in the affected area. Vaccination was instituted for backyard and village-type poultry. It is unknown whether VVND appeared first in village or commercial poultry. The role of fighting cocks and pet birds in the outbreak cannot be eliminated.

Other ND Issues – Definition of ND. The definition of the forms of ND requiring regulatory action differ in the U.S. and the European Union (EU) and an OIE redefinition more like the EU version has been approved. In the U.S. exotic ND is any velogenic Newcastle disease described as an acute, rapidly spreading, and usually fatal viral disease of birds and poultry. In the EU ND means an infection of poultry caused by a strain of avian paramyxovirus-1 (APMV-1), i.e. Newcastle disease virus (NDV), with an intracerebral pathogenicity index (ICPI) in day-old chicks of 0.7 or greater. The OIE definition was similar to the U.S. definition until a new OIE definition of ND was approved in 1999 (1). The conclusion of the OIE Commission was that viruses with an ICPI of 0.7 or greater are pathogenic or have the potential to become pathogenic. The new OIE definition is now similar to the current EU definition based on the ICPI to define virulence with the addition of an alternative molecular criterion that viruses with multiple basic amino acids at the cleavage site of the fusion protein are also a cause of ND. Vaccine-like lentogenic NDV are excluded as a cause of ND in all definitions. The EU definition and the proposed OIE definition both go beyond the U.S. definition by establishing regulatory action for virus strains presently classified as mesogens and would thereby include pigeon NDV isolates. Although mesogenic strains haven't been recovered from U.S. chickens and turkeys since the 1970s, the periodic isolation of ND viruses from pigeon lofts in poultry production areas does occur. The new OIE definition of ND poses some potential concerns for U.S. poultry industries because following the OIE guidelines would increase the number of reports of ND. However, it is understood that implementation of the new OIE definition requires the revision of the ND chapter of the *International Animal Health Code* and that revision is in process as stated in the following paragraph.

The ND chapter in the *International Animal Health Code* is currently being reviewed. The committee may submit revisions for adoption at the 69th general session of OIE in May 2001. Since there were several versions of the definition of ND before the current version was adopted, it is not known if revising the Code will also require multiple revisions. A portion of the currently proposed wording that addresses the situation of pigeon NDV isolates is summarised here. That wording places birds into one of three compartments: 1) domestic birds - commercial poultry: all birds raised or held in captivity for the purposes of meat or table egg production or for restocking supplies of game, or other commercial products including the breeding stock for the aforementioned birds; 2) domestic birds - other than commercial poultry: birds kept in captivity, including racing pigeons and captive feral and wild birds; and 3) free-living birds**:** feral and wild birds that live without supervision, control by or dependence on humans. The diagnosis of ND in commercial poultry would affect international trade whereas a diagnosis of ND in domestic birds other than commercial poultry or in free-living birds would not. Although the proposed definition essentially resolves the issue of ND isolates from racing pigeons, it does not resolve the issue of back yard birds.

Other ND Issues – ND Vaccination. The recent (March 2000) ND outbreak in Mexico has raised a concern about the level of protection to ND in U.S. broiler flocks. This concern followed the observation that the majority of flocks affected by the ND outbreak in Mexico had been vaccinated with live Newcastle-infectious bronchitis vaccines applied as a fraction of the vaccine label field dose typical of programs used in the U.S. broiler industry. In contrast, flocks vaccinated with full dose live virus as well as inactivated vaccines were not similarly affected (USDA, APHIS).

It is known that an efficacious dose of commercially available ND vaccines in a responsive host will induce protection against the morbidity and mortality of a velogenic NDV infection. Protection of vaccinated birds against infection is usually less effective and is usually seen as a reduction in the amount of virus shed by an infected bird, a reduction that will diminish but not eliminate virus transmission to other susceptible birds (3). It was learned during the ND outbreak in California in 1971-1973 that vaccination reduced losses but it didn't prevent flocks from becoming infected and shedding virus (25). Results from a serological survey of broilers (12) and from a challenge study of field vaccinated broilers (26) are evidence that a level of immunity necessary for protection against ND mortality was not being attained in most of those flocks when the samples were collected. It is anticipated, although not specifically known, that the current level of protection of field vaccinated birds has not changed since those earlier studies. However, there are arguments against increasing the ND vaccination program. For flocks not

exposed to endemic velogenic NDV, the intensity of the vaccination program necessary to attain and maintain protection against VND may reduce flock productivity because of increased post-vaccinal reactions, airsacculitis, and/or reduced feed conversion as well as increasing the cost of vaccination. Further during the ND outbreak in California vaccination was shown to mask clinical disease and decrease lesions therefore making ND diagnosis more difficult (25).

Acknowledgements. The authors thank Dennis A. Senne, Max Brugh, Alex Bermudez, Peter Kirkland, T.J. Meyers, Peter Woolcock, and Juan Garcia Garcia for providing information through personal communications. Information on the VVND outbreak in Mexico was obtained from OIE website and Mexico Visit-Executive Summary by USDA, APHIS, VS (Dennis Senne, John Hahn and Martin Smeltzer).

REFERENCES

1. OIE Official Acts. Resolution No. XIII-Newcastle disease. OIE Bulletin. 111:266-267. 1999.

2. Disease outbreaks reported to OIE in March-April 2000. Newcastle disease. OIE Bulletin. 112:137-143. 2000.

3. Alexander, D.J. Newcastle disease and other avian Paramyxoviridae infections. Calnek, B. W. Diseases of Poultry. 10th, 541-569. 1997. Ames, Iowa State University Press.

4. Cauthen, D. E. Swayne, S. Schultz-Cherry, M. L. Perdue, and D. L. Suarez. Continued circulation in China of highly pathogenic avian influenza viruses encoding the hemagglutinin gene associated with the 1997 H5N1 outbreak in poultry and humans. Journal of Virology. 74:6592-6599. 2000.

5. Charlton. Antibodies to selected disease agents in translocated wild turkeys in California. Journal of Wildlife Diseases. 36:161-164. 2000.

6. Davidson, H. W. Yoder, M. Brugh, and V. F. Nettles. Serological monitoring of eastern wild turkeys for antibodies to Mycoplasma spp. and avian influenza viruses. Journal of Wildlife Diseases. 24:348-351. 1988.

7. Davison, D. Galligan, T. E. Eckert, A. F. Ziegler, and R. J. Eckroade. Economic analysis of an outbreak of avian influenza, 1997-1998. JAVMA. 214:1164-1167. 1999.

8. Fitch, R. M. Bush, C. A. Bender, and N. J. Cox. Long term trends in the evolution of H (3) HA1 human influenza type A. Proc.Natl.Acad.Sci.USA. 94:7712-7718. 1997.

9. Halvorson, C. J. Kelleher, and D. A. Senne. Epizootiology of avian influenza: Effect of season on incidence in sentinel ducks and domestic turkeys in Minnesota. Applied.Environ.Microbiol. 49:914-919. 1985.

10. Hopkins, J. K. Skeeles, G. E. Houghten, D. Slagle, and K. Gardner. A survey of infectious diseases in wild turkeys (Meleagridis gallopavo silvestris) from Arkansas. Journal of Wildlife Diseases. 26:468-472. 1990.

11. Kawaoka, T. M. Chambers, W. L. Sladen, and R. G. Webster. Is the gene pool of influenza viruses in shorebirds and gulls different from that in wild ducks? Virology. 163:247-250. 1988.

12. King. Serological profiles of commercial broiler-breeders and their progeny. 2. Newcastle disease virus. Avian Diseases. 30:724-727. 1986.

13. Kirkland. Virulent Newcastle Disease Virus: in through the 'back door'. Australian Veterinary Journal. 78:331-333. 2000.

14. Lin, M. Shaw, V. Gregory, K. Cameron, W. Lim, A. Klimov, K. Subbarao, Y. Guan, S. Krauss, K. Shortridge, R. Webster, N. Cox, and A. Hay. Avian-to-human transmission of H9N2 subtype influenza A viruses: Relationship between H9N2 and H5N1 human isolates. Proc.Natl.Acad.Sci.U.S.A. 97:9654-9658. 2000.

15. Schultz, W. M. Fitch, S. Ludwig, J. Mandler, and C. Scholtissek. Evolution of pig influenza viruses. Virology. 183:61-73. 1991.

16. Scott, H. Westbury, R. L. Reece, and G. G. Arzey. Review of Newcastle disease virus in Australia. Proceedings of the Western Poultry Disease Conference. 48:94-95. 1999.

17. Sharp, Y. Kawaoka, D. A. Jones, W. J. Bean, S. P. Pryor, V. Hinshaw, and R. G. Webster. Coinfection of wild ducks by influenza A viruses: Distribution patterns and biological significance. Journal of Virology. 71:6128-6135. 1997.

18. Slemons, D. C. Johnson, J. S. Osborn, and F. Hayes. Type-A influenza viruses isolated from wild free-flying ducks in California. Avian Diseases. 18:119-124. 1974.

19. Stallknecht, D.E. Ecology and epidemiology of avian influenza viruses in wild bird populations: waterfowl, shorebirds, pelicans, cormorants, etc. Swayne, D. E. and Slemons, R. D. Proceedings of the Fourth International Symposium on Avian Influenza. 61-69. 1998. United States Animal Health Association.

20. Stallknecht, S. M. Shane, P. J. Zwank, D. A. Senne, and M. T. Kearney. Avian influenza viruses from migratory and resident ducks of coastal Louisiana. Avian Diseases. 34:398-405. 1990.

21. Suarez. Evolution of avian influenza viruses. Veterinary Microbiology. 74:15-27. 2000.

22. Suarez, 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. Journal of Virology. 73:3567-3573. 1999.

23. Suarez and D. A. Senne. Sequence analysis of related low-pathogenic and highly pathogenic H5N2 avian influenza isolates from United States live bird markets and poultry farms from 1983-1989. Avian Diseases. 44:356-364. 2000.

24. Turek, B. Tumova, V. Mucha, and A. Stumpa. Type A influenza virus strains isolated from free living ducks in Czechoslovakia during 1978-1981. Acta Virol.Praha. 27:523-527. 1983.

25. Utterback, W.W. Epidemiology of VVND in Southern California. Proceedings of the 76th Annual Meeting of the U.S. Animal Health Association. 280-287. 1972.

26. Villegas, P., G.E.Avellaneda, and R.K.Page. Immune status of Georgia broilers against Newcastle disease. Program of the Annual Meeting of the Am.Vet.Med.Assoc. 1994.

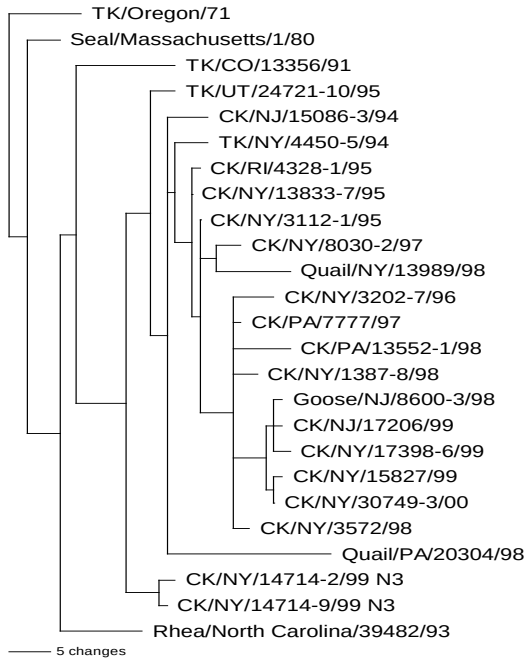
27. Webster, W. J. Bean, O. T. Gorman, T. M. Chambers, and Y. Kawaoka. Evolution and ecology of influenza A viruses. Microbiol.Rev. 56:152-179. 1992.

28. Xu, K. Subbarao, N. J. Cox, and Y. Guo. Genetic characerization of the pathogenic influenza A/Goose/Guangdong/1/96 (H5N1) virus: Similarity of its hemagglutinin gene to those of H5N1 viruses from the 1997 outbreaks in Hong Kong. Virology. 261:15-19. 1999.

Table 1. Isolation of H5 and H7 influenza in poultry in the United States since 1990

1991	Colorado	H7N3NSA	Turkeys
1992	Minnesota	H5N2	Turkeys
1993	Texas	H5N2NSA	Emus, Rheas
1993	Pennsylvania, New York, New Jersey, Florida, Texas	H5N2NSB	Chickens, Pheasants, Rheas, Guinea Fowl, Ducks, Emus
1993	Maryland	H5N2NSA	Pheasants
1993	North Carolina	H7N1NSA	Rheas
1994	Arkansas	H7N3NSA	Quail
1994-2000	Live Bird Markets NJ, NY, PA, RI	H7 N2-3 NSA-B	Chickens, Turkeys, Guinea Fowl, Quail, Pheasants
1995	Minnesota	H5N2NSA	Turkeys
1995	Texas	H7N2	Emus
1995	Utah	H7N3NSA	Turkeys
1998	New Jersey, New York, Minnesota	H5N2NSB	Pheasants, Env., Chukkars
1999	Delaware, New York	H7N2	Chickens, Quail
2000	New Jersey, New York	H5N2	Chickens, Turkeys

Figure 2. Phylogenetic tree of hemagglutinin gene from U.S. H7 avian influenza viruses



AN OVERVIEW ON THE AVIAN INFLUENZA AND NEWCASTLE DISEASE EPIDEMICS IN ITALY DURING 1999 AND 2000

Ilaria Capua[A], Franco Mutinelli, Giovanni Cattoli and Nicola Pozzato[B]

[A]National Reference Laboratory for Newcastle Disease and Avian Influenza,
[B]Centro Regionale per l'Epidemiologia Veterinaria, Istituto Zooprofilattico Sperimentale delle Venezie,
Via Romea 14/A, 35020 Legnaro, (PD), Italy

INTRODUCTION

Avian Influenza (AI) and Newcastle disease (ND) are two viral diseases of poultry included in OIE List A. In the European Union their control is imposed by EU Directives 92/40/EEC and 92/66/EEC, respectively (5, 6). These two diseases may have devastating effects on the poultry industry particularly following the high mortality rates they determine in susceptible birds, but also their presence in a given territory results in restrictions on animal movements, marketing and trade of poultry and poultry products.

North-eastern Italy has been affected by a devastating epidemic of highly pathogenic avian influenza (HPAI), caused by a type A influenza virus of the H7N1 subtype that originated from the mutation of a low pathogenicity avian influenza (LPAI) virus of the same subtype (2). The LPAI epidemic and the subsequent HPAI epidemic occurred in the Veneto and Lombardia regions, which raise 65% of Italy's industrially reared poultry. Furthermore, some areas affected by the epidemics (particularly south of Verona province), are densely populated livestock areas (DPLA), which count (in some municipalities of Verona province) 70,000 birds raised per square kilometer.

The HPAI epidemic caused directly or indirectly the death or culling of over 14 million birds that inevitably determined the disruption of the marketing system and great economic losses to the poultry industry and to the social community. The dramatic economic and social problems determined by the epidemic indirectly led to the introduction of Newcastle disease that, for obvious reasons, led to additional losses and trade restrictions.

Following depopulation and restocking of the HPAI infected areas, LPAI re-emerged, thus determining the poultry industry to request and obtain vaccination against avian influenza of the H7 subtype. The events, which connect the four epidemics mentioned above, are, in our opinion, linked together, and are reported below.

Low pathogenicity avian influenza epidemic. On the 29th of March 1999 the first isolation of a type A, H7 avian influenza virus was officially notified. The virus was further characterized, in accordance to EU Directive 92/40/EEC (5) as a LPAI virus, by the EU Reference Laboratory for Avian Influenza and Newcastle Disease, Weybridge UK, as a type A avian influenza virus of the H7N1 subtype. The intravenous pathogenicity index (IVPI) of the isolate, in 6 week-old SPF chickens was of 0.0, and the deduced amino acid sequence of the genome segment which encodes for the cleavage site of the precursor of the hemagglutinin molecule was typical of LPAI viruses since it did not contain multiple basic amino acids (8, 9).

Following the first official notification a significant number of outbreaks were diagnosed and notified for a total of 199 infected flocks. The highest number of outbreaks affected meat turkeys (164), and only a limited number of turkey breeder flocks were affected (6). Infection also affected chickens (12 outbreaks in layers, 11 in broiler breeders and four in broilers) and two guinea fowl flocks. From the epidemiological inquiry it appeared that at the moment of the first submission approximately 60-70 turkey farms had already been infected. Infection was particularly severe in the turkey industry, causing severe losses to farmers (4).

Nevertheless, this virus could not be defined as "avian influenza" since it did not have the characteristics listed in EU Directive 92/40/EEC; therefore, no compulsory stamping out policy could be implemented, and it was not possible at the time to stamp out such a consistent number of flocks on a voluntary basis. Moreover, since LPAI is not considered in Italian veterinary legislation, there were no legislative tools to prevent its spread. However, the regional authorities of the two affected regions implemented restriction orders with the aim of reducing the number of new outbreaks. The main strategies of these orders were to avoid movement of viremic birds, and to avoid movement of dead birds and infected litter, which were identified as being among the primary sources of infection. These policies, aided by the oncoming warm season, determined a decrease in the number of outbreaks during the summer that inevitably increased from the month of September.

Highly pathogenic avian influenza epidemic. On the 13th of December 1999 a private practitioner submitted pathological samples from a meat turkey flock exhibiting high mortality rates. The outbreak was confirmed as HPAI on the 17th of December with the characterization of an H7N1 isolate with an IVPI index of 3.0 and a deduced amino acid sequence containing multiple basic amino acids, typical of highly pathogenic viruses (3).

Due to the complex field situation (isolation of an H7 virus was not unusual at the time) it was not possible to suspect immediately the presence of HPAI virus when it first appeared and to promptly implement eradication measures, thus resulting in spread of infection. Furthermore, the holiday season was approaching and high slaughter levels resulted in a further spread of the virus with complete loss of control of infection. Four hundred thirteen outbreaks were diagnosed involving 177 meat turkey flocks, 121 table-egg layer flocks, 39 broiler flocks, 29 broiler breeder flocks, 25 backyard flocks, nine guinea fowl flocks, six turkey breeder flocks, three ostrich farms, two pheasant flocks, one Pekin duck flock and one quail flock; and death of over 14,000,000 birds. The last outbreak was notified on April 5, 2000.

As a result of mass mortality, (stamping out policy and pre-emptive slaughter), several establishments such as hatcheries, feed mills, abattoirs, processing plants and other connected activities were forced to interrupt their activity, causing unemployment and heavy economic losses to the poultry industry and to the social community due to disruption of the marketing system. Further economic losses were also determined by the export bans imposed on the infected regions and by the depopulation of the infected area.

Eradication of HPAI. Following the implementation of directive 92/40/EEC (5) infected flocks were stamped out, and cleaning and disinfection of infected premises was carried out. To improve eradication procedures, a complete depopulation of the infected area was imposed. An area of 5500 square kilometers was depopulated, including intensive, semi-intensive and backyard flocks that remained empty for a minimum period of 60 days. Restocking began on June 15, 2000.

Newcastle disease epidemic. Due to mass mortality caused by the HPAI epidemic and to the consequent unavailability of chicks on the Italian market, day-old chicks and hatching eggs were imported from several European and non-European countries. These imported batches originated from different countries with different sanitary statuses and were mingled in the same hatcheries. Veterinary controls were reduced on imports due to the urgent and rising demand.

Furthermore, in AI free areas, stocking densities were increased, resulting in poor environmental conditions for the birds. In addition, due to the high stocking densities, and to the fact that the immune status to ND of the imported chicks was generally unknown, vaccination programs for ND were reduced or abandoned.

The first industrial flocks that were affected by ND originated from a single broiler hatchery that had imported from several foreign countries. Infection spread to other industrial flocks, to dealers (who sell all sorts of poultry to backyard farm owners), and subsequently to backyard flocks, for a total of 17 outbreaks in industrial farms, 17 in dealers, 219 in backyard flocks and one ostrich flock.

The ND strain involved ids a highly virulent virus, with an intracerebral pathogenicity index of 1.8 and a deduced amino acid sequence at the cleavage site of the F protein of …SGGRRQ*RRF…., which contains multiple basic amino acids, a feature typical of virulent viruses. It was subsequently typed with a panel of 28 monoclonal antibodies as a virus belonging to the C1 group (1).

Following the implementation of Directive 92/66/EEC, all infected flocks were stamped out, and at present we only have occasional outbreaks in backyard poultry.

Re-emergence of LPAI. On the 14th of August 2000 a clinical suspicion of LPAI was forwarded in a turkey flock located in the DPLA, and was confirmed by the laboratory on August 20, 2000. The Italian Ministry of Health ordered the eradication of infection with a stamping out policy imposed by an extraordinary act. Fifty-two outbreaks were diagnosed and stamped out.

A vaccination policy against avian influenza was, at this point, strongly requested by the farmers and by the poultry industry, and a vaccination program was drawn up and approved by the European Commission.

Vaccination policy. The vaccination program began on November 15, 2000 and will last until May 2002. Six million birds [only meat type birds and table-egg layers (that apply the all-in all-out system)] raised in a restricted zone (1155 km^2) south of Verona are involved in the vaccination program. No vaccinated live birds or poultry products that originate from the vaccination zones will be authorized for intra-community trade.

The vaccine that has been used does not contain a homologous H7N1 virus, but has been prepared from an inactivated H7N3 virus (A/CK/Pakistan/95/H7N3). The reason for this is the possibility of using it as a natural "marker" vaccine, or more correctly a DIVA [Differentiating Infected from Vaccinated Animals] vaccine. In fact, the presence in the vaccine of an H7 antigen ensures protection against clinical signs and the

reduction of virus shedding, since it is well known the neutralizing antibodies to influenza A viruses are induced primarily by the hemagglutinin molecule (7). The presence of a different neuraminidase (N) subtype, which will induce specific antibodies (against N3 rather than N1) will enable us, with the aid of an *"ad hoc"* diagnostic kit, to discriminate between infected and vaccinated flocks, and to monitor and follow the evolution of the situation.

DISCUSSION

A few considerations can be made from this experience. Firstly, farmers and private companies should bear well in mind that within the current European legislation there is no financial aid from local or national governments or from the European Union in case of LPAI. Therefore, on one hand permanent surveillance programs should be implemented in order to allow the prompt diagnosis of infection by H5 and H7 LPAI viruses to allow the stamping out of infected flocks until this is economically feasible. In the spring of 1999 we were faced with 60-70 outbreaks, and it was not possible to stamp out infected flocks without compensation.

The spread of infection was also a result of the structure and organization of the local poultry industry. In several areas worldwide, the poultry industry has substantially grown in an often irrational way, particularly where the system has developed as a semi-vertical integration. The latter (i.e. house owned by the farmer and day-old chicks and feed supplied by private company), has the disadvantage that there is no planning behind the spatial distribution of the units that are involved in the system, and furthermore, there are a sensible number of contacts between establishments. In fact, frequently feed trucks and other vehicles (e.g., abattoir delivery), visit a number of farms daily, regardless of the species reared and of the type of production, and basic biosecurity measures are rarely respected. The concentration of poultry houses, hatcheries, abattoirs, litter processing plants, and other establishments in a restricted area is definitely convenient from an organizational point of view, but has a series of drawbacks from the sanitary point of view, that dramatically emerge when an epidemic of a highly contagious disease is faced.

The disruption of the marketing system determined social consequences, forcing farmers out of business and in some instances favoring the use of illegal vaccines. This practice most probably determined the re-emergence of LPAI, through the movement of infected litter collected from farms containing clinically healthy carriers.

Furthermore, the commercial pressure posed on the companies determined imports at risk, which associated to insufficient veterinary controls, managing inaccuracies, and weak vaccination programs led to the emergence of ND.

With reference to the vaccination program, this is being used as a last resort, although there are conflicting opinions on its effectiveness as an eradication tool.

In conclusion, the Italian experience with avian influenza shows that it is extremely difficult to control avian influenza in densely populated areas, especially if infection with LPAI is already widespread in the area, and therefore in order to avoid similar situations prevention systems should be implemented.

Besides a structural change in the industrial system, which must inevitably take place in order to reorganize production circuits, veterinary surveillance, quarantine and controlled marketing particularly in restocking procedures are also essential to prevent sanitary emergencies. In addition to this, education of farmers and staff to the basic concepts of biosecurity is also a critical point to the eradication of avian influenza and Newcastle disease and are fundamental to the management of intensively reared poultry.

ACKNOWLEDGEMENTS

The authors wish to thank the staff of the Virology and Histopathology and Epidemiology Departments of the Istituto Zooprofilattico Sperimentale delle Venezie and of the Istituto Zooprofilattico della Lombardia e dell'Emilia Romagna. D.J.Alexander, R.J.Manvell and J.Banks of the EU Reference Laboratory for Avian Influenza and Newcastle Disease, Weybridge UK are also gratefully acknowledged for their support and technical assistance.

REFERENCES

1. Alexander, D. J., R.J. Manvell, J.P. Lowings, K.M. Frost, M.S. Collins, P. H. Russell and J.E. Smith. Antigenic diversity and similarities detected in avian paramyxovirus type 1 (Newcastle disease virus) isolates using monoclonal antibodies. Avian Pathology 26: 399-418. 1997.

2. Capua, I., and S. Marangon. Avian influenza in Italy (1999-2000) a review. Avian Pathology 29: 289-294. 2000.

3. Capua, I., F. Mutinelli, S. Marangon, and D. J. Alexander. H7N1 Avian Influenza in Italy (1999-2000) in intensively reared chickens and turkeys. Avian Pathology, 29: 537-543. 2000.

4. Capua, I., S. Marangon, M. Dalla Pozza, F. Mutinelli, G. Vincenzi, and U. Santucci. The low pathogenicity avian influenza (H7N1) epidemic in the Veneto region, Italy. Proceedings of the Sixth Joint Annual Meeting of the EU Reference Laboratories for

Newcastle Disease and Avian Influenza Bruxelles 29-30 November 1999. pp 66-72. 1999.

5. CEC. Council Directive 92/40/EEC of 19 May 1992 introducing Community measures for the control of avian influenza. Official Journal of the European Commission, L167: 1-15. 1992.

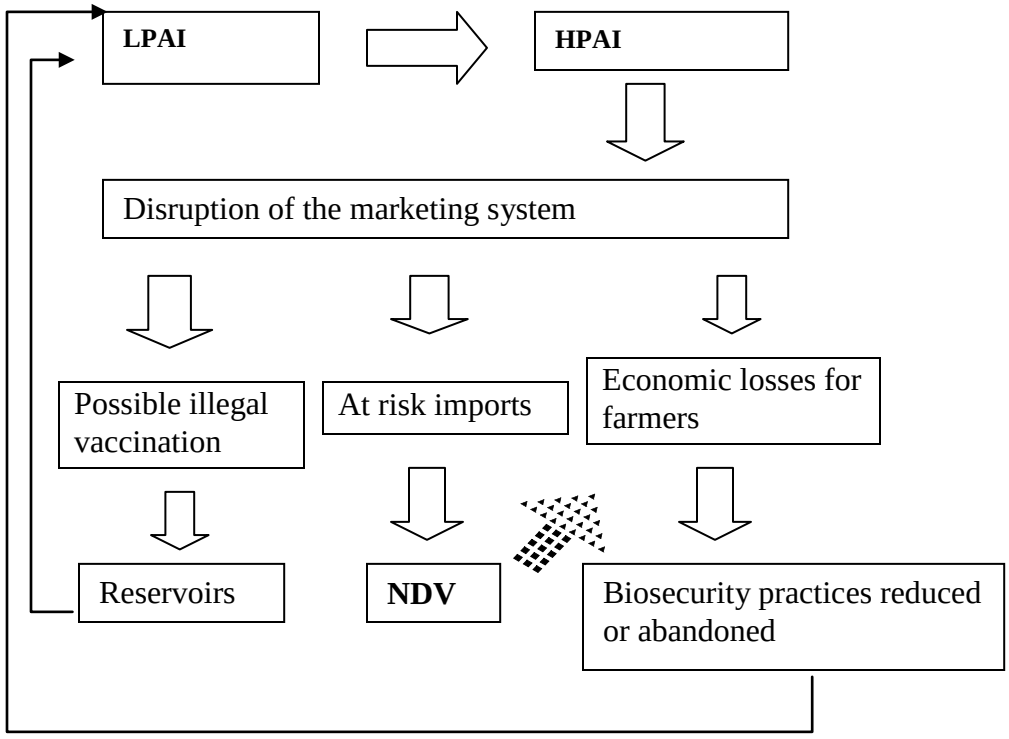
6. CEC. Council directive 92/66/EEC of 14 July 1992 introducing Community measures for the control of Newcastle disease. Official Journal of the European Communities, L260: 1-20. 1992.

7. Swayne, D. E., J. R. Beck, M. Garcia and H. D. Stone. Influence of virus strain and antigen mass on the efficacy of H5 avian influenza inactivated vaccines. Avian Pathology 28:245-255. 1999.

8. Wood, G.W., J. Banks, J.W. McCauley, and D. J. Alexander. Deduced amino acid sequences of the hemagglutinin of H5N1 avian influenza virus isolates from an outbreak in turkeys in Norfolk, England. Archives of Virology 134: 185-194. 1994.

9. Wood, G.W., J. Banks, I.H. Brown. I. Strong and D. J. Alexander. The nucleotide sequence of the HA1 of the hemagglutinin of an H1 avian influenza isolate from turkeys in Germany provides additional evidence suggesting recent transmission from pigs. Avian Pathology, 26: 347-355. 1997.

Fig. 1 Connections between the LPAI and HPAI and NDV epidemics in Italy, 1999-2000



CLINICAL, GROSS AND MICROSCOPIC FINDINGS IN DIFFERENT AVIAN SPECIES NATURALLY INFECTED BY TYPE A, HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS OF THE H7N1 SUBTYPE

Franco Mutinelli[A], Ilaria Capua [A], Calogero Terregino[A] and Giovanni Ortali[B]

[A]National Reference Laboratory for Newcastle Disease and Avian Influenza, Istituto Zooprofilattico Sperimentale delle Venezie, Via Romea 14/A, 35020 Legnaro (PD), Italy,
[B]Gruppo Veronesi, Quinto di Valpantena, Verona, Italy

INTRODUCTION

Avian influenza viruses may be classified on the basis of the clinical condition they determine in susceptible birds. Low pathogenicity avian influenza (LPAI), may be caused by viruses belonging to all 15 hemagglutinin types (H1-H15) and determine a mild disease in susceptible poultry, characterized by respiratory and enteric signs that are often associated in breeders and table-egg layers to reproductive abnormalities. Highly pathogenic avian influenza (HPAI) that is caused by only certain viruses of the H5 and H7 subtypes is instead a devastating disease of poultry with mortality that approaches 100% in gallinaceous birds.

Evidence collected from recent influenza outbreaks indicates that LPAI viruses belonging to the H5 and H7 subtypes may mutate and become HPAI, probably after introduction to poultry (9,12) resulting in extremely complex situations, which may have dramatic effects on the poultry industry.

Italy has been affected by both HPAI and LPAI throughout the years. However, in recent years a minor epidemic of HPAI caused by a virus of the H5N2 subtype in semi-intensive and backyard farms in 1997-1998 occurred in northeastern Italy (2). Also, a limited number of isolations of LPAI have been recorded from 1990 (10,11).

During 1999 northeastern Italy has been affected by an epidemic of LPAI due to a virus of the H7N1 subtype. The epidemic involved 199 outbreaks and caused considerable losses to the poultry industry (7). In the month of December 1999 the H7N1 LPAI virus mutated to a HPAI virus, which rapidly spread, causing 413 outbreaks, and determining direct or indirect death of over 14 million birds of different species (3).

Intensively reared chickens, turkeys, ostriches, guinea fowl, quail, ducks, geese, and pheasants were affected by the epidemic; and in the present paper we report on the clinical, gross and microscopic findings recorded in affected birds during the epidemic.

MATERIALS AND METHODS

Laboratory investigations. Birds of different species exhibiting clinical signs were submitted for laboratory investigations including post-mortem examination, bacteriology, histopathology and attempted virus isolation.

Following the implementation of Directive 92/40/EEC (8), samples were collected from all infected and suspect flocks for virological investigation. Virological investigations were performed in accordance with the guidelines reported in Directive 92/40/EEC, and the isolates were characterized as reported by Alexander and Spackman (1). The virulence of the isolates was determined through the intravenous pathogenicity index test [IVPI] (8) and by nucleotide sequencing in the region of the genome coding for the cleavage site of the hemagglutinin molecule (13,15).

For histopathology, selected organs were sampled and immediately fixed in 10% phosphate-buffered formalin. Tissues were embedded in paraffin, sectioned at 3 μm and stained with hematoxylin & eosin. Unstained paraffin embedded sections were immunohistochemically examined for presence of influenza A nucleoprotein. The primary antibody was a monoclonal antibody against type A influenza virus nucleoprotein (kindly supplied by Dr. D.E. Swayne, USDA, ARS, Athens, GA, USA). Briefly, an antigen retrieval step was performed by pressure-cooking for 25 min. in citrate buffer pH 6; the primary antibody was applied at 1:2000 dilution, using the En Vision AP (DAKO K1396) detection system and Nuclear Fast Red (DAKO K1396) as chromogen. Routine bacteriology was performed on the viscera of the affected birds.

RESULTS

Virological investigations. Virus isolation attempts yielded hemagglutinating agents on first passage, often accompanied by early embryo mortality (within 48 hours). Viruses were characterized

serologically and all influenza isolates were characterized as type A influenza viruses of the H7N1 subtype.

The IVPI test performed on a number of isolates scored 3.0, and deduced amino acid sequence of the cleavage site of the hemagglutinin molecule was …PEIPKGSRVRR*GLF……., which contains multiple basic amino acids, a feature typical of highly pathogenic viruses.

Bacteriology. Routine bacteriological tests constantly yielded a negative result.

Clinical investigations. In chickens, turkeys, and guinea fowl reared on litter, 100% mortality was observed 48-72 hours from the onset of the first clinical signs. Anorexia and depression were followed by nervous signs characterized by tremors and incoordination. Similar clinical signs were recorded in pheasants although mortality rates appeared to be lower. In a limited number of broiler breeder flocks, cyanosis of the comb and wattles and petechial hemorrhages on the hock could be seen.

A different situation was noted in caged birds, such as layers and quail, in which the disease eventually killed all the birds, but moved within the flock in a much slower way. At first, severe depression or mortality could be seen in only one bird per cage in a restricted area of the house, but slowly spread to neighboring cages. This different behavior in spread between caged and litter-reared birds was probably related to the amount of infected feces in direct contact with the birds.

In free ranging ostriches (5), the first clinical signs observed were anorexia and depression in a limited number of the young birds (7-9 months) that in the following days spread to a significant number of young birds. Common clinical findings, apart from depression and anorexia, were a swollen appearance of the throat and neck associated with nervous signs such as incoordination, paralysis of the wings, and tremors of the head and neck. A consistent clinical sign was the production of brilliant green urine, which was also rich in urates and of hemorrhagic feces. Following the onset of clinical signs, a total of 44 (30%) birds died. The remaining birds recovered (to normality) within a week from the onset of the clinical condition. The adults (breeders) appeared healthy throughout the episode.

Quite unexpectedly, mortality also affected Muscovy ducks and geese in a backyard flock (4). The ducks had exhibited an abnormal gait associated with incoordination prior to death.

Post mortem findings. On post mortem, a lesion which was common to all affected birds was pancreatitis. The gross finding was most severe in chickens and turkeys. Besides this finding, in chickens occasionally, the spleen presented necrotic foci on its surface, and the cecal tonsils appeared hemorrhagic. Generally speaking, internal organs appeared congested, and in a limited number of cases, affecting both turkeys and chickens urate deposits in the kidney could be seen.

On the contrary, the gross findings seen in the ostriches resembled infection by *Clostridium spp*. In fact, apart from edema of the head and upper part of the neck, and presence in the oral cavity and oesophagus of bile-green mucous liquid, the most striking lesions were observed in the intestine and in the liver. Most of the intestine was affected by a severe hemorrhagic enteritis, and its lumen contained a hemorrhagic exudate and blood clots. The liver appeared enlarged with rounded margins and its surface exhibited whitish and dark brown irregular areas. As previously mentioned, in a number of birds the pancreas appeared hemorrhagic, enlarged and hardened. The kidneys also appeared enlarged, friable and contained urate deposits. The spleen also was increased in size. The lung and trachea appeared congested and the epicardium exhibited petechial hemorrhages.

With reference to the affected waterfowl, on post mortem examination both geese exhibited pancreatic lesions. In particular, in one of them the pancreas appeared enlarged, hardened and yellowish in color. Its surface exhibited a foamy appearance with small rounded greyish vesicles. The duodenum appeared congested, and on opening it, it contained hemorrhagic material. The spleen appeared reduced in size, and an inflammation of the proventriculus was also present. The heart appeared congested and enlarged. No other lesions were detected in other organs. No gross lesions were detected in the two Muscovy ducks.

Histopathology and immunohistochemistry. The histopathological findings recorded in chickens and turkeys were very similar. Pancreatitis with severe, focal to diffuse necrosis of acinar cells was the main finding. Pancreatic lobes exhibited strong irregular eosinophilic staining caused by acinar necrosis. The most severe necrotic foci were lined by a thin rim of inflammatory cell debris. Interstitial edema was also present, associated with fibrinous peritonitis affecting both pancreas and intestine. Spleen lesions were of vascular wall fibrinoid necrosis. Brain and cerebellum showed focal necrosis in affected turkeys. Lymphocytic choroiditis was also observed. No other relevant lesion was detected in other districts. Avian influenza virus nucleoprotein was identified in necrotic acinar epithelium of the pancreas, nervous and heart tissues.

In the dead ostriches, focal to diffuse coagulative necrosis of spleen, kidney and liver were detected. Inflammation and fibrinoid necrosis of the arterioles were prominent in the spleen and brain. The pancreas was affected in a limited number of birds and exhibited

focal coagulative necrosis of acinar cells, restricted mononuclear infiltration and mild to severe fibrosis surrounding a few small rounded lobules, which appeared compressed and atrophic. Brain and cerebellum sections revealed foci of malacia and neuronophagia. Lymphocytic choroiditis was also present. Necrotic and hemorrhagic lesions were present in the intestine. No other relevant alterations were detected histologically. Type A avian influenza virus nucleoprotein was detected by immunohistochemistry in necrotic lesions.

Focal to diffuse necrosis of the pancreas occasionally associated with lymphohistiocytic infiltration and sclerosis was observed in guinea fowl and quail. No other significant lesion was evident on histology. Necrotic lesions were immunohistochemically positive for avian influenza virus nucleoprotein.

Interestingly, different lesions were observed in the ducks and geese. In contrast to diffuse necrosis of affected organs observed in other birds, the main histological lesion was inflammation of the affected organs in these birds. Only a limited number of necrotic foci of the acinar cells of pancreas were observed in the geese and, to an even lesser extent, in the Muscovy ducks. Mild hemorrhagic duodenitis was observed in both geese and in the Muscovy ducks, while necrosis of the cecal tonsils was observed in the geese only. The latter also presented with congestion, mild hydropic degeneration and focal granuloma in the liver. Mild to moderate lymphocytic encephalitis with perivascular cuffing was observed in the brain of geese and Muscovy ducks.

Mild positive immunohistochemical reaction against the viral nucleoprotein antigen was detected in the acinar cells of pancreas of the geese. Similarly, nuclei and cytoplasm of the neurons and astrocytes in the grey matter of the central nervous system of geese showed an intense, positive immunohistochemical reaction, while in the Muscovy ducks, a positive reaction was restricted to a few individual neurons and glial cells. Lymphocytic perivascular cuffing never showed a positive reaction on immunohistochemistry. The remaining organs for both species were negative by immunohistochemistry.

DISCUSSION

Clinical and pathological data collected during the epidemic confirm that gallinaceous birds are highly susceptible to HPAI, and that all efforts should be made to control this disease, which may have devastating consequences on the poultry industry and on the social community.

Furthermore it appears that ostriches are also susceptible to HPAI, although it should be stated that only the young birds exhibited clinical signs while adults remained clinically healthy. This evidence should stimulate further research aiming to establish whether adult ostriches could behave as carriers of this infection when imported for breeding purposes into countries that are free from HPAI.

Of particular interest are the findings observed in the infected waterfowl. In fact, although waterfowl are considered refractory to HPAI, from the evidence collected, it appears that in some instances they may exhibit clinical signs and experience viremia that results in viral replication in vital organs. It should be stated that isolation of HPAI from the brain of experimentally infected Pekin ducks has been reported in the past (14).

In addition to this, from the gross, histopathological and immunohistochemical data collected in the epidemic, it appears that the pancreas has a crucial role in the pathogenesis of HPAI, possibly due to the presence of proteolytic enzymes that are involved in virus activation. Further studies are necessary to elucidate the role of this organ in the pathogenesis of HPAI.

ACKNOWLEDGEMENTS

The authors wish to thank the staff of the Virology and Histopathology Departments of the Istituto Zooprofilattico Sperimentale delle Venezie and Ruth Manvell, CVL Weybridge for their precious technical assistance. Jill Banks, CVL Weybridge, UK, and Ernesto Tisato Biotechnology Laboratory Istituto Zooprofilattico Sperimentale delle Venezie are gratefully acknowledged for sequencing the isolates. The authors also wish to thank Dr. D.E. Swayne for kindly supplying the monoclonal antibody.

REFERENCES

1. Alexander, D.J., and D. Spackman. Characterization of influenza A viruses isolated from turkeys in England during March-May 1979. Avian Pathology, 10: 281-293. 1981.

2. Capua, I., Marangon, S., L. Selli, D.J. Alexander, D.E. Swayne, M. Dalla Pozza, E. Parenti and F.M. Cancellotti.Outbreaks of highly pathogenic avian influenza (H5N2) in Italy during October (1997) to January (1998). Avian Pathology, 28: 455-460. 1999.

3. Capua I., and S. Marangon. The avian influenza epidemic in Italy (1999-2000) a review. Avian Pathology 29: 289-294. 2000.

4. Capua I. and F. Mutinelli. Mortality of Muscovy ducks (Chairina moschata) and domestic geese (Anseranser var.domestica) following natural

infection with highly pathogenic avian influenza of the H7N1 subtype. Avian Pathology, in press. 2000.

5. Capua, I., F. Mutinelli, M.A. Bozza, C. Terregino, G. Cattoli. Highly pathogenic avian influenza (H7N1) in ostriches (Struthio camelus). Avian Pathology 29: 645-648. 2000.

6. Capua, I., F. Mutinelli, S. Marangon, and D.J. Alexander. H7N1 Avian Influenza in Italy (1999-2000) in intensively reared chickens and turkeys. Avian Pathology, 29: 537-543. 2000.

7. Capua, I., S. Marangon, M. Dalla Pozza, F. Mutinelli, G. Vincenzi, and U. Santucci. The low pathogenicity avian influenza (H7N1) epidemic in the Veneto region, Italy. Proceedings of the Sixth Joint Annual Meeting of the EU Reference Laboratories for Newcastle Disease and Avian Influenza - Bruxelles 29-30 November 1999. pp 66-72. 1999.

8. CEC Council Directive 92/40/EEC of 19 May 1992 introducing Community measures for the control of avian influenza. Official Journal of the European Commission, L167: 1-15. 1992.

9. Garcia, M., J.M. Crawford, J.W. Latimer, E. Rivera-Cruz, and M.L. Perdue. Heterogeneity in the haemagglutinin gene and emergence of the highly pathogenic phenotype among recent H5N2 avian influenza viruses from Mexico. Journal of General Virology 7: 1493-1504. 1996.

10. Papparella, V., A. Fioretti, and L.F. Menna. The epidemiological situation of avian influenza in Italy from 1990 to 1993 in feral bird populations and in birds in quarantine. Proceedings of the Joint First Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Communities, Brussels, 1993. pp 19-21. 1994.

11. Papparella, V., A. Fioretti, and L.F. Menna. The epidemiological situation of avian influenza in Italy 1994. Proceedings of the Joint Second Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Brussels, 1994. pp 14-15. 1995.

12. Perdue, M.L., M. Garcia, D. Senne, M. Fraire. Virulence-associated sequence duplication at the hemagglutinin cleavage site of avian influenza viruses. Virus Research, 49: 173-186. 1997.

13. Wood, G.W., J. Banks, J.W. McCauley, and D.J. Alexander. Deduced amino acid sequences of the haemagglutinin of H5N1 avian influenza virus isolates from an outbreak in turkeys in Norfolk, England. Archives of Virology 13: 185-194. 1994

14. Wood, G.W., G. Parsons, and D.J. Alexander. Replication of influenza A viruses of high and low pathogenicity for chickens at different sites in chickens and ducks following intranasal inoculation. Avian Pathology, 24: 545-541. 1995.

15. Wood, G.W., J. Banks, I.H. Brown, I. Strong, and D.J. Alexander. The nucleotide sequence of the HA1 of the haemagglutinin of an H1 avian influenza isolate from turkeys in Germany provides additional evidence suggesting recent transmission from pigs. Avian Pathology, 26: 347-355. 1997.

VIRULENCE OF U.S. PIGEON NEWCASTLE DISEASE VIRUS ISOLATES FOR CHICKENS

Daniel J. King[A], Glaucia D. Kommers[B], Corrie C. Brown[B], and Bruce S. Seal[A]

[A]USDA, ARS, Southeast Poultry Research Lab, 934 College Station Road, Athens, GA 30605
[B]University of Georgia, College of Veterinary Medicine, Department of Pathology, Athens, GA 30602

Pigeons are the most common source of Newcastle disease virus (NDV) isolates of moderate virulence. Viruses of similar virulence, classically defined as mesogens, have not been isolated from poultry in the U. S. since the 1970s. In 1999 the definition of Newcastle disease (ND) recognized internationally was changed by the Office International des Epizooties. ND was formerly defined as a disease of birds caused by a virus significantly more virulent than vaccine strains like B1 and La Sota, a definition very similar to that utilized in the U. S. In the new definition ND is an infection of birds with a virus of avian paramyxovirus serotype 1 (APMV-1), i.e. NDV, with an intracerebral pathogenicity index (ICPI) of 0.7 or greater or with multiple basic amino acids in the fusion protein cleavage site. Most pigeon APMV-1 isolates are characterized as virulent by the new definition, but their potential to cause disease in chickens is generally unknown. In the present study selected isolates were characterized for virulence before and after serial passage in specific-pathogen-free white leghorn chickens.

Seven NDV isolates, 6 from pigeons and 1 from doves, were included in the study. The virus isolates were from 2 ND outbreaks in pigeons in 1984, 2 from ND outbreaks in racing pigeons in Texas and Georgia in 1998, and 3 from quarantine station or regulatory actions, 2 from pigeon in 1975 and 1 from quarantined

doves in 1998. Serial passage was done in 2-wk-old chickens inoculated intramuscularly. A homogenate of spleens harvested at 2 days post-inoculation was inoculated intramuscularly for the subsequent passage. Virus pathotyping was completed on the initial passage inoculum and chicken embryo propagated virus from the fourth or final passage.

A pigeon NDV specific monoclonal antibody inhibited 4 of the isolates, the 1984 and 1998 pigeon isolates, in a hemagglutination-inhibition test and differentiated those 4 isolates from the 1975 pigeon and 1998 dove viruses that were not inhibited by that antibody. Viruses inhibited by the pigeon specific antibody are typical of those classically identified as pigeon paramyxovirus serotype 1 (PPMV-1). Increased virulence from chicken passage was evident in the results of the pathotyping tests of the 4 PPMV-1 isolates. The mean death time (MDT) of inoculated embryos decreased and the ICPI and intravenous pathogenicity index (IVPI) increased for all 4 viruses. Although all of the pathotyping test results indicated an increased virulence after chicken passage, an increase beyond moderate virulence was evident for only one of the viruses. There was however no virulence difference among the 4 passaged PPMV-1 viruses when they were inoculated by eye drop in 4-wk-old chickens. Lesions of the brain and heart were a consistent finding even though clinical disease was mild and limited to depression and some nervous signs in no more than 33% of the birds inoculated with each of the isolates. Mortality and greater morbidity did occur in 2-wk-old birds inoculated eye drop with 1 of the passaged PPMV-1 isolates.

In contrast to the PPMV-1 results, the isolates that were not inhibited by the pigeon specific monoclonal antibody were either of high virulence or acquired high virulence by chicken passage. A viscerotropic velogenic form of ND was produced by the initial passage inoculation of the two 1975 isolates. The MDT, ICPI, and IVPI of the 1998 dove isolate indicated it was of moderate to high virulence initially and became more virulent during passage. Intracloacal inoculation of the initial dove isolate produced no clinical disease but after passage a viscerotropic velogenic form of ND occurred.

Passage of PPMV-1 isolates in chickens increased their virulence but not to the level inherent in those 3 isolates that produced viscerotropic velogenic ND before or after passage. Further, the observation that the tested isolates induce different clinical forms of ND are evidence that all isolates from birds of the *Order Columbiformes* are not of the classical PPMV-1 type. The increased virulence in pathotyping tests of passaged PPMV-1 was not reflected in an increased severity of clinical disease. However, the fact that some clinical disease was evident, which included mortality in younger birds, is an indication that these isolates are a greater hazard to chickens than the low virulence NDV strains routinely recovered from poultry. NDV infected pigeons are a potential hazard for infection of commercial poultry and every effort should be made to prevent those infections.

(A full report of the study will be submitted for publication in *Avian Diseases*)

A NOVEL PATHOGENIC REOVIRUS STRAIN (ERS) THAT INDUCES FIELD PROBLEMS

A.A.W.M. van Loon[A], H.C. Koopman[A], W. Kosman[A] and A. Malo[A]

[A]Intervet International BV, P.O. Box 31, 5830 AA Boxmeer, The Netherlands

SUMMARY

This paper describes the isolation and identification of a novel virulent reovirus, the so-called Enteric Reovirus Strain (ERS). Virulence, dissemination, and reaction patterns with different monoclonal antibodies are reported. Screening of field isolates revealed that reovirus strain ERS is also present in other countries, being isolated from cases of malabsorption syndrome (MAS). Furthermore, efficacy data after the use of a new monovalent inactivated reovirus ERS vaccine is presented.

INTRODUCTION

Avian reovirus infections in chickens cause major economic problems for commercial poultry producers throughout the world. Mainly in heavy breeds, avian reoviruses have been associated with a variety of diseases, including viral arthritis/infectious tenosynovitis, stunting, malabsorption syndrome, growth retardation, pericarditis, myocarditis, enteritis and immunosuppression. Recently, high mortality has been reported in commercial broilers from well-vaccinated parents in Poland. Difficulty in walking and high mortality in the flock are the most common signs

in affected flocks. White spots on the liver and pericarditis are seen at post-mortem. Later on the causative agent: a so-called Enteric Reovirus Strain (ERS) was isolated from affected chickens. In this paper the properties of this novel reovirus strain and the work that led to the production of an inactivated vaccine are presented.

MATERIALS AND METHODS

Virus isolation. Organs (liver, kidney, thymus ceacal tonsils, spleen, intestine and heart) from clinically affected chickens were individually homogenized and used for virus isolation. For the isolation attempt primary chicken embryo fibroblasts (CEF) or primary chicken-embryo-liver-cells (CELi) were used.

VIRUS CHARACTERIZATION

Inmunofluorescence test. Different avian reovirus strains were characterized compared by means of IFT using polyclonal and different monoclonal antibodies (MCA). The virus strains investigated were 1133, 2408, 2177, UM203, Olson, 1733 and CO8 (all described in literature). Also commercially available live vaccines Enterovax (MBL, USA) and Tensynvac (Intervet Inc., USA) and reovirus strains recently isolated in different countries were included in the comparison.

Plaque reduction test. The antigenic relatedness between the reovirus isolates was determined by the plaque reduction test. The amount of antibodies against reovirus present in different sera was quantified by means of IFT against reovirus strain 1133 before being used in the plaque reduction test. Antibodies against reovirus induced by different reovirus strains (1733, 2408 and 2177) and 4 commercially available vaccines were compared in their ability to neutralize reovirus strain ERS in the plaque reduction test. The capacity of antibodies induced by reovirus strain ERS to neutralise reovirus strains 1733, 2408, 2177 and ERS was included in the study.

Pathogenicity and dissemination studies of reovirus strain ERS. In a first experiment, one day old SPF chickens were orally inoculated with reovirus strain ERS. In a second experiment, 3 weeks old SPF birds were inoculated with reovirus strain ERS via the foot pad or subcutaneously. The presence of virus in liver, heart, spleen, bursa, cecal tonsils, tendons, pancreas, whole stomach (proventriculus and gizzard) and intestine was investigated at 3, 7, 14 and 21 days after inoculation in both experiments. In a third experiment, 9 weeks old SPF birds were inoculated with reovirus strain ERS via the foot pad route and animals were observed daily for mortality.

Growth retardation induced by reovirus strain ERS in commercial broilers. Two groups of 15, one day old commercial broilers (with maternal antibodies against reovirus) were orally or subcutaneously inoculated with reovirus strain ERS (from Poland) using a dose of 5 log10 $TCID_{50}$/bird. Two other groups of 15 commercial broilers per group of the same origin, were orally or subcutaneously inoculated with reovirus strain ERS (from Poland) at 8 days of age. Fifteen animals of the same age and source were not inoculated and served as negative controls. Animals were weighed each week for a period of 7 weeks to investigate growth retardation. At each weighing point, the average weight of the uninoculated animals was set at 100%.

Protection studies using an inactivated monovalent ERS vaccine. SPF animals of 3-4 weeks old were intramuscularly vaccinated (0.5ml/bird) with an inactivated monovalent reovirus ERS water-in-oil emulsion. Not vaccinated animals of the same age and source served as challenge control. Three and 5 weeks after vaccination sera were investigated for the presence of antibodies against reovirus with the IFT-test. Five weeks after vaccination animals were challenged via the foot pad route with pathogenic reovirus strain ERS. Mortality and morbidity were scored daily. The average lesion score of the degree of inflammation and discoloration of the foot pad and shank was measured for a period of 14 days after challenge. The score ranges from 0 to 3.5 in which 0.0 = no swelling and 3.5 = severe swelling in foot pad and swelling in whole shank with discoloration.

RESULTS

Isolation of reovirus from clinically affected chickens. Enteric reovirus strain (ERS) was isolated from liver, kidney, thymus, ceacal tonsils, spleen and heart. The isolated virus was characterized as a reovirus by the immune-fluorescence-technique (IFT) using rabbit polyclonal anti reovirus serum. The isolated reovirus was plaque purified and further characterized.

Characterization of reovirus strains using monoclonal antibodies. The results of the characterization of different reovirus strains by means of the IFT using polyclonal and different monoclonal antibodies are presented in Table 2. Reovirus strain ERS from Poland and isolates from different geographical parts of the world show a distinct panel pattern with different MCA's when compared to reovirus strains that have been described in literature.

Plaque reduction test. Antibodies against reovirus induced by different reovirus strains (1733, 2408 and 2177) and different commercial vaccines were unable to neutralize reovirus strain ERS in a

plaque reduction test. In contrast, antibodies induced by reovirus strain ERS could neutralize reovirus strains 1733, 2408, 2177 and ERS. The data is summarized in Table 1.

Pathogenicity and dissemination of reovirus strain ERS. In the first experiment, reovirus strain ERS caused 100% mortality within 7 days after oral inoculation of one day old SPF birds. Three and 7 days after inoculation, virus was reisolated in all animals from the following organs: liver, intestine, tendons, pancreas, ceacal tonsils, spleen, bursa of Fabricius, whole stomach (proventriculus and gizzard) and heart. In the second experiment, reovirus strain ERS caused 53 or 12% mortality when applied to 3-weeks-old SPF-birds via the foot pad or subcutaneous (SC) route, respectively. Virus was present in all above mentioned organs, investigated 3 and 7 days after inoculation. Furthermore, in all animals at 2 weeks of age and in 3 out of 5 animals one week later reovirus strain ERS was isolated from the pancreas. In the third experiment, reovirus strain ERS caused 12% mortality when applied to 9-weeks-old SPF-birds via the foot pad route.

Growth retardation induced by reovirus strain ERS in commercial broilers. At the age of 7 weeks, animals infected at one day of age via the oral or subcutaneous route showed a growth retardation of approximately 34% compared to the non-infected control animals. At 7 weeks of age, the weight for non-infected control animals was 2469 ± 261g versus 1635g ± 272g for the infected animals. At the age of 7 weeks, animals infected at one week of age via the oral or subcutaneous route showed a growth retardation of approximately 25% compared to the non-infected control animals. At 7 weeks of age, the weight for non-infected control animals was 2469 ± 261g versus 1842 ± 376g for the infected animals.

Protection studies using a inactivated monovalent ERS vaccine. After challenge none of the vaccinated animals died or showed signs of disease. In contrast, 87.5% of the animals were ill and 12.5% of the animals in the challenge control group died. Post-mortem examination revealed as cause of death exposure to the challenge virus. The serological response induced by the inactivated monovalent ERS vaccine 3 and 5 weeks after vaccination was respectively 9.6 and 9.2 (log2). Fourteen days after challenge the average foot pad and shank scores were 1.4 for the vaccinated group and 3.1 for the none-vaccinated challenge control group, indicating that all vaccinated animals were protected, whereas none of the animals in the control group were protected.

DISCUSSION

Recently, high mortality has been reported in broiler flocks in Poland. Even the progeny from well-vaccinated parents was affected. The causative agent could be isolated and was identified as a reovirus, designated as “Enteric Reovirus Strain” (ERS). After plaque purification it was shown that the reovirus is highly pathogenic, causing high (100%) mortality in one day old SPF birds infected orally. Dissemination studies in 3 weeks old SPF birds infected via the SC route revealed that the virus could be reisolated from many different organs at 3 and 7 days after infection. Furthermore, the virus was still present in the pancreas 3 weeks after infection.

Characterization of the virus isolate from Poland with a panel of monoclonal antibodies revealed that the isolated reovirus had a distinct panel pattern when compared with reovirus strains that have been described in literature. Furthermore, the results of the plaque reduction test showed that reovirus strain ERS could not be neutralized with antibodies directed against reovirus strains 1733, 2408 and 2177 nor with antibodies induced by different inactivated commercial reovirus vaccines. In contrast, antibodies induced by reovirus strain ERS could neutralize reovirus strains 1733, 2408, 2177 and ERS. It was concluded that the Polish isolate was different from the strains investigated in this study. Further studies using MCA have demonstrated that field isolates from different countries (see table 2) show an identical reaction pattern as reovirus strain ERS as well as cross plaque reduction test (data not shown) and may be considered as belonging to the same group. This indicates that reovirus strains of the ERS type are present in different geographical regions and awareness for the problems they may cause (malabsorption) should be considered. Therefore, the need for a homologous vaccine based on the new reovirus strain ERS was obvious. In order to evaluate the efficacy of such a product challenge experiments were carried out.

Animal experiments show that the monovalent inactivated reovirus vaccine based on the Polish isolate can induce a serological response in animals, protects vaccinated animals from mortality and clinical signs, and protects chickens from severe lesions in the foot pad and shank.

REFERENCES

1. Hieronymus DRK, Villegas P, and Kleven SH. Identification and serological differentiation of several reovirus strains isolated from chickens with suspected Malabsorption syndrome. Avian Diseases 1983; 27: 246-54.

2. Johnson DC. Diagnosis, pathology, and etiology of tenosynovitis in broilers and broiler breeders. Avian Diseases 1972; 16: 1067-72.

3. Olson NO, Shelton DC, and Muro DA. Infectious synovitis control by medication-effect of strain differences and pleuropneumonia-like organisms. Am J Vet Res 1957; 18: 735-9.

4. Roessler DE, and Rosenberger JK. In vitro and in vivo characterisation of avian reoviruses. III Host factors affecting virulence and persistence. Avian Diseases 1989; 33: 555-65.

5. Rosenberger JK. Characterization of reoviruses associated with runting syndrome in chickens. Int. Union Immunol Soc, Sydney, Australia. 1983; Proc No 66: 141-152.

6. Rosenberger JK, Sterner FJ, Botts S, Lee KP, and Margolin A. In vitro and in vivo characterization of avian reoviruses. Pathogenicity and antigenic relatedness of several avian reovirus isolates. Avian Diseases 1989; 33: 535-44.

7. Rosenberger JK, and Olson NO. Reovirus infections. In BW Calnek (Ed.), Disease of Poultry. Ames: Iowa State University Press. 1991 (9th edn, pp. 639-47).

8. Vakharia VN, Read-Connole EL, Frana MF, and Edwards GH. Preparation and characterization of monoclonal antibodies for diagnosis of avian reovirus infection. Published in: "Proceedings of the International symposium on adenovirus and reovirus infections in poultry", Rauischholzhausen, Germany, 1996: 295-304.

9. Van der Heide L, Geissler J, and Bryant ES. Infectious tenosynovitis: serological and histopathological response after experimental infection with a Connecticut isolate. Avian Diseases 1974; 18: 289-96.

10. Van der Heide L, Lutticken D, and Horzinek, M. Isolation of avian reovirus as a possible etiologic agent of osteoporosis ("Brittle bone disease"; "Femoral head necrosis") in broiler chickens. Avian Diseases 1981; 25: 847-56.

Table 1. Identification of reovirus strain ERS by means of plaque reduction.

	Chicken serum →				
	1733	2408	2177	ERS	Negative serum
Virus↓					
1733	99	ND	ND	96	-
2408	ND	95	ND	87	-
2177	ND	ND	90	83	-
ERS	0	0	0	97	-

Chicken sera were directed against different reovirus

ND = not done.

Table 2. Identification of different reovirus strains by means of IFT

			Monoclonal antibodies →					
Strain	Identification	Country	Rabbit 68A	154	14-67 INT	INT 14-11	INT13-6	15-1-INT
S-1133	L. van der Heide	USA	+	+	+	+	+	+
2408	Rosenberg	USA	+	+	+	+	+	+
UM 203	Johnson	USA	+	+	+	+	+	+
WVU 1675	Olson	USA	+	+	+	+	+	+
Olson Enterovax	MBL	USA	+	+	+	+	+	+
Tensynovac	Intervet	USA	+	+	+	+	+	+
1733	Rosenberg	USA	+	+	+	+	+	-
2177	Rosenberg	USA	+	+	+	-	-	+
CO8	Hieronymus, Villegas	USA	+	+	+	-	+	+
ERS	Isolate 1	Poland	+	+	+	-	-	-
ERS	Isolate 2	Netherlands	+	+	+	-	-	-
ERS	Isolate 3	United Arab Emirates	+	+	+	-	-	-
ERS	Isolate 4	Indonesia	+	+	+	-	-	-
ERS	Isolate 5	United Kingdom	+	+	+	-	-	-
ERS	Isolate 6	South Africa	+	+	+	-	-	-
ERS	Isolate 7	Germany	+	+	+	-	-	-
ERS	Isolate 8	Belgium	+	+	+	-	-	-
ERS	Isolate 9	Argentina	+	+	+	-	-	-

INCLUSION BODY HEPATITIS/HYDROPERICARDIUM SYNDROME: VERTICAL INDUCTION WITH FOWL ADENOVIRUS AND CHICKEN ANEMIA VIRUS

H. Toro[A], O. González[A], C. Escobar[A], L. Cerda[B], M. A. Morales[A], and C. Gonzalez[A]

[A]Faculty of Veterinary Sciences, University of Chile, Casilla 2, correo 15, Santiago, Chile
[B]Veterinary Science Division, Ministry of Agriculture

ABSTRACT

The hypothesis that fowl adenovirus (FAV) and chicken anemia virus (CAV), transmitted vertically and simultaneously, induce the inclusion body hepatitis/hydropericardium (IBH/HP) syndrome in progeny chickens was tested. Thus, 35 wk old light brown layer breeders, showing absence of antibodies against FAV and variable titers against CAV, were intramuscularly singly infected with the FAV serotype 4 isolate 341 or dually infected with CAV (isolate 10343) and FAV. In the present study, both infective viruses FAV and CAV were isolated from progenies obtained as early as 5 days after infection of their breeders. Hematochemical values showed few significant differences between the chicken groups. Most of these differences were detected in the dually infected progeny chickens. These changes could be interpreted as the result of a more severe damage in the livers of this group. However, almost all values met reference ones obtained by other authors for the species. Therefore, the values obtained do not strongly help to support our hypothesis. The pathological findings showed that progeny chickens obtained from both singly and dually infected breeders developed macroscopic and histopathological changes of IBH/HP. Additionally, chickens whose parents received CAV and FAV showed lymphocyte depletion and apoptosis in the spleen. These lesions were attributed to the CAV infection. There is conflicting evidence concerning the role of adenoviruses as the primary etiologic agent of IBH/HPS. Although a few reports have underlined the role of FAV's as primary etiologic agents of IBH/HPS, most studies have suggested that outbreaks of IBH/HPS in chickens involve concurrent marked immunodepression. These findings have been corroborated using Chilean FAV isolates. Previous results showed the use of FAV-4 isolate 341 alone via the intramuscular route in 20-day-old SPF chickens induced 10% mortality due to IBH/HPS. The oral infection with this isolate determined neither mortality nor IBH/HPS lesions. Interestingly, in the present study, the progeny of breeders receiving FAV isolate 341 alone reproduced the IBH/HP syndrome. This result was not expected since neither synergisms nor prior immunodepression by CAV was concurrent. Accordingly, the present results do not allow the confirmation of the hypothesis that the association of FAV and CAV is necessary for the successful induction of the IBH/HP syndrome in chickens when transmitted vertically.

Financed by the Chilean National Science Foundation FONDECYT (1990816)
(Full article will appear in *Avian Diseases*)

PRACTICAL STRATEGIES TO CONTROL SUBCLINICAL INFECTIOUS BURSAL DISEASE (IBD) IN BROILER FLOCKS EXPERIENCING IMMUNOSUPPRESSION DUE TO HIGH IBD FIELD CHALLENGE

N.L. Tablante[A], F.N. Hegngi[B], D.A. Bautista[A], and E.M. Odor[C]

[A]Virginia-Maryland Regional College of Veterinary Medicine, Maryland Campus, Salisbury Facility, 27664 Nanticoke Road, Salisbury, MD 21801
[B]Maryland Department of Agriculture, P.O. Box 2599, Salisbury, MD 21802
[C]University of Delaware, Lasher Diagnostic Laboratory, R.D. 6, Box 48, Georgetown, DE 19947

ABSTRACT

Two broiler farms that had been experiencing production problems on the Delmarva Peninsula were monitored weekly through flock observations, post-mortem examinations, serology using Enzyme-Linked Immunosorbent Assay (ELISA), histopathological examination of sections of bursa of Fabricius, and virus isolation. Field and laboratory findings indicated high IBDV field challenge. Management changes such as vaccination of broilers at 7 and 14 days of age with an intermediate strain of IBD, placement of chicks from not more than three mid-production breeder sources, application of litter acidifiers and insecticides, and reinforcement of biosecurity, hygiene, and sanitation were initiated. Better feed conversion and livability were observed after proper implementation of management changes on one farm. This provides further evidence that, while eradication of IBD may be unrealistic and impractical, significant improvements in broiler performance can be achieved through proper vaccination and timing, good chick uniformity, strict biosecurity, and good hygiene and sanitation.

(A full-length article will be submitted for review and consideration for publication in the *Journal of Applied Poultry Research*)

VERY VIRULENT INFECTIOUS BURSAL DISEASE VIRUS IN EGYPT: ISOLATION, RESISTANCE OF LOCAL BREEDS AND IMMUNOGENICITY OF CLASSIC VACCINE

Mohamd K. Hassan[A], Manal Afifi[B] and Mona M. Aly[A]

[A]Animal Health Research Institute
[B]Faculty of Veterinary Medicine, Cairo, Egypt

Infectious bursal disease (IBD) is still an important economic problem facing the Egyptian poultry industry. In 1999, six commercial poultry flocks, located in lower and upper Egypt, were diagnosed with severe bursal lesions and high mortalities (8% - 28%). All flocks were vaccinated with live classic vaccines. On post mortem examination, typical IBD lesions were noticed; detection of IBDV antigen was performed by agar gel precipitation test and inoculation of SPF embryos. Six continuous cell lines and one primary cell culture were tested for their ability to support replication of very virulent IBDV (VVIBDV). None of the six VVIBDV isolates could be adapted to BGM-70, Vero, BHK, RK-13 and MDBK cell lines or chicken embryo fibroblast cells after 6 blind passages.

Susceptible chickens from five Egyptian and one foreign breeds were evaluated for genetic resistance to VVIBDV. Birds of the 6 breeds were divided into vaccinated and nonvaccinated, and exposed by eye drop route to VVIBDV. Five parameters were monitored: mortality, relative bursa and spleen weight, bursal lesion score, antibody titer and the response of blood lymphocytes to mitogens. In the pathogenicity experiment, birds of the Mandara breed were significantly more resistant than chickens of Gimmizah, Golden, Fayoumi, Balady, and foreign white leghorn breeds. In the immunogenicity experiment, chickens were vaccinated with a live classic vaccine (Bursine 2) and challenged 10 days later with the VVIBDV. Mortality, following

challenge, was not significantly different among the 6 breeds. The vaccinated birds were partially protected against challenge with VVIBDV. The challenged birds were protected against clinical disease and death but not bursal damage and atrophy. The correlations of the five parameters with resistance to VVIBDV were inconsistent, indicating that nonimmunogenic factor(s) might play a critical role in IBD resistance. The study illustrated the importance of IBD resistance evaluation in both vaccinated and nonvaccinated chickens.

In another experiment, the protection induced by graded doses of classic-intermediate vaccine in both IBD resistant (Mandara) and susceptible (Gimmizah) local chickens was investigated. The immunogenicity of the tested doses was evaluated by measurement of the serological response and resistance to VVIBDV challenge 10 days post vaccination. Results showed that similar immune responses to the vaccine could be generated over a wide (100 fold) dose range. It was concluded that single vaccination, by eye drop route, with live intermediate vaccine (1x) could protect chickens against clinical disease and mortality. However, the immune responses generated by 1x, 10x or 100x vaccine doses did not protect against VVIBDV super infection of the bursa following challenge. This finding point out the highly invasive nature of the prevailing VVIBDV in Egypt. Further investigations are needed to evaluate the antigenic and immunogenic relatedness of the VVIBDV and commercially available vaccines.

ACKNOWLEDGMENT

This work was funded by the International Foundation of Science (IFS), Sweden. Under research grant No. B/904-1.

EFFICACY OF A BURSAL DISEASE - MAREK'S DISEASE VACCINE IN BROILERS ADMINISTERED THROUGH IN OVO VACCINATION

Tarcisio Villalobos, Alexandra Camacho, Carlos Zamora, Jose Elizondo, Rafael Fernández, Ricardo Bonilla

Merial Avian Global Enterprise, PO Drawer 2497, 1112 Airport Parkway, Gainesville, GA 30503

Two hundred twenty-five thousand, eighteen day-old embryonated commercial broiler eggs were injected *in ovo* with Bursal Disease - Marek's Disease Vaccine Serotype 3 Live Virus (S-706 + HVT). The chickens were observed daily for forty-two days. Serological and production parameters were evaluated. The results were then compared against a control group (HVT *in ovo* + S-706 by spray at one day old). The results showed the safety and efficacy of *in ovo* vaccination with Bursal Disease - Marek's Disease Vaccine Serotype 3, Live Virus was satisfactorily demonstrated in commercial broiler chickens.

MAREK'S DISEASE: RECENT DEVELOPMENTS AND CURRENT STATUS

R. L. Witter[A] and R. W. Morgan[B]

[A]USDA, Agricultural Research Service, Avian Disease and Oncology Laboratory, East Lansing, MI
[B]Department of Animal and Food Sciences, University of Delaware, Newark, DE

INTRODUCTION

In 1978, an international symposium was held in Berlin to address current concerns and research developments in Marek's disease. Twenty-two years later, in August 2000, the 6th symposium in this series was held in Montreal. This current symposium, organized by K.A. Schat, featured 56 presentations and 17 posters by prominent scientists in the field from many countries. The symposium was held in conjunction with the XXI World's Poultry Congress. Like its predecessors, this symposium provided an excellent snapshot of the state of the disease and documented many new developments, some of which will surely alter the course of future activity in the disease. This summary is based largely, but not entirely, on results presented in the symposium. No attempt is made to cover all the presentations. The

proceedings will be published early in 2001 and should be consulted for more detailed information. Two excellent summary reports on the symposium by B.W. Calnek and Robin Morgan, respectively, are published in the proceedings (3, 14) and provided both information and inspiration for this report.

STATE OF THE DISEASE

Broiler condemnations and layer mortality from Marek's disease (MD) are at an all time low. MD condemnations for the United States in 1999 were 0.012% and, for the first 9 months of 2000, were 0.009%. This level is about 10-fold less than in 1961 when statistics were first recorded and about 150-fold less than peak levels in 1970. These favorable trends also are reflected in many other countries although we learned from one of the MD Symposium workshops that sporadic outbreaks have been seen recently in Pakistan, Israel, United States, Vietnam and Korea. Subsequently, additional reports of excessive MD losses have been received from Nepal and Brazil. The low passage CVI988 vaccine is widely used and appears highly effective in most cases. In ovo vaccination is now widely practiced for broilers, at least in the United States. Revaccination is also widely practiced, mostly outside the United States, but specific protocols vary and benefits remain controversial.

Diagnostic criteria for MD are not applied uniformly by all laboratories. Although traditional pathology still forms the basis for differential diagnosis of MD lymphomas, some laboratories tend to rely on polymerase chain reaction (PCR) assays even though it seems clear that MD virus (MDV) can be present in chickens in the absence of overt disease. The issue of differential diagnosis is reemerging and is being currently addressed by a subcommittee of the AAAP Tumor Virus Committee. However, it seems likely that a diagnostic scheme based on traditional criteria of clinical presentation, gross and microscopic pathology, and epidemiology will be most useful, especially if supplemented with tests to demonstrate T-cell markers or the presence of MDV and its antigens in tumor cells.

Despite the favorable situation with MD, there are valid concerns that the virus will continue to mutate to greater virulence and that current vaccines will no longer provide adequate protection. In fact, some recently isolates strains have greater virulence for CVI988-vaccinated chickens than earlier strains. Witter (20) addressed this concept in depth at the MD Symposium and concluded that the virus will not easily be defeated and that some kind of commensal relationship with its host may be both inevitable and beneficial. Such an outcome could be advantageous, especially if the industry cannot support the costs of complete eradication, but is not yet assured.

HIGHLIGHTS FROM MD SYMPOSIUM

The MD Symposium covered virtually all aspects of the virus and the disease. The proceedings should be consulted for details. The intent here is to showcase a few carefully selected accomplishments that may have special relevance to increasing our understanding of the disease and improving methods for its control. By necessity, many interesting presentations are omitted from this discussion.

Genomic sequences. During the past year, complete DNA sequences have been obtained for 5 virus isolates representing all 3 serotypes. Of particular interest were the sequences for two strains of serotype 1 MDV that differ in relative virulence, i.e., GA (10) and Md5(19). This is a landmark accomplishment that will have a far reaching impact on the course of MD research. Lee (11) presented the GA sequence at the MD Symposium and compared the genomic organization to that of herpes simplex virus. Of particular interest are the several open reading frames that appear to have no counterparts in other herpesviruses. This information will permit a more systematic and focused approach to identify MDV genes that may have important roles in replication, oncogenicity and immunogenicity.

Techniques for mutant viruses. The second landmark accomplishment revealed in the symposium was the development of not one but two improved methods for making directed mutations in the MD viral genome. The first method developed by Reddy (17) utilized a series of five overlapping cosmid clones derived from the Md5 strain to produce recombinant viruses. In one of these viruses, the pp38 gene was deleted to demonstrate the utility of the technique. The technique permitted construction of the mutant as well as a recombinant control virus without the specified mutation. This strategy allows direct comparisons between mutant and recombinant control virus, thereby facilitating the association of the mutation with changes in biological properties. The pp38-deleted mutant virus replicated in chickens but less vigorously than the recombinant control and did not cause gross tumors.

The second method (presented by Osterreider in an informal workshop) involved production of a bacterial artificial chromosome (BAC) that contained an infectious clone of a high passage serotype 1 virus derived from vv+ strain 584A. This technique was used to produce a gB deletion mutant that replicated on quail cells previously transfected with the gB gene. Since complementation by the gB cell line was needed for replication, one could conclude that gB is essential for replication in cell culture.

These two methods, taken together, represent a quantum leap in MDV technology. Mutants represent

the most direct way to identify the function of specific viral genes. Mutation (or deletion) of genes associated with virulence represent a strategy for molecular attenuation of serotype 1 MDV strains and the production of candidate vaccine strains. On the other hand, traditional but elegant technologies continue to be used to develop additional mutants. For example, Parcells (16) reported on a group of mutants of the RB1B strain where green fluorescent protein was fused to various genes. Interpretation of the biological data is difficult in the absence of rescuants but new information was obtained for several genes that are apparently not essential for oncogenicity, e.g., US2, pp38 and vIL8.

Induction of host genes. DNA microarrays or gene chips is a powerful new technology that was used to identify a number of host genes that were apparently induced by infection with MD virus. Morgan reported that several specific host genes such as NK-lysin are consistently induced by MDV infection in vivo (15); these genes may represent targets for the virus and in some cases may be linked to previously identified quantitative trait loci (QTLs). In most cases, the viral gene responsible for the induction is not known. However, Liu and Cheng (12), using the yeast two-hybrid system, identified a unique interaction between the viral SORF2 gene and the cellular gene for growth hormone (GH). The association of GH with lymphoma susceptibility in one of two commercial lines of chickens was especially intriguing and provides impetus to further efforts to link specific viral genes with specific host genes in ways that determine disease susceptibility.

MHC down regulation. Flow cytometry was used by Hunt (6) to show that the cell surface class I MHC molecules are down regulated in MSB-1 cells or MDV-infected OU2 cells following treatment with BUDR. In both cases, down regulation was associated with the onset of productive infection. Kent (8) used confocal microscopy to demonstrate down regulation of MHC class I on MDV-infected chicken embryo fibroblasts. Gimeno (5) also showed down regulation of class I Mhc in the brain subsequent to MDV infection, although in this system there was very limited productive infection. Down regulation of the Mhc may be a mechanism for the virus to evade cellular immune responses, but more information is needed to determine how this functions in practice.

Pathogenesis and immunity. Due to the development of new technologies, studies on pathogenesis are now often supplemented by data obtained by quantitative PCR assays. Such measures of "viral load" are potentially a useful measure of viral activity in MDV-infected chickens. However, some reports at the MD Symposium provided PCR data that did not correlate well with assays for infectious virus, prompting concerns about the relative sensitivity of the two techniques. Quantitative PCR has a huge potential to add valuable knowledge, but more work will be needed to standardize procedures and to properly interpret data. Similarly, it is perhaps disconcerting that techniques for viral isolation and propagation worked out and used successfully for 30 years are, in some cases, modified in ways that appear to decrease their sensitivity. It may be a challenge to retain the knowledge developed previously in MD research and transfer it to new investigators.

Macrophage function and cytokines in MD infection were the subject of several reports. Schat (18) reported that suppressor macrophages induced by MDV infection can decrease, through nitric oxide production, the mitogenic responsiveness of lymphocytes as well as the replication of MDV in permissive cells. Schat also reported that gamma interferon produced by macrophages upregulated IL8 expression on T cells. Kaplan and Heller found that interferon down regulated early viral proteins while Jarosinski (7) showed interferon decreased NK cytotoxicity.

Immune responses in MD infection, studied by Markowski (13), identified gE and gI but not gD as potential targets for cytotoxic T cell responses. Some target antigens seemed to be more important in specific genetic strains of chickens.

Neurotropism of MDV was discussed by Gimeno (5) who presented evidence of viral DNA and antigens, indicating at least a limited productive infection in the brain 9-24 days post infection. Effects differed with viral strain and with chicken strain.

Evolution of virulence. Continued surveillance of field isolates for virulence has not revealed evidence of major changes in the past 4 years, although modest incremental changes are likely. However, some recent isolates may be selectively more virulent in chickens vaccinated with CVI988/Rispens although this impression is based on limited data and requires confirmation (20).

Association of viral properties with virulence. Whereas information on relative virulence of MDV isolates is important and of general interest, there is no agreement on the methods to obtain biological data on viruses or on the criteria to classify viruses by pathotype. Buscaglia (2) reported that a relationship between lymphoid organ atrophy with virulence was apparent with some but not all field isolates and concluded that this method would not be a substitute for traditional pathotyping. However, the immunosuppressive ability varies significantly among different serotype 1 MDV isolates and represents an opportunity to better understand the role of immunosuppression in the disease.

An interesting report by Barrow (1) described the unique tropism of a contemporary MDV isolate, C12/130, for replication in macrophages. Moreover, the authors implied that this property may be linked to increased virulence of MDV strains and may even be a marker for vv+ or highly virulent pathotypes. Surely this will be tested soon on a larger collection of strains.

Vaccines - recombinant and conventional. Interestingly, few papers discussed development of new MD vaccines and no breakthroughs were apparent. The next generation of more effective products is not yet apparent.

Dudnikov (4) conducted cross protection studies with attenuated serotype 1 vaccine strains to show that autologous vaccines were no more protective than heterologous vaccines. This finding suggests it may not be important to use contemporary, highly virulent strains as the starting point for future serotype 1 vaccine development and supports the absence of antigenic variation among serotype 1 isolates.

The review talk by Witter (20) reported that two new attenuated serotype 1 strains provided significant protection but were less effective than CVI988/Rispens. However, the RM1 strain was superior to all others tested. There was some indication that partially attenuated vaccines provided superior protection compared to fully attenuated viruses, but the mechanisms involved have not been determined. Partial attenuation, along with synergism and revaccination, were identified as classical strategies that warranted additional effort.

APPLICATIONS OF THE NEW TECHNOLOGY

Recent developments in MD research are substantial and exciting. Elucidation of the complete DNA sequences for all 3 serotypes and the development of cosmid clone technology that can be used to create specific mutations are major breakthroughs. We should now be able to determine what genes are the most likely to have important functions and, moreover, be able to create the mutants to confirm the biological functions of specific genes. The implications are huge. Through this technology we will not only advance our understanding of basic viral properties and host response but also the possibilities of novel vaccines. Deletion of oncogenicity in low passage viruses may be an attractive strategy for new vaccines since such vaccines, compared to those attenuated by serial cell culture passage, may replicate better and produce a more robust immune response.

Additional information on differences among serotype 1 strains continues to be an attractive area for research. As biological properties and DNA sequence variations are identified, these can be screened in panels of field isolates representing different pathotypes or other characteristics. Such panels are available and deserve more frequent use. We also need to continue to monitor the evolution of field strains for virulence and other properties since a thorough understanding of evolution may be essential to long term control strategies.

Immune response mechanisms and innate resistance are areas that are slowly yielding information that will be useful to immunization strategies. This difficult area should be substantially assisted by the technological breakthroughs described above. Similarly, host genes and genetic resistance represent an arena on the verge of significant breakthroughs. However, it is important that this resistance be compatible and additive with that of vaccines.

Also promising is the ability to determine the host genes that interact with the virus and that may determine disease resistance. As QTLs and specific host genes are identified, some of these may well prove valuable to breeders in improving resistance of commercial lines to MD challenge. Evaluations of some genetic loci are being conducted in commercial chicken stocks and more such studies may be beneficial. It remains to be determined, however, how best to utilize this information in breeding programs and how much resistance can be added by such techniques. It is also important that host gene-derived resistance be additive to the immunity induced by vaccination since it is unlikely that any genetic resistance will be sufficient in the absence of vaccines.

The development of improved vaccines has languished in recent years but should be stimulated by the advent of new molecular technologies. It is not enough to produce an effective vaccine - it will need to confer some improvement over existing products to have commercial value. New vaccines are indeed needed, since viral virulence continues to increase and other control mechanisms are either too costly or require long periods for development. Also, any new vaccine must be compatible with in ovo administration and the limited profit margins of the poultry industry.

A VISION FOR THE FUTURE

In conclusion, the MD situation appears stable and quiet at present. No major virulence shifts or failures of existing vaccines have been noted. Nonetheless, the acquisition of virulence through selection pressure by increasingly effective vaccines remains a major concern. This prompts and justifies continued efforts to identify new sources of genetic host resistance and to develop new vaccines. The role of industry, however, is critical because even now cost factors restrict the options that industry is willing to consider.

At this point there is an urgent need to focus, i.e., to judiciously select the most productive avenues among the myriad of possibilities created by the new technologies discussed previously. Equally important is to strengthen the interface between molecular and classical virologists to unravel the biology of the expected plethora of new recombinant strains.

Witter (20) previously invoked a battlefield metaphor to describe the evolutionary conflict between MDV and the host chicken. The virus wins by mutating and because man abdicates responsibility. The chicken wins because man invests the funds needed to produce better vaccines and to provide barriers to limit the evolutionary drift of the virus. With enough resources, one might even consider eradication through SPF housing, transgenes or sterilizing immunity. Failing this, however, a compromise may be the most practical solution where the host moderates but does not extinguish primary viral infection and the virus inhibits competing infections, as described by Lederberg (9). Such a solution is evolutionarily correct and appears to serve the needs of both virus and host. Perhaps man should attempt to facilitate such an outcome.

REFERENCES

1. Barrow, A.D., S. C. Burgess, and K. Venugopal. Infection of avian macrophages by highly virulent MDV strain C12/130 represents a "tropic shift" in MDV pathogenesis. In: Proc. 6th Intl. Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amer. Assn. Avian Pathologists, New Bolton Center, PA. pp. (in press) 2001.

2. Buscaglia, C., M. A. Risso, M. V. Prio, E. del Barrio, and M. C. Villat. Immunosuppressive potential of two Argentine pathotypes of Marek's disease virus isolates. In: Proc. 6th Intl. Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amer. Assn. Avian Pathologists, New Bolton Center, PA. pp. (in press) 2001.

3. Calnek, B.W. Summary presentation. In: Proc. 6th Intl. Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amer. Assn. Avian Pathologists, New Bolton Center, PA. pp. (in press) 2001.

4. Dudnikov, L.A. and R. L. Witter. A comparison of autologous and heterologous vaccination against Marek's disease. In: Proc. 6th Intl. Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amer. Assn. Avian Pathologists, New Bolton Center, PA. pp. (in press) 2001.

5. Gimeno, I., R. L. Witter, H. D. Hunt, L. F. Lee, S. M. Reddy, and U. Neumann. Chronological study of brain alterations induced by a very virulent plus (vv+) strain of Marek's disease virus (MDV). In: Proc. 6th Intl. Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amer. Assn. Avian Pathologists, New Bolton Center, PA. pp. (in press) 2001.

6. Hunt, H.D., B. Lupiani, M. M. Miller, I. Gimeno, L. F. Lee, and M. S. Parcells. Marek's disease virus down regulates surface expression of MHC (B complex) class I (BF) glycoproteins. In: Proc. 6th Intl. Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amer. Assn. Avian Pathologists, New Bolton Center, PA. pp. (in press) 2001.

7. Jarosinski, K.W., J. Wei, and K. A. Schat. Decreased natural killer cell cytotoxicity in chickens treated with a high dose of recombinant chicken interferon-a. In: Proc. 6th Intl. Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amer. Assn. Avian Pathologists, New Bolton Center, PA. pp. (in press) 2001.

8. Kent, J., E. L. Bernberg, and R. W. Morgan. Major histocompatibility complex (MHC) expression is down-regulated on the surface of Marek's disease virus (MDV)-infected chicken embryo fibroblasts but up-regulated on the surface of adjacent uninfected cells. In: Proc. 6th Intl. Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amer. Assn. Avian Pathologists, New Bolton Center, PA. pp. (in press) 2001.

9. Lederberg, J. Infectious history. Science 288:287-293. 2000.

10. Lee, L.F., P. Wu, D. Sui, D. Ren, J. Kamil, H.J. Kung, and R.L. Witter. The complete unique long sequence and the overall genomic organization of the GA strain of Marek's disease virus. Proc. Natl. Acad. Sci. USA 97:6091-6096. 2000.

11. Lee, L.F., P. Wu, D. Sui, D. Ren, J. Kamil, H. J. Kung, and R. L. Witter. Comparison of genomic organization of GA strain of Marek's disease virus with herpes simplex virus. In: Proc. 6th Intl. Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amer. Assn. Avian Pathologists, New Bolton Center, PA. pp. (in press) 2001.

12. Liu, H.C., H. H. Cheng, L. Sofer, and J. Burnside. Identification of genes and pathways for resistance to Marek's disease through DNA microarrays. In: Proc. 6th Intl. Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amer. Assn. Avian Pathologists, New Bolton Center, PA. pp. (in press) 2001.

13. Markowski, C., P. O'Connell, and K. A. Schat. Cytotoxic T lymphocyte responses to Marek's disease virus (MDV)-encoded glycoproteins. In: Proc. 6th Intl. Symposium on Marek's Disease. K.A. Schat, R.W.

Morgan, M.S. Parcells and J.L. Spencer, eds. Amer. Assn. Avian Pathologists, New Bolton Center, PA. pp. (in press) 2001.

14. Morgan, R.W. Summary presentation. In: Proc. 6th Intl. Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amer. Assn. Avian Pathologists, New Bolton Center, PA. pp. (in press) 2001.

15. Morgan, R.W., L. Sofer, A. M. Miles, A. Anderson, E. L. Bernberg, J. Cui, and J. Burnside. Microarray analysis of Marek's disease virus: the virus and the host cell. In: Proc. 6th Intl. Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amer. Assn. Avian Pathologists, New Bolton Center, PA. pp. (in press) 2001.

16. Parcells, M.S., R. L. Dienglewicz, J. T. Prigge, and V. Majerciak. Construction and characterization of recombinant Marek's disease viruses) MDVs) having the green fluorescent protein gene fused to MDV genes. In: Proc. 6th Intl. Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amer. Assn. Avian Pathologists, New Bolton Center, PA. pp. (in press) 2001.

17. Reddy, S.M., B. Lupiani, R.F. Silva, L.F. Lee, and R.L. Witter. Genetic manipulation of a very virulent strain of Marek's disease virus. In: Proc. 6th Intl. Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amer. Assn. Avian Pathologists, New Bolton Center, PA. pp. (in press) 2001.

18. Schat, K.A. Specific and nonspecific immune responses to Marek's disease virus. In: Proc. 6th Intl. Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amer. Assn. Avian Pathologists, New Bolton Center, PA. pp. (in press) 2001.

19. Tulman, E.R., C.L. Afonso, Z. Lu, L. Zsak, D.L. Rock, and G.F. Kutish. The genome of a very virulent Marek's disease virus. J. Virol. 74:7980-7988. 2000.

20. Witter, R.L. Marek's disease vaccines - past, present and future (Chicken vs virus - a battle of the centuries). In: Proc. 6th Intl. Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amer. Assn. Avian Pathologists, New Bolton Center, PA. pp. (in press) 2001.

MOLTING AND SALMONELLA ENTERITIDIS: ANY CAUSE AND EFFECT?

Peter S. Holt, USDA/ARS

Southeast Poultry Research Laboratory, 934 College Station Road, Athens, GA 30605

Induced molting is an important economic tool used by the egg industry to recycle an aging layer flock. As a laying flock ages, its ability to lay eggs decreases (15, 13) and the decline reaches a point where it is no longer economically feasible to keep the flock in lay. At this time, the producer must decide whether to retire the flock and bring a new flock into production, or recycle the current flock to achieve a second, or even third, lay from the hens. In 1987, an estimated 60% of laying flocks nationally and 90% in California were recycled annually (3) and use of the procedure is currently estimated at 70% for flocks nationwide and almost 100% in California. Considering that there are approximately 240 million hens in production in the United States, a rough estimate of the numbers of hens molted every year would be between 144 and 168 million birds, a substantial number. Industry estimates that fully one-third of the profits from a flock are derived from molted hens (Dr. Don Bell, University of California, Riverside).

There are many methods to induce molt but feed removal until the hens drop a specific weight is the most prevalent molt strategy in the U.S. (38). Increased body weight loss correlates with increased postmolt egg production and egg quality (2). The flocks require 9-10 weeks before resuming optimum egg lay which is generally 80-90% of the maximum lay achieved during the initial cycle (3, 6, 7).

While feed removal provides the benefit of extending the effective egg-laying life of the flock, the potential stress in which the hens may be subjected during the molt may negatively affect their health and wellbeing. Research over the past 30 years has shown that exposure of mammals (11, 14) and birds (5; 1981) to deficient diets results in diminished immune responsiveness in those individuals. Experiments in our laboratory showed that molting hens through feed withdrawal altered their immune responsiveness. Antibody responses remained largely unaffected while cell-mediated immunity, as indicated by three different tests, was significantly depressed (17). Total peripheral blood lymphocyte numbers were

significantly decreased in molted birds (17) and a flow cytometric examination of the lymphocyte subsets showed that the CD4+ T cells, the helper T cell subset, was significantly decreased in these birds (18). This lymphocyte population is very important in protecting the body against infectious agents as witnessed by the devastating consequences when viruses infect and destroy this cell in diseases such as AIDS.

The discovery that the immune system in molted hens was compromised therefore prompted an investigation into the effect of the procedure on the progression of an infection. *Salmonella enteritidis* (SE) was the organism chosen for the studies since SE is primarily a layer industry problem (8, 9, 10, 16, 34), molting is primarily a layer industry procedure (3), and cell-mediated immunity is important in protecting against a *Salmonella* infection (12, 27). The organism therefore appeared to be the most appropriate choice for the study, directly addressing potential industry problems.

As was observed with the immune system, responses of molted birds to SE infection were also affected - hens exposed to an exogenous source of SE concomitantly during molt induction exhibited a much more severe infection as compared to their unmolted counterparts. The intestinal shed rate was higher in these birds (24, 25, 20), these hens also shed more organisms (19,24, 25,21,20), exhibited significantly more intestinal inflammation, primarily in the colon and cecum, due to the infection (1, 24, 20, 28, 29), were 100-1000-fold more susceptible to an SE infection (19, 21), and readily transmitted SE horizontally to birds in adjacent cages (20) and to birds via the airborne route (23). Molting also caused the recurrence of a previous SE infection (26) indicating that the procedure can significantly affect an SE infection at different times in the infection cycle.

The realization that a very important economic tool of the layer industry exacerbated an SE infection in hens prompted research into methods to ameliorate the situation through alternate methods of inducing a molt. A low-energy, low-calcium diet, shown previously to cause an egg-lay pause in hens (31), was the first molt procedure, alternative to feed removal, which was investigated with respect to their relative effects on an SE infection. Birds on the molt diet shed less SE, were less susceptible to an infection by this pathogen, and exhibited less intestinal inflammation, compared to the fasted birds (21). These results indicated that low-nutrition diets may allow producers to recycle their aging hens without the radical exacerbation of an SE problem observed during feed removal. A comparison of effects of several other molt procedures alternative to full feed withdrawal have since been conducted. The emphasis of the studies was to test whether providing hens full access various feed ingredients, which lack the energy and nutrients necessary to maintain egg lay, would provide the necessary bulk to help keep the intestine full. Soybean hulls, cracked corn, or wheat middlings were administered ad libitum to hens and reduced SE levels were observed in all 3 groups. However, hens administered wheat middlings, a by-product of wheat processing, exhibited the most pronounced SE reductions. Fecal excretion of SE was significantly decreased in these hens compared to the full feed withdrawal group, and this was observed by 3 days post challenge and continued at days 10 and 17 (33). Significantly lower liver/spleen and ovary levels were observed at day 7 post challenge in the wheat middling group of hens and, in one trial, 62% of ovaries from full feed withdrawal hens were culture positive for SE while no SE could be detected in the wheat middlings group of hens. Egg lay generally ceased within 7 days of wheat middlings administration, indicating that use of this product may have commercial applications, but this will have to await full-scale field trials to determine just how economically feasible the use of wheat middlings may be in a commercial setting. Intervention strategies such as antibiotics (32) and vaccination (Holt, unpublished data 2000) have also been shown to reduce intestinal shedding and internal organ contamination by SE in hens undergoing molt.

Much attention has been paid by government and nongovernment groups in recent years to the possible link molting may have with human SE outbreaks. The FSIS SE Risk Assessment task force listed molting as a significant variable in its risk assessment document (37), and Objective 7.1.1 of the President's Council on Food Safety Action Plan (1999) identified the need for more research on molting and its relation to SE prevalence. The animal welfare groups have weighed in heavily against molting. United Poultry Concerns and the Association of Veterinarians for Animal Rights submitted petitions to the USDA and the FDA to ban molting based on both welfare and food safety issues. Further, a bill was recently presented before the California legislature to ban molting based on similar grounds. Two areas continue to come to the forefront with regards to molting: welfare and food safety. The welfare aspects of molting, while an important concern and possibly germane to the susceptibility of hens to infectious agents such as SE, will not be addressed in this paper. The question that arises then "Is molting a food safety issue?" The answer is maybe, maybe not. Experimental studies provide overwhelming evidence that molting has a significant impact on an SE infection in hens. However, caution should be exercised before extrapolating the results derived from the laboratory to on-farm experience. Actual results from the field will provide a more complete picture and some recent studies do provide insight into possible effects of

molting on commercial flocks. Swayne et al (35) observed that intestinal spirochete infections were more severe in molted hens, indicating that, similar to what was observed experimentally for SE, molting upset the equilibrium normally attained between the host and that parasite. A study conducted by the SE Pilot Project in Pennsylvania (36) showed that the production of eggs contaminated with SE increased during the molt and prompted the authors to categorize molting as a risk factor for SE. The recent USDA/APHIS National Animal Health Monitoring System Layers 99 study showed a 9-fold increase SE recovery in the environment of molted hens 1-16 weeks post molt (39). However, this study only provides a snapshot of molting as it relates to an SE infection - no prior knowledge of the SE situation in the houses will be known. It is therefore crucial for the egg industry, the consumer, and the regulatory agencies to ascertain the role, if any, molting plays in the current egg-borne outbreaks of human SE infections occurring in this country. This can only be determined through in-depth surveillance on the farm.

So what needs to be done? First, determining whether molting does indeed have an effect on the flock SE situation is a major priority. Along with this, the type of molt needs to be defined: short feed withdrawal with a possible skip-feed regimen, full feed withdrawal for 1-2 weeks, or feed restriction to achieve a particular body weight loss. This will tell us whether 1) molting affects an SE infection and 2) the relative effect each procedure has on the SE situation. Second, if molting does impact a flock SE problem, it is important to determine what risk factors/prevention strategies may affect the SE outcome: house size, flock age, manure handling, rodent and fly situation, vaccination, and biosecurity, to name a few. If these can be identified, it may be possible to implement intervention strategies to reduce or eliminate SE problems which may occur during molt.

In closing, it should be emphasized that the SE and molting question will not go away on its own. More pressure will be brought to bear by the welfare groups and consumer groups will inevitably become involved in the future. Sooner or later the FDA will be forced to implement rules to regulate molting. These regulations can either be based on facts obtained from earlier studies, the bulk of which are experimental and reflect poorly on molting, or from actual studies on the farm under real-life commercial settings. It is crucial that this food safety issue be resolved soon or the layer industry stands to lose an important management tool.

REFERENCES

1. Arnold, J.W., and P.S. HOLT. Cytotoxicity in chicken alimentary secretions as measured by a derivative of the tumor necrosis factor assay. Poult. Sci. 75:329-334. 1996.

2. Baker, M., J. brake, and G.R. McDaniel. The relationship between body weight loss during an induced molt and postmolt egg production, egg weight, and shell quality in caged layers. Poult. Sci. 62:409-413. 1983.

3. Bell, D. Is molting still a viable replacement alternative? Poult. Trib. 93(5):32-35. 1987.

4. Ben-Nathan, B, N. Drabkin, and D. Heller. 1981. The effect of starvation on the immune response of chickens. Avian Dis. 25:214-217.

5. Ben-Nathan, B., E.D. Heller, and M. Perek.The effect of starvation on antibody production of chicks. Poult. Sci. 56:1468-1471. 1977.

6. Brake, J. Recent advances in induced molting. Poult. Sci. 72:929-931. 1993.

7. Brake, J., 1994. Feed removal remains predominant method of molt induction. Poult. Times, June 20, Vol. 42:6,9.

8. Centers for Disease Control. Update: Salmonella enteritidis infections and shell eggs -- United States, 1990. Morbid. Mortal. Weekly Rep. 39:909-912. 1990.

9. Centers for Disease Control. Outbreak of Salmonella enteritidis infection associated with consumption of raw shell eggs, 1991. Morbid. Mortal. Weekly Rep. 41:369-372.1992.

10. Centers for Disease Control. Outbreaks of Salmonella serotype Enteritidis infection associated with consumption of raw shell eggs -- United States, 1994-1995. Morbid. Mortal. Weekly Rep. 45:737-742. 1996

11. Chandra, R.K., and P.M. Newberne. Nutrition, immunity, and infection mechanisms of interactions. Plenum Press, New York. Pp. 127-180. 1977.

12. Collins, F.M. Vaccines and cell-mediated immunity. Bacteriol. Rev. 38:371-402. 1974.

13. Cunningham, F.E., O.J. Cotterill, and E.M. Funk. The effect of season and age of birds. 1. On egg size, quality, and yield. Poult. Sci. 39:289-299. 1960

14. Depasquale-Jardieu, P. and P.J. Fraker. The role of corticosterone in the loss in immune function in the zinc-deficient A/J mouse. J. Nutr. 109:1847-1855. 1979.

15. Etches, R.J. The ovulatory cycle of the hen. Crit. Rev. Poult. Biol. 2:293-318. 1990.

16. Gast, R.K. Understanding Salmonella enteritidis in laying chickens: the contributions of experimental infections. Int. J. Food Microbiol. 21:107-116. 1994.

17. Holt, P. S. Effects of induced moulting on immune responses of hens. Br. Poult. Sci. 33:165-175. 1992a.

18. Holt, P. S. Effect of induced molting on B cell and CT4 and CT8 T cell numbers in spleens and

peripheral blood of white leghorn hens. Poult. Sci. 71: 2027-2034. 1992b.

19. Holt, P. S. Effect of induced molting on the susceptibility of white leghorn hens to a Salmonella enteritidis infection. Avian Dis. 37:412-417. 1993.

20. Holt, P. S. Horizontal transmission of Salmonella enteritidis in molted and unmolted laying chickens. Avian Dis. 39:239-249. 1995.

21. Holt, P. S., R.J. Buhr, D.L. Cunningham, and R.E. Porter, Jr. Effect of two different molting procedures on a Salmonella enteritidis infection. Poult. Sci. 73:1267-1275. 1994.

22. Holt, P. S., N.P. Macri, and R.E. Porter, Jr. Microbiological analysis of the early Salmonella enteritidis infection in molted and unmolted hens. Avian Dis. 39:55-63. 1995.

23. Holt, P. S., B. W. Mitchell, and R. K. Gast. Airborne horizontal transmission of Salmonella enteritidis in molted laying chickens. Avian Dis. 42:45-52. 1998.

24. Holt, P.S. and R. E. Porter, Jr. Microbiological and histopathological effects of an induced molt fasting procedure on a Salmonella enteritidis infection in chickens. Avian Dis. 36:610-618. 1992a.

25. Holt, P.S. and R. E. Porter, Jr. Effect of induced molting on the course of infection and transmission of Salmonella enteritidis in white leghorn hens of different ages. Poult. Sci. 71:1842-1848. 1992b.

26. Holt, P.S. and R. E. Porter, Jr. Effect of induced molting on the recurrence of a previous Salmonella enteritidis infection. Poult. Sci. 72:2069-2078. 1993.

27. Killar, L. and T.K. Eisenstein. Strain dependent variation of delayed-type hypersensitivity in Salmonella typhimurium infected mice. Adv. Exp. Med. Biol. 162:297-302. 1983.

28. Macri, N.P., R. E. Porter, Jr., and P.S. Holt. The effects of induced molting on the severity of acute intestinal infection caused by Salmonella enteritidis. Avian Dis. 41:117-124. 1997.

29. Porter, R. E., Jr and P. S. Holt. Effect of induced molting on the severity of intestinal lesions caused by Salmonella enteritidis in chickens. Avian Dis. 37: 1009-1016. 1993.

30. President's Council on Food Safety. Egg Safety from Production to Consumption: An Action Plan to Eliminate Salmonella enteritidis Illnesses Due to Eggs. p. 27. 1999.

31. Rolon, A., R. J. Buhr, and D. L. Cunningham. Twenty-four-hour feed withdrawal and limited feeding as alternative methods for induction of molt in laying hens. Poult. Sci. 72: 776-785. 1993.

32. Seo, K.-H., P.S. Holt, R.K. Gast, and C.L. Hofacre. Combined effect of antibiotic and competitive exclusion treatment on Salmonella Enteritidis fecal shedding in molted laying hens. J. Food Prot., in press. 2000.

33. Seo, K.-H., P.S. Holt, R.K. Gast. Comparison of Salmonella enteritidis levels in hens subjected to different molt procedures. Poult. Sci. 79 (Supp): 24. 2000.

34. St. Louis, M. E., D. L. Morse, M. E. Potter, T. M. Demelfi, J. J. Guzewich, R. V. Tauxe, and P. A. Blake. The emergence of Grade A eggs as a major source of Salmonella enteritidis infections: new implication for the control of salmonellosis. J. Am. Med. Assoc. 259:2103-2107. 1988.

35. Swayne, D. E., A. J. Bermudez, J. E. Sagartz, K. A. Eaton, J. D. Monfort, J. W. Stoutenburg, and J. R. Hayes. Association of cecal spirochetes with pasty vents and dirty eggshells in layers. Avian Dis. 36:776-781. 1992.

36. USDA, 1995. Effect of molting on the prevalence of SE in layer flocks. Salmonella enteritidis Pilot Project Progress Report, p. 66-68. USDA/APHIS, Hyattsville, MD.

37. USDA, 1998. Salmonella Enteritidis Risk Assessment: Shell Eggs and Egg Products. Washington, DC.

38. USDA, 2000a. NAHMS Layers >99. Part II: Reference of 1999 table egg layer management in the U.S. p. 13-14.

39. USDA, 2000b. NAHMS Layers >99. Salmonella enterica serotype Enteritidis in table egg layers in the U.S. p. 17.

SALMONELLA PRESENCE IN POULTRY SAMPLES AND RESULTS OF SEROTYPING

A. S. Dhillon; Parimal Roy; Dennis Schaberg; Daina Bandli and Sylvia Johnson

Avian Health Laboratory, Washington State University –Puyallup 7613 Pioneer Way East, Puyallup, WA 98371-4998 Email: asdhillon@wsu.edu

Two thousand eight hundred and fifty four samples of poultry meat, rinse water of chicken carcasses, fluff, environmental samples, and samples collected from diagnostic accessions were tested for the

presence of *Salmonella* over a period of 15 months during 1999 and the first quarter of 2000. Three hundred and seventy seven samples were found to be positive (13.21%) and 87.79% were negative for the *Salmonella*. Of all *Salmonellas* about 98% belong to somatic serogroup B & C and the remaining to D, E, and K. One hundred *Salmonella* specimens were serotyped. Of those 26% were *S. heidelberg*; 23% *S. kentucky*; 12% *S. montevideo*; 5% each of *S. infantis* and *S. enteritidis*; 4% each of *S. typhimurium*, *S. ohio*, *S. thompson*, *S. cerro* and *S. hardar*; 3% *S. mbandaka*; 2% *S. seftenberg* and 1% each of *S. indiana*, *S. saint paul*, *S. instanbul* and *S. berta*. To further summarize, 15 species of *Salmonella* were identified from poultry in the proportion given above.

TRENDS IN SALMONELLA DECONTAMINATION OF POULTRY FEEDS IN EUROPE

Chris Morrow, PhD BVSc

Ross Breeders Ltd, Midlothian Scotland EH28 8SZ

Feeds traditionally have been a high-risk source of *Salmonella* contamination for poultry. In Europe the trend now the use of heat to decontaminate finished feeds and this is mainly driven by consumer (supermarket) demand. For "Salmonella Kill" two main processes are being used; long term conditioning (LTC) and anaerobic pasteurization conditioning (APC). Typically LTC is 86ºC for 5 minutes and APC 82ºC for greater than 2 minutes for parent stock feeds. Technical difficulties include quality controlling these processes, nutrient and digestibility changes, throughput and the prevention of recontamination until presentation to the chicken. Initial investment is high but the processes are now considered proven and reliable. Initial validation of the ability of a process/plant to kill salmonella kill is usually done by microbiological enumeration studies on feed. Processes are quality controlled by monitoring feed temperature time conditions. Short term conditioning (STC) of feeds is used for "Salmonella knockdown" at broiler level although some producers are now using *Salmonella* kill processes for broiler feeds developing meat products that are marketed directly to consumers (via television advertisements) as *Salmonella* free (and even *Campylobacter* free).

TRIALS ON THE EFFICACY OF SALMONELLA ENTERITIDIS LIVE AND INACTIVATED VACCINE IN LAYER FLOCKS UNDER FIELD CONDITION

H.M. Hafez, A. Mazaheri and A. Edel

Institute of Poultry Diseases, Free University Berlin, Koserstr, 21, 14195 Berlin, Germany

The animal and public health problems related to Salmonella in poultry have increased to such an extent that they have become major political issues, of which the general public has become very aware. This problem has indicated to industry and government agencies an increasing requirement for effective measures to control the infection. In Germany according to Poultry Salmonella Order from 1994, the commercial pullets should be obligatorily vaccinated against *Salmonella*. Till mid 2000 only live *Salmonella typhimurium* (ST) as well as ST and *S. enteritidis* (SE) inactivated vaccines were licensed in Germany and are being used extensively in the field. The protection against SE in many cases, where ST live vaccines were used alone was not sufficient enough. This forced the industry to develop SE live vaccine. The present investigation was carried out to estimate the efficacy of SE live and inactivated vaccine in layer flocks under field condition.

In the present investigation 12 layer flocks, with 35000 to 75000 birds per flock, kept on farms with floor and/or free range rearing form, with SE infections history, although ST live vaccines were used for several years before were included. The birds were vaccinated twice with SE live vaccine (Salmovac SE®,

DT Impfstoffwerk, Dessau-Tornau GmbH, Germany) at 2nd and 16th days of age via drinking water (the vaccine is licensed in Germany since mid 2000) and then once between 15th and 16th weeks of age with inactivated SE vaccine (Salenvac®, Intervet). Fecal samples were collected at 3 to 5 week intervals starting from first day of age till 72nd week of age (end of the production) and bacteriologically examined for *Salmonella*. In addition, 60 birds at 20th and some flocks at 30th and 40th weeks of age were collected from each flock, humanly killed, and examined for *Salmonella*.

SE vaccinal strain could be detected in some flocks till 21 days post vaccination. From the first 7 vaccinated flocks SE could be detected in two flocks at week 20, re-examination of those flock at several intervals till the end of production period revealed negative results.

In 4 out 5 flocks (still in production) SE could be detected. In flock 9, *S. agona* as well as SE were detected at week 55. In flock 10, SE was isolated at first day of age and the flock remained positive by week 53. In the flock 11, SE was isolated at first day of age. At 20th weeks of age *S. agona* could be isolated but SE could not be redetected till 46th week of age. In flock 12, SE could be isolated at 30th and 40th weeks of age.

The results indicate, that the application of live and inactivated vaccines together with improvement in the production hygiene, have been remarkably successful and have resulted in a marked decline in salmonella at least in the first 7 vaccinated flocks. In birds previously infected with a field *Salmonella* strain (at first day of age), the vaccine did not eliminate the infection. It seems to be, that the use of the vaccine might send the wrong message, since the salmonella detection rates were increased in the last four vaccinated flocks. In conclusion, vaccination must be considered as an important support to good hygiene and husbandry practices in all production systems.

A COMMERCIAL LAYER STUDY COMPARING VACCINATION PROGRAMS FOR THE PREVENTION OF SALMONELLA ENTERITIDIS

Jerry D. Maiers, DVM

Fort Dodge Animal Health, 9401 Indian Creek Parkway, Overland Park, KS 66210

INTRODUCTION

Vaccination of commercial layers to aid in control of *Salmonella enteritidis* (SE) has been an option for the layer industry for a number of years. The commercial egg producer in the United States now has both SE bacterins and modified live *Salmonella typhimurium* (ST) vaccines available with which to immunize leghorn pullets. Experimental studies have shown that both SE bacterin (4) and live ST vaccinated birds (6) have less fecal shedding, less colonization of the internal organs, and reduced egg contamination. This large commercial study compared three different commercial *Salmonella* vaccines (two SE bacterins and one live ST vaccine). Serum and egg yolk SE antibody levels were monitored throughout lay with the enzyme-linked immunosorbent assay (ELISA from IDEXX, Westbrook, ME) for the detection of antibodies to *Salmonella enteritidis*. The challenged birds consisted of 25 hens from the various vaccination groups, including non-vaccinated controls. The hens were removed from the lay houses at 63 weeks of age, taken to a laboratory and orally challenged with a phage type 13a SE inoculum. Bacteriological sampling of ceca, intestines and internal organs for the presence of SE was conducted.

MATERIALS AND METHODS

Study design. A large U.S. commercial layer company cooperated in this study. The company uses an in-house vaccination crew for all of their vaccine administrations. Eighty commercial leghorn pullets were assigned to each vaccination group in a tiered cage pullet house. Cages were assigned randomly throughout the house for the respective vaccination groups. The pullet farms were confirmed to be SE negative on environmental testing prior to vaccination of the pullets. The pullets were moved to SE negative lay houses in several geographic regions.

Vaccines evaluated. Two commercial oil emulsion SE bacterins and one live gene-deleted ST vaccine were evaluated. Bacterin A was administered once intramuscular in the leg and Bacterin B was given once subcutaneous in the inguinal fold. Both bacterins were administered at 13 weeks of age. The live ST vaccine was administered by coarse spray at 2, 4, and 14 weeks of age.

Serology. Twelve blood serum samples were taken from each of the vaccinated groups at various times throughout lay to assay their serum SE antibody levels. The bacterin vaccinated groups were sampled at 12 weeks (pre-vac sample), and at 16, 22, 28, 34, 40, 52, and 62 weeks of age. The live ST vaccinated group was sampled at 13 weeks (prior to the 14 wk vac), then at 16, 22, 28, 34, 52, and 70 weeks of age.

Egg Yolk Antibody Assay. Yolk was assayed from 12 eggs per treatment group at 40 and 62 weeks of age. The yolk was diluted 1:2 upon harvesting to prevent gelation during extended low-temperature storage. Samples were then sent to IDEXX Laboratories for the FlockChek SE assay. The ELISA test is a g.m. flagellin-based test utilizing the microtiter wells coated with purified SE antigen. The yolk sample was diluted 1:10 with sample diluent and mixed well before pipetting into the microtiter wells.

Challenge study. At 63 weeks of age 25 hens from each of the three vaccinated groups along with 25 non-vaccinated hens (controls) were transported to the Fort Dodge Animal Health laboratory at Fort Dodge, IA. The four groups of commercial hens along with another control group of 25 SPF leghorns were challenged by oral gavage with a 1.32×10^7 cfu per dose of a nalidixic acid resistant strain of SE phage type 13a (FDAH 1105-03). All birds were observed for 7 days post-vaccination for any clinical signs of disease. At the end of the 7-day period all surviving chickens were necropsied and examined grossly for the presence of visible lesions.

Bacteriological examination of tissue samples. Approximately one gram each of the ovary, oviduct (infundibulum, magnum, isthmus and uterus), spleen kidney, and liver were aseptically obtained from each bird. Tissues obtained from each bird were pooled (ovary and oviduct in pool 1; spleen, kidney and liver in pool 2). Pools were placed in sterile whirl pak bags and then transferred to the lab. Ten milliliters (10 mL) of BGTB (Brilliant Green Tetrathionate) Broth) was added to each bag and the contents were macerated in a Stomacher blender for a period of 30 seconds. The bags were incubated for 24 hours at 37^0 C.

A 10 mm sample of the duodenum (bottom of the duodenal loop below the pancreas), jejunum (region of the yolk sac diverticulum), and the ileum (anterior to the ileocecal junction) was aseptically collected from each bird, flushed internally and externally with sterile PBS (pH 7.2), pooled (pool 3), and processed similarly. The bags were incubated for 24 hours at 42^0 C.

After 24 hours of incubation, a loopful from each whirl pak bag culture was streaked onto brilliant green agar containing a 100ug/mL nalidixic (BGAN) and onto xylose-lysine tergitol 4 agar (XLT4). Plates were examined after 24 hours of incubation at 37^0 C. If SE grew on either of the plate media, the pool was considered positive. If plates were negative on the initial culture, plates were restreaked from the whirl pak bag culture (48 hrs. incubation) and incubated at 37^0 C.

Cecal contents were collected from each bird (pool 4) and placed in a whirl pak bag. The net weight of the contents of each bag was determined by weighing on a tare-adjusted balance. Sterile PBS (pH 7.2) was added to each bag in the ratio of 100 ml PBS per gram of cecal contents. The contents were plated on duplicate plates of BGAN. The plates were incubated for 24 hours at 37^0 C. Growth of SE on either of the plate media was considered positive.

An agglutination test with group D *Salmonella* antiserum was performed on at least one colony per plate from all positive plates to confirm the presence of a group D Salmonella species.

RESULTS

Serum antibody assay. The live ST group which had been vaccinated by coarse spray at 2 and 4 weeks were serum antibody negative at 13 weeks prior to their 3rd spray vaccination at 14 weeks (Table1). This group was 91% positive at 16 weeks, 2 weeks after their 3rd vaccination with live ST. Antibody titers continued to drop from this point in time throughout lay in the live ST vaccinated birds. At 34 weeks (peak production) 50% of the ST vaccinated birds were SE positive and at 70 weeks only 17% of the birds were SE positive. The Bacterin A and Bacterin B groups were 100 and 91% positive respectively at 16 weeks (3 weeks post vaccination) and remained 80 to 90% SE positive through 63 weeks of age.

Table 1. 12 birds from each group were assayed for serum antibodies at various times throughout lay.

Age Wks	13	16	22	28	34	40	52	62	70
Bact A	0	100	100	92	90	82	92	83	NT
Bact B	0	91	92	83	83	90	92	100	NT
Live ST	0	91	83	58	50	NT	21	NT	17

% Positives on SE ELISA Test

Detection of Egg Yolk SE antibodies. Egg yolk was tested at 42 and 62 weeks of age from each vaccination group as well as the non-vaccinated controls (Table 2). Both bacterin vaccinated groups were 80 to 90% ELISA positive at 42 and 62 weeks of age while the live ST group were 27% and 53% ELISA positive at the same respective ages.

Table 2. Yolk from 12 eggs in each group was assayed twice during lay.

Age	42 wks	62 wks
Bacterin A	80	87
Bacterin B	80	93
Live ST Vac	27	53
Non-Vac Controls	0	0

% Positives on SE ELISA Test

Ref. Table 3.

Recovery of SE post challenge. SE isolation rates from the ceca, intestines, organs, and reproductive tracts from five groups of birds were analyzed. Birds vaccinated with either Bacterin A or Bacterin B had significantly lower isolation rates from the intestines than the Live ST product, significantly lower isolation rates from the intestines and organs than the field controls, and significantly lower isolation rates from the ceca, intestines, and organs than the SPF controls. There was no significant difference between Bacterin A or Bacterin B across all comparisons. There was no significant difference in isolation rates from the reproductive tract across all five groups. A description of the complete analysis follows.

Recovery of SE from ceca. The isolation rates from the ceca were significantly lower in the bacterin vaccinates compared to the SPF controls (RR 0.04, 95%CI 0.01, 0.30). There was a borderline non-significant difference in the SE isolation rates between the bacterin vaccinates and the Live ST vaccinates (RR 0.14, 95%CI 0.02, 1.08). There was no significant difference in the rate of SE isolation between the two bacterin vaccinates or the bacterin vaccinates and the field controls.

Recovery of SE from intestines. The isolation rates from the intestines were significantly lower in the bacterin vaccinates compared to the Live ST vaccinates (RR 0.33, 95%CI 0.12, 0.89), the field controls (RR 0.26, 95%CI 0.10, 0.67), and the SPF controls (RR 0.17, 0.07, 0.43). There was no significant difference in the rate of SE isolation between the two bacterin-vaccinated groups.

Recovery of SE from organs. The isolation rates from the organs were significantly lower in the bacterin vaccinates compared to either the field controls (RR 0.28, 95%CI 0.11, 0.72) or the SPF controls (RR 0.18, 95%CI 0.07, 0.45). There was a borderline non-significant difference in the SE isolation rates between the bacterin vaccinates and the Live ST vaccinates (RR 0.40, 95%CI 0.14, 1.11). There was no significant difference in the rate of SE isolation between either bacterin vaccinated groups.

Recovery of SE from the reproductive tract. There was no difference among any of the groups in the rate of SE isolation from the reproductive tract.

Table 3. Birds were orally challenged with pathogenic SE at 63 weeks of age.

		% SE Culture Positive Birds			
Group No.	Treatment	Ceca	Intest Pool	Organ Pool	Reprod Pool
1	Bacterin A	1/25 (4%) a	4/25 (16%) a	4/25 (16%) a	0/25 (0%) a
2	Bacterin B	1/25 (4%) a	3/25 (12%) a	2/25 (8%) a	1/25 (4%) a
3	Live ST Vac	7/25 (28%) a	12/25 (48%) b	10/25 (40%) a	2/25 (8%) a
4	Field Control	3/26 (12%) a	16/26 (62%) b	15/26 (58%) b	3/26 (12%) a
5	SPF Controls	23/25 (92%) b	23/25 (92%) b	22/25 (88%) b	3/25 (12%) a

DISCUSSION

The bacterin vaccinated birds elicited a strong and long lasting humoral antibody response, maintaining 80-90% positive on the ELISA through 62 weeks. The antibody response to the live ST vaccine was much weaker and titers depleted much more rapidly. Since immunity against *Salmonella* infection also includes a cell-mediated or mucosal component, measurement of circulating antibodies alone can be an uncertain value as indicators of overall protection. Therefore as an important part of this study it was necessary to evaluate

protection of each vaccinated group in the face of an SE challenge in late lay and compare that protection to both non-vaccinated field controls (same house) and SPF controls.

Egg yolks are an alternative source of antibodies that can be measured by methods similar to those applied in serum (2,3). In addition, obtaining egg yolk samples requires less labor and eliminates stress on the birds. Sampling egg yolks could be another way to either monitor proper vaccine administration or in non-vaccinated flocks, to detect field exposure to wild strains of SE. In this study, the ELISA test was used to measure the level and duration of specific SE antibodies in the yolk in response to vaccination. Since the ELISA is a g.m. flagellin based test, other *Salmonella* serotypes that share the epitopes of g.m. flagellin can potentially yield positive results. To assure that the non-vaccinated birds did not have elevated anti-SE antibodies, eggs were sampled from the controls at 42 and 62 weeks of age. Egg yolk antibody levels in this study were found to closely mirror serum antibody levels throughout lay in both the live ST and killed SE groups.

Holt and co-workers have shown in the laboratory the inhibitory effect of SE growth in egg yolk from hens that were vaccinated with a SE bacterin (6). The inhibitory effect of SE antibodies was found to be specific for the SE organism and could potentially serve as a protection to the consumer should eggs be mishandled before consumption. The effect of vaccination on growth inhibition of SE in egg yolk was not included in this study.

As the commercial layer goes beyond peak production and into late lay it becomes more difficult to infect her with *Salmonella enteritidis*. This resistance could be a result of exposure to various non-pathogenic *Salmonella* during lay and the production of cross-reactive antibodies that may neutralize SE to some degree. Therefore, the commercial layer in late lay becomes a poor model for a challenge study. To demonstrate differences in protection between the controls and the vaccinated birds an unusually high challenge of highly pathogenic SE at a dose of 1.32 x log 10^7 cfu was given by oral gavage.

The live ST vaccinated group did not protect against challenge as well as the bacterin vaccinated birds but this extremely high artificial challenge is unlike that which occurs in a commercial lay house. One would expect the live ST vaccinated birds to protect better in a lower challenge setting. It is important to note that the SE bacterin birds protected against intestinal shed significantly better than the live ST vaccinated group in the face of severe challenge. The bacterins similarly protected better against SE recovery from the ceca and organs except for a borderline non-significant difference between the bacterins and the live ST group. One could presume that less fecal shedding and contamination of the environment would occur in the bacterin vaccinated birds in the face of challenge. Davison and co-workers showed that SE positive farms could be returned to SE negative status by vaccination of flocks with a SE bacterin (1). Recovery from the reproductive tract was low in all groups (0-12%) whether the birds were vaccinated or not. This demonstrates why recovery from SE in the eggs as a means of monitoring SE status in a flock is not a very reliable method.

Vaccination of flocks as a preventative method in the control of *Salmonella enteritidis* in commercial layers has shown to be a sound approach. A vaccination program will only be successful if all of the other intervention measures are in place; including biosecurity, clean chick source, clean feed and water, proper dead bird disposal, and good rodent and insect control. The convenience and ease of administration of live vaccines by mass application through spray or drinking water is appealing as compared to the injection of SE bacterins. It would appear from this study that to get similar protection from live vaccines as compared to bacterins, one would be required to give multiple booster vaccinations throughout lay to maintain the same level of protection. Some studies have shown that administering live ST vaccine early in the bird's life to prime the immune system followed by the SE bacterin prior to lay could be a valid approach. (7). The live/killed vaccination program was not evaluated in this study. More responsibility will certainly be put on the producer of meat and egg products to assure a safe and pathogen-free product to the consumer. Vaccination programs to prevent SE will become an integral part of the S*almonella* prevention management on commercial layer farms.

REFERENCES

1. Davison, S., Benson, C.E., Henzler, D.J., and Eckroade, R.J. Field observations with Salmonella entritidis
bacterins. Avian Diseases. 43:664-669, 1999.

2. Gast, R.K., and Beard, C.W. Detection of Salmonella serogroup D-specific antibodies in the yolks of eggs laid by hens infected with Salmonella enteritidis. Poul. Sci. 70:1273-1276. 1993

3. Gast, R.K., Porter, R.E., Jr., and Holt, P.S. Applyng tests for specific antibodies to predict contamination by Salmonella enteritidis in eggs from experimentally infected laying hens. Avian Diseases 41:195-202, 1997.

4. Gast, R.K., Stone, H.D., and Holt, P.S. Evaluation of the efficacy of oil-emulsion bacterins for reducing shedding of Salmonella enteritidis by laying hens. Avian Diseases 37:1085-1091, 1993.

5. Hassan, J.O. and Curtis, R. III. Efficacy of live avirulent salmonella typhimurium vaccine in preventing colonization and invasion of laying hens by Salmonella typhimurium and Salmonella enteritidis. Avian Diseases 41:783-791, 1997.

6. Holt, P.S., Stone, H.D., Gast, R.K., and Porter, R.E. Jr. Growth of Salmonella enteritidis (SE) in egg contents from hens vaccinated with SE bacterin. Food Microbiology. Vol. 13, pp 417-426. 1996.

7. Zacek, D. Vaccination to prevent Salmonella enteritidis in table eggs. Egg Industry. Pp 11-12. July 2000.

ECONOMIC IMPACT OF THE SALMONELLA ENTERITIDIS CONTROL PROGRAM TO THE EGG INDUSTRY IN CALIFORNIA

H. Kinde[A], D. H.Read[A], A. Ardans[B], R. Breitmeyer[C], D. Bell[D], D. Kuney[D], G. Cutler[E]

[A]California Animal Health and Food Safety Laboratory San Bernardino
[B]Davis, School of Veterinary Medicine, University of California, Davis
[C]California Department of Food and Agriculture, Sacramento
[D]University of California Cooperative Extension, Riverside
[E]8450 Happy Camp Rd., Moorpark CA 93020

Salmonella enteritidis is not considered to be a significant cause of morbidity or mortality in commercial layer flocks. The major threat to the producer is that the farm will be implicated in a trace back investigation following a human illness due to *S. enteritidis* and the associated cost in fulfilling the regulatory compliance. For the industry as a whole there is the negative publicity from the public perception that eggs are linked to human salmonellosis. This risk associated public perception could translate in to a financial loss for the egg industry. Alternatively, if the producer chooses to implement a *S. enteritidis* control program, there may be a reduced risk of human salmonellosis, improved consumer confidence and the industry will benefit from sustained product demand and financial gain. However, implementing a *S. enteritidis* control program in a commercial layer flock is not simple and it could certainly increase production costs. There is no previous documentation dealing with the economic impact of the *S. enteritidis* control program. The purpose of this paper is to present two cost estimates: 1) a *S. enteritidis* control program for the California Egg Industry and 2) the loss of income incurred by a producer in the absence of a *S. enteritidis* monitoring program based on a retrospective study.

COST ESTIMATES FOR CONTROL PROGRAM

The limitations in estimating costs for a *S. enteritidis* control program stem from the many variations in flock sizes, age, type of housing and equipment, environmental conditions, and management practices. However, the great majority of California producers have joined the California Egg Quality Assurance Plan (CEQAP) and subscribe to common basic core program components. Therefore in estimating costs, components such as cleaning and disinfection, rodent control and testing for *S. enteritidis* etc. are required by the plan and assumptions are made that they are practiced by all participants. It is recognized that these farm practices existed long before the *S. enteritidis* problem but producers became more vigilant following the implementation of CEQAP. Cost estimates for rodent control and cleaning and disinfection were based on 2 companies' expenditures: a large company with a capacity of 1,776,900 birds, and a mid size company with a capacity of 63,000 birds.

Assumptions. There are 32 companies, 100 farms, 1000 houses with 23 million laying hens in California with an average of 23,000 birds per house. Eighty five percent of the companies hire veterinarians; and 25% of the annual veterinary services, 50% of the rodent control and 25% of the cleaning and disinfection efforts are estimated for a *S. enteritidis* control (monitoring) program. Eight percent of the laying hens are vaccinated.

The cost of not monitoring for *S. enteritidis*. When shell eggs are implicated in human salmonellosis, a trace back investigation is initiated by regulatory agencies. The cost of compliance with the regulatory protocol is very expensive because eggs are diverted to pasteurization or the infected flock is depopulated. Unlike some of the zoonotic diseases such as brucellosis and tuberculosis in cattle, there are no indemnities paid if the producer chooses to depopulate the infected flock. If the producer chooses to divert eggs for pasteurization the shell egg premium

will be lost (by as much as 50%). Indirect income losses to the producer include lack of replacement pullets for example, if replacement pullets are not planned several weeks in advance the producer may incur cost of idle capital; other indirect costs may include the cost of purchasing eggs in the open market to fulfill existing contract obligations. Other costs include laboratory testing, liability claims, increased insurance premiums, decreased consumer confidence, etc.

INCOME LOSS ASSOCIATED WITH S. ENTERITIDIS PHAGE TYPE 4 OUTBREAK IN COMMERCIAL LAYER CHICKENS (19 MONTHS FOLLOW UP) – A RETROSPECTIVE STUDY

In May of 1994 *S. enteritidis* phage type 4 (Se PT4) was isolated from five of six 27-week old layer chickens submitted for necropsy from a flock of 43,000. Bacteriologic and epidemiological investigations on the farm revealed that 5 of the eight flocks (n=176,000) were infected. The prevalence of Se PT4 in randomly selected healthy birds ranged from 1.7% (cage birds) to 50% (free range birds) and the prevalence in culled birds (kept in dirt floor houses) ranged from 14 to 42%. The estimated overall prevalence of group D *Salmonella* was 2.28 per 10,000. The estimated prevalence of group D *Salmonella* in eggs from caged birds in three infected houses ranged from 1.5 to 4.1 per 10, 000, whereas in 2 houses of free range birds, prevalence was 14.9 to 19.1 per 10,000. Three of the 8 flocks on the farm remained negative for *Salmonella* throughout the observation period (May 1994 to December 1995). The producer voluntarily diverted eggs for pasteurization and there was no human illness associated with this outbreak and no trace back investigation was initiated.

Estimated loss of income associated with Se PT4. The different age layer flocks were kept in 8 different houses and produced regular or specialty type eggs. For estimating loss, one price was chosen for all the eggs (large price +50 Cents) produced on the farm. At the start of the outbreak egg production was about 92% and a mortality of 0.2% per week was estimated thereafter until the flock was removed or marketed. Three flocks were never infected during the observation period (19 months). The other flocks became positive intermittently and eggs were treated according to protocol. This entailed testing 1000 eggs from a positive flock four times (every 2 weeks). During this testing period, eggs were diverted to pasteurization. If eggs became negative for four consecutive times, the producer was allowed to sell shell eggs and the flock was monitored for the rest of the production life by sampling 480 eggs every 3 months. If at any time eggs became positive, diversion to pasteurization would resume and eggs would be tested every 2 weeks (1000 eggs per house). The net loss from each flock was calculated by subtracting the sum of the sampling cost and the income from pasteurized eggs from the original value. Diverted eggs were estimated to be 50% of the shell egg price (Table 1). At the start of the outbreak the producer opted to hire a veterinarian and additional cost was incurred.

Annual Se monitoring cost estimates for the state of California Egg Industry

Cost of *S. enteritidis* testing 16 swabs per house (4 pools), 10 houses per farm $45/per pool, for 1000 houses = $45 X 4 X 1000	$180,000
Cleaning and Disinfection $1927 per house (25% estimated for the Se program) = $1927 X 1000 (25%) = $481,750 (18 months) For 12 months = 67% ($481,750)	$322,772
Rodent Control $1327 per Farm (50% estimated for the Se program) = $1327 X 100 (50%)	$66,350
Vaccination for *S.enteritidis* 14 Cents per bird, 8% of 230,000,000 birds $0.14 X 1,840,000	$257,600
Consultation/Professional fee, $12,000/year/company (25% est., for SE) 32 companies, 85% hire veterinarians = 27 $3000 X 27	$81,000
Total	$907,722

Table 1. Estimated Loss of Income (US $) Associated with Se PT4 Infection of Laying Flocks

Flock #	Original Value of Eggs	Cost of eggs used for testing	Net Value of eggs	Income from Eggs	Net Loss
1	1,493,957	1,253	1,492,704	156,250	1,337,706
2	761,830	844	760,986	94,344	667,486
3 Not infected	387,147	407	386,740	386,740	407
4 Not infected	246,842	329	246,513	246,513	329
5 Not infected	1,200,175	329	1,199,846	1,199,846	329
6	305,779	1,274	304,505	32,212	273,567
7	76,061	960	75,101	12,026	64,034
8	63,953	1,148	62,805	10,400	53,553
Total	**4,535,744**	**6,544**	**4,529,200**	**2,138,331**	**2,397,413**

Total Net Loss= Original value-income from diversion=(4,535,744-2,138,331)=2,397413

Other Costs:

Flock Vaccination Cost (SE)	$ 2,195
Professional fee	$ 4,500
Grand Total	**$2,404,108**

Laboratory costs related to the outbreak:

Collection of Samples (time)	
31 trips, 3hrs each trip, $100/hr = $300 X 31	$9,300
Cost of necropsy and *Salmonella* culture of laying hens:	
655 birds @ $30 per bird	$19,650
@ 5% positive for group D *Salmonella*	
33 isolates serotyped ($12 each) and phage typed ($11 each) = 33 X $23 =	$759
Cost of environmental testing:	
Drag swabs 180@ $45 each	$8,100
13% (23 isolates) serotyped at $12 each	$276
Rodent and other feral animal sample culture 58@ $30 each	$1,740
Group D *Salmonella* was serotyped ($12 each) and phage typed ($11 each)	
12 isolates @ $23 = 12 X 23	$276
Feed Samples 7 @ $30 each	$240
One isolate of *Salmonella* serotyped @ $12 each	$12
Tank Water samples 8 @ $30 each	$240
Cost of serotyping 6 isolates ($12 each)	$72
Cost of testing eggs	
85,360 eggs, pool of 20 eggs	
4268 pools @ $17 per pool	
4268 X $17 =	$72,556
Cost of serotyping and phage typing = $23 each	
58 group D *Salmonella* isolates = $23 X 58	$1,334
Cost of testing Moore swabs (creek water) for *Salmonella*	
40 swabs @ $30 each =	$1,200
68 *Salmonella* isolates were serotyped 68 @ $12 each	$816
Total 4 *Salmonella enteritidis* were phage typed 4 @ $11 each	$44
Total	$116,585
Grand Total	**$2,520,693**

CONCLUSION

The *S. enteritidis* monitoring cost estimates outlined in this study would pale in to insignificance should the egg industry experience negative consumer confidence like what occurred in Europe in the 1980's. The 1994 Se PT4 outbreak in California caused significant financial loss to a producer and resulted in an estimated additional $5 million loss to the industry due to a short-term trade embargo. It has also alerted the California Egg Industry to reinforce the importance of the Se monitoring program. It is imperative for the producer to weigh all the decision-making factors whether to divert eggs or not. In this study the producer took a great risk and allowed the sampling process to continue in the hope that eggs would be negative. The loss would have been greatly reduced should he discontinued sampling of eggs and decided to depopulate the flocks.

RESULTS OF A ONE YEAR STUDY OF SALMONELLA LEVELS IN BROILER HOUSES AS DETECTED BY DRAG SWABS

John Schleifer[A], W. D. Waltman[B]

[A]Intervet Inc., P.O. Box 205, Gillsville, GA 30543
[B]Georgia Poultry Laboratory, P.O. Box 20, Oakwood, GA 30566

INTRODUCTION

The US poultry industry continues to have a heightened awareness of the importance of reducing the level of *Salmonella* contamination of broiler carcasses. It is well established that a significant step in reducing the level of *Salmonella* contaminated carcasses, is to reduce the contamination of broilers arriving at the processing plant. Numerous studies support this hypothesis and have been reviewed (1).

One area of focus has been the development of a non-invasive monitoring technique in order to best determine possible levels of broiler flock infection or colonization. Most particularly, studies have shown that there is a relationship with environmental contamination and bird colonization (3,5). Currently many techniques are under investigation. However, the technique most often used to monitor *Salmonella* presence in a poultry house environment is the drag swab technique. The drag swab technique was first introduced by Kingston in 1981 (2). This technique was further endorsed by the NPIP and protocols have been established (4). Waltman showed in 1998 that the drag swab technique was one of the most sensitive indicators of the presence of *Salmonella* in a broiler type environment (7).

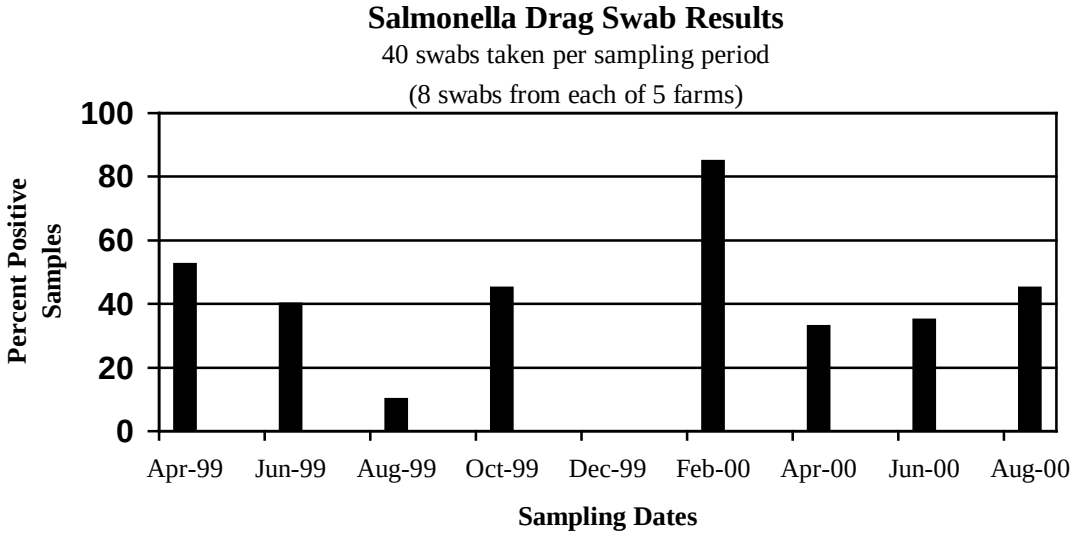
The current method for the detection of *Salmonella* on broiler carcasses as specified by the USDA-FSIS inspection code is the carcass rinse method. Conventional discussions within the industry state that *Salmonella* contamination levels are dependent on several things. One of those items is the season of the year. To date, there is no referencable research that indicates that there is a correlation between drag swab contamination levels and the carcass contamination level in birds from this environment. The purpose of this study was to monitor the level of *Salmonella* contamination of commercial broiler farms, as detected by the drag swab method over a 12-month period of time. Possible influences and correlation of the environmental contamination level is hoped to shed some light on what might influence carcass contamination levels.

STUDY DESIGN

An integrated operation in the Southeastern US was identified as the trial location. This operation processes 1 million broilers per week. Sampling occurred on either the first or second Wednesday of each month starting on April 7, 1999 and ending August 9, 2000. Five farms were selected for testing at each sampling time. The bird age range was between 34 to 37 days of age at the time of sampling. The grower profiles for the farms was to include two of the better producing farms, two average producing farms and two underperforming farms. Two houses per farm were sampled. All farms and houses were selected by the company personnel, and most generally, the farms differed with each sampling time. The December, 1999 sampling was missed because of weather irregularities.

The litter drag swabs were prepared by Dr. W.D. Waltman, Georgia Poultry Laboratory and stored frozen in double strength skim milk transport media. The sampling of broiler houses were conducted according to NPIP protocol (4) with four swabs obtained for each identified house. With the completion of the sampling, all swabs were shipped via next day courier to Dr. W.D. Waltman. Isolation and

identification and confirmation of *Salmonella* spp. were performed by the Georgia Poultry Laboratory according to previously described sampling techniques (6).



Serogrouping of the *Salmonella* positive samples revealed that serogroup B *Salmonella* was identified on 40 of the 64 farms sampled. Serogroup C1, C2 and E were identified on 3, 6 and 1 of the farms sampled, respectively. Of the farms identified as being contaminated with serogroup C1 all were also contaminated with serogroup B. Of the farms contaminated with C2 all but 2 farms were also contaminated with serogroup B. One of the farms was only contaminated with serogroup C2 and one farm was contaminated with a combination of serogroup C2 and E. None of the 64 farms sampled during the 17 month study were contaminated with more than two serogroups of *Salmonella* and none were contaminated with a combination of serogroups C1 and C2.

DISCUSSION

Based on the *Salmonella* drag swab results it appears that indeed there may be some seasonal variation in *Salmonella* contamination in the environment of broiler houses. The highest recovery rates occurred in the winter months, February and April, with the lowest recoveries during the summer months. It is unclear how this data correlates with carcass wash data at this operation. Live production and processing plant quality assurance personnel stated that the drag swab results graph resembled the variations they see in the carcass wash data.

The significance of the serogrouping results is unclear. It is the hope of this author that this data may further the discussion of what significance serogroup isolation results may have on carcass wash data, as well as advance discussions on appropriate methods in monitoring broiler house environments and their correlation on carcass wash data.

REFERENCES

1. Hargis, B.M., D.J. Caldwell, J.A. Byrd, D.E. Corrier, and L. H. Stanker. Preharvest crop contamination and salmonella recovery from broiler carcasses at processing. International Symposium on Food-Borne Salmonella in Poultry. Baltimore, MD. July 1998.

2. Kingston, D.J. Research note: A comparison of culturing drag swabs and litter for identification of infections with Salmonella spp. in commercial chicken flocks. Avian Dis 25:513-516. 1981.

3. Mutalib, A., P. McDonough, S. Shin, V. Patten and D. Lein. Salmonella enteritidis in commercial layer farms in New York state; environmental survey results and significance of available monitoring tests. J Vet Diagn Invest 4:416-418. 1992.

4. National Poultry Improvement Plan and Auxiliary Provisions. USDA, Animal and Plant Health Inspection Service, Veterinary Services, Publication APHIS 91-55-021. U.S. Government Printing Office. June 1994.

5. Poppe, C., R.P. Johnson, C.M. Forsberg and R.J. Irwin. Salmonella enteritidis and other Salmonella in laying hens and eggs from flocks with Salmonella in their environment. Can J Vet Res 56:226-232. 1992.

6. Waltman, W.D., A.M. Horne, C. Pirkle, and T. G. Dickson. Use of delayed secondary enrichment for

the isolation of salmonella in poultry and poultry environments. Avian Dis 35:88-92. 1991.

7. Waltman, W.D. Isolation of Salmonella from poultry environments. International Symposium on Food Borne Salmonella in Poultry. Baltimore, MD. July 1998.

CELLULITIS IN LEGS AND CAUDAL THORACIC AREA OF MARKET WEIGHT TURKEYS, IN WESTERN CANADA: HISTOPATHOLOGICAL AND BACTERIOLOGICAL EVALUATIONS

Gomis, S.[A], Amoako, K.[A], Ngeleka, M.[B], Belanger, L[C], Althouse, B.[C], Kumor, L.[D], Waters, E.[B], Stephens, S.[E], Riddell, C.[E], Potter, A.[A], and Allan, B.[A]

[A]Veterinary Infectious Disease Organization, 120 Veterinary Road, Saskatoon, SK. Canada, S7N 5E3
[B] Prairie Diagnostic Services, 52, Campus Dr. Saskatoon, SK., Canada, S7N 5B4
[C]Canadian Food Inspection Agency, Wynyard Veterinary District Office, Box 1719, Wynyard, SK., Canada, S0A 4T0
[D]Canadian Food Inspection Agency, Food Production and Inspection Branch, 2162, Airport Dr., Saskatoon, SK. Canada, S7L 6M6
[E] Department of Veterinary Pathology, WCVM, University of Saskatchewan, 52, Campus Drive, Saskatoon, SK. Canada, S7N 5E3

The objective of this study was to identify the causative agent of cellulitis in turkeys. Eighteen flocks from 9 producers were sampled at the local processing plant and 37 birds with cellulitis on legs or caudal thoracic area were obtained. None of the 37 birds with cellulitis had lesions in other organs. On gross examination, lesions were categorized into two groups (a) cellulitis with open skin lesions and (b) cellulitis with unopened skin lesions. Histopathologically, cellulitis with open skin lesions had chronic granulomatous/granulation tissue type reaction associated with foreign materials but cellulitis with unopened skin lesions had dermal necrosis with underlying fibrin and inflammatory exudate. A complete bacteriological study was conducted on 25 of 37 birds. Bacteria were isolated from 12 of the 25 birds with cellulitis lesions. No aerobic, microaerophilic or anaerobic bacteria were isolated from the remaining 13 birds with cellulitis lesions. *E. coli* was the organism isolated frequently from the lesions in low numbers from 9 of 12 but in mix cultures with *Proteus mirabilis, Lactobacillus* spp, *Klebsiella, Staphylococcus* spp. The remaining few cases yielded *P. mirabilis* in pure cultures or mixed with *Pseudomonas aeruginosa*. These types of cellulitis lesions [(a) and (b)] in turkeys could be associated with primary skin abrasions and contact dermatitis, respectively. Their occurrence is likely associated with different management practices.

UTILITY OF EPIDEMIOLOGIC RISK ASSESSMENT APPROACH IN POULTRY DISEASES: A CASE STUDY, RISK OF CAMPYLOBACTER JEJUNI IN LIVE POULTRY MARKETS

Hussni O. Mohammed

Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

SUMMARY

In the recent history there has been global interest in quantitative risk assessment (QRA) approach as evidenced by the number of publications on this topic. The interest is driven by the public concern regarding the safety of the food they consume and environment in which they live. Epidemiologic approaches to QRA combine deterministic and stochastic mathematical modelling. As such, epidemiologic risk assessment has

a great potential for utility in poultry diseases. We demonstrate its utility in assessing the potential risk of *Campylobacter jejuni* infection associated with the consumption of meat from live-poultry markets. Scenario pathway and event tree approaches were used to model the sequences of events which take place at the live-poultry market and determine the consequent hazard of contamination of carcasses. The probabilities in the events were derived as function of events whose events were not known. Monte Carlo sensitivity analysis was used to assess the risk associated with the *C. jejuni*. Initial bacterial load and the sanitary level of the defeathering machine had significant impact on the hazard of carcass contamination. Knowledge gained through epidemiologic risk assessment can be used in the development of cost-effective hazard analysis of critical control points.

INTRODUCTION

Risk assessment is one of the three complementary components of risk analysis (Molak 1997). The other two components are risk communication and risk management. Several national and international organizations have developed their own documentation on how to carry out risk analysis (Codex Alimentarius 1997; ICMSF 1996). All these documentations agree on the general principles of the risk analysis and admitted the complexity of the issue. They agreed on three components but they disagree on how to perform each and to integrate these components in a complementary way. The difference between the proposed approaches in the risk analysis and risk assessment among different disciplines stems from the confusion in the taxonomy. The term risk has been defined differently by different scientists in different disciplines and sometimes within the same discipline. Furthermore, instances of application of the traditional approaches in risk analysis can be criticized for having several pitfalls including ignoring variability, uncertainty, and science.

On the other hand, the discipline of epidemiology has been poorly understood by a variety of scientists. Epidemiology is the study of determinants of health in a population. The epidemiologic approaches have always emphasized understanding, prediction, and evaluation (Kleinbaum et al., 1983). This process entails systematic data collection, coalition and analysis. Analyzing these helps the epidemiologist to predict the emergence of new situations, evaluate the factors that influence the situation (either worsen it or improve it), design strategies to control and manage the situation, and continuously evaluate the efficacy of these interventions and revise them as more knowledge becomes available. As such, the epidemiological approach accounts for the variability and addresses the uncertainty in the occurrence of the risk. Epidemiologic approaches in a sense encompass two major components of risk analysis: risk assessment and risk management. Traditionally, the epidemiologic approaches have not emphasised risk communication.

There are many similaraties between epidemiologic methodologies and risk analysis approaches. In this manuscript I will present my perspective of epidemiologic risk assessment and demonstrate its utility in the poultry industry. A case study of the risk of *Campylobacter jejuni* in live poultry market wll be used to illustrate the utility of the approach. In the the process I will demonstrate similarities and differences between the two approaches, highlights the points of confusion, and suggest where collaboration could be beneficial to both.

Covello and Merkhoffer (1993) defined risk as “a characteristic of a situation or action wherein two or more outcomes are possible, the particular outcome that will occur is unknown, and at least one of the possibilities is undesired”. In the epidemiologic context, risk is defined as “A probability that an event or phenomenon will ocurr” (Last 1983). Close examination of these two definitions highlight the conceptual similarity. Therefore, in principle, “risk” has been perceived similarly by risk assessors and epidemiologists.

Traditional risk assessment consist of four steps: hazard idenfication, dose-response assessment, exposure assessment, and risk characterization (Haas et al., 1999). The epidemiological approaches have always emphasized understanding, prediction, and evaluation in a population. These are three complementary approaches that were designed to address a scenario objectively and constructively. If the ultimate goal is to make an objective decision about a phenomenon, there should a better understanding of the factors that led to its introduction, transmission, and perpetuation. These factors that played a role in the manifestation of the problem in a population are agent, environment,and host dependent. The epidemiologic approach emphasizes collecting information on the factors, analyze the data, and make the correct inference regarding their role in manifesting the phenomenon. In doing so, epidemiologists have adopted systematic and standardized methodologies. These methods varied from observational studies (where there is no manipulation in the study factor, Kelsey et al., 1999), to experimental studies (where the study factor is manipulated, Rothman 1986) or a combination of both. The common theme among these studies is the validity and reliability of the estimates and inferences.

Validity of the study refers to the bias in the estimate(s) made in a study. Bias is a systematic error

that might have resulted from the way the study units are assembled (selection bias), information on exposure or other factors are collected (information bias), or the effect of other factors (confounder or effect modifier) is ignored. On the other hand, reliability of a study refers to the precision in the estimate made or the reproducibility of the study. The reliability of the study can be measured in terms of the variability around the estimated parameters. All epidemiologic studies, if done correctly, are expected to take into consideration the issues related to validity and reliability. These studies are expected to be free of bias and have precise estimators (Rothman 1986).

The epidemiologic approach being holistic in nature doesn't separate between the risk assessment and the risk management process. In the process of risk factor identification the epidemiologists feature elements of the risk management. For example, in studying the risk of *C. jejuni* that is associated with the consumption of contaminated meat, the epidemiologist report a risk model as follows:

P(C) = *f*(level of exposure (frequency of eating) ↑, storage temperature ↓ , shelf life ↓, immune status ↓)

where P(C) is the likelihood of *C. jejuni* and it is modeled as a function of the frequency of meat consumption which reflects the level of exposure, storage temperature of the processed meat, shelf life, and the immune status of the consumer. The epidemiologist concludes that the risk of *C. jejuni* increases with the amount of contaminated meat consumed, but decreases with the decrease in the storage temperature, the shelf life of the meat, and the immune status of the consumer. The latter two factors can be used in the risk management to control the risk of *C. jejuni* associated with poultry meat. These are the factors that modify or eliminate the risk. Combining elements of program evaluation (i.e., intervention evaluation, decisions analysis, and hazard analysis of critical control points) with risk identification, the epidemiologist would be able to communicate to the consumer, stakeholders, and decision makers an integrated picture of the risk and the alternative strategies to control this risk.

On the other hand, the main tool of the traditional risk assessment is the qualitative or quantitative description of the chain of events leading to the disease. The model inputs (ie, hazard identification, dose-response, and exposure assessment) are generally obtained from published studies and/or expert opinion and incorporated in a model as stochastic distribution. And hence, the risk characterization is presented using an approach as Monte Carlo simulation. In principle, infected (prevalence) with *C. jejuni* is estimated to be 0.45.

Infected birds pose a potential hazard to their immediate environment (killing platform) by shedding the organism in their feces (right arrow, P_1). Several factors would influence the survival of *the C. jejuni* in the killing platform. These include the contamination rate and the sanitary level. However, if the modeling approach is not only used for risk distribution it can also provide valuable information on the uncertainty associated with the inputs and control options to be suggested to the risk managers.

An example of epidemiologic risk assessment: *C. jejuni* in live-poultry market. To illustrate the utility of epidemiologic risk assesment in poultry industry, we carried out a study to evaluate the potential hazard associated with the presence of *C. jejuni* in poultry at live-poultry markets in New York State. The live-poultry market is one point in a complex process of production of safe food. Knowledge regarding the potential hazards at this point will help in devising hazard analysis of critical control point (HACCP) plan to mange the associate risk.

Campylobacter jejuni is known to be prevalent in chickens and turkeys (Acuff et al., 1987; Izat et al., 1988). The organism was detected in 67 (74%) of 91 chicken products purchased from 16 retail markets representing 11 franchises in the Minneapolis-St Paul metropolitan area (Smith et al., 1999). *C. jejuni* was isolated from surrounding environment, including sands, housefly, litters, drinking water containers, water, air, dogs and cats. Such a prevalence has the potential to pose risk to human health. It is estimated that the incidence of campylobacteriosis in human is 2%, mean duration of the disease of 10 days, and a case-fatality of 1% (Sobel et al., 1996). Thirty percent of the foodborne outbreaks in human during 1980-1982 were attributed to the consumption of contaminated poultry products (Finch and Blake, 1985). The majority of these outbreaks are sporadic in nature and attributed to mishandling or the consumption of undercooked poultry (Deming et al., 1987; Harris et al., 1986). A lesser source of exposure that has been incriminated in these outbreaks is the cross contamination of foods by raw poultry (Harris et al., 1986).

We carried out an epidemiologic risk assessment using a scenario pathway and tree event model – one of the quantitative risk analysis methodologies. The conceptual framework for the pathway model is presented in Figure 1. This scenario represents a chain of events that measured by conditional probabilities (P_i). The probability of occurrence of an event is conditional on the occurrence of the previous events. The initial hazard is at the bird level (event 1). The first event in this scenario is that the birds are infected with *C. jejuni* before they are brought to the live-bird market and they have a potential to shed the organism in the environment. If the birds are not infected then there is no risk of contamination to the environment, down arrow (shaded arrow). The probability that a bird is

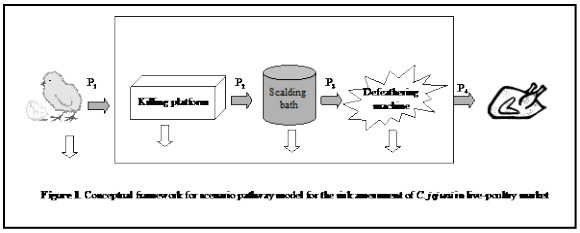


Figure 1. Conceptual framework for scenario pathway model for the risk assessment of *C. jejuni* in live-poultry market

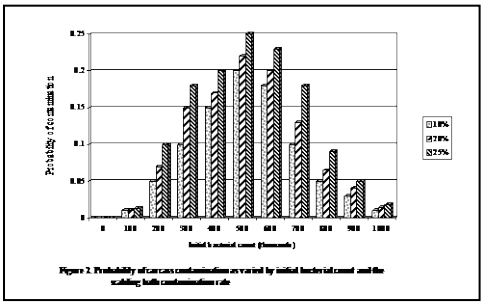
circumstances are not conducive for the survival or contamination, then the organism will die and the killing platform would not pose a risk (right arrow at event 2). This risk was assumed to follow the triangular distribution with the minimum of 0.0, most probable is 0.1, and a maximum of 0.3. If the organism survives on the killing platform it will be transmitted through fomites (utensils, handlers, and other structures in the habitat) and hence, contaminate the scalding bath (P_2). The probability distribution of the contamination at the scalding bath was assumed to follow the beta-distribution with the parameters (35, 210.5). If the organism survived the conditions at the scalding bath, the birds (carcasses) in the bath will be contaminated and have a potential to contaminate birds in the defeathering machine (P_3). The likelihood for this scenario to happen is rare, therefore, the probability distribution is assumed to follow the Poisson with the parameter (1.2). The final probability outcome (end-state), risk of presence of *C. jejuni*, on finished carcasses, was estimated using simulation models. Because of the uncertainty associated with some of the $P_{I's}$ several iterations were performed using the Monte Carlo simulations in @RISK software (Palisade Corporation, Newfield, NY).

RESULTS AND DISCUSSION

Our analysis showed that the hazard of the presence of *C. jejuni* in processed carcasses at the live-poultry market depends largely on the initial contamination rate of birds as they arrive in the market (Figure 2). The cumulative probability graph predicted, with 95% confidence, that the risk of carcass contamination is one in a thousand if the prevalence of *C. jejuni* in birds arriving at the market is below 1 %. Figure 2 shows in addition to the initial bacterial load on the bird, three different levels of contaminations at the scalding bath (10, 20, and 25%, respectively. As the initial bacterial load increases and the rate of contamination in the scalding bath increases, the likelihood of carcass contamination increases (Figure 2). The level of contamination of the carcass is maximum, under our assumptions, when the initial bacterial load is $5X10^5$ and the contamination rate at the scalding bath is at 25%. At these levels, the probability that the carcass would be contaminated with *C. jejuni* is 25%. It appears that there is a diminishing return effect for the initial bacterial load on the likelihood of carcass contamination as shown in Figure 2. The likelihood of carcass contamination starts to drop beyond an initial bacterial load of $5X10^5$. One plausible explanation for this observation is the constraint we built in the model by assuming that the contamination rate at the killing platform is triangular which has a minimum, most probable, and a maximum values. By definition of the distribution, the likelihood of a higher bacterial load will drop after the most probable number. Another plausible explanation would be the implementation of good sanitary practices at the killing platform which will have an impact on the bacterial count in the scalding bath.

To account for the uncertainty in the estimates of the parameters of the model at different levels of the processing plant and their impact on the total count of *C. jejuni* on the carcass, we ran the simulation using different parameters. Figure 3 shows the impact of the frequent changing of water in the scalding bath on the number of organisms on the carcass. Frequent changing of the water in the scalding bath is three times more likely to reduce the number of organisms on the carcass in comparison to not changing the water frequently.

This study demonstrated the application of epidemiologic risk assessment to an important part of the poultry study. One could think of many other situations where such an approach would be applicable, that strict risk mitigation efforts have e.g., a decision to implement vaccination strategies on a farm to control a potential risk. Although our approach is a simulation approach, which favors a worse case scenario, it was evident significant impact on the hazard of carcass contamination and would provide knowledge to be used in a HACCP program.



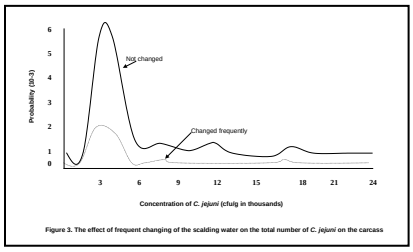


Figure 3. The effect of frequent changing of the scalding water on the total number of *C. jejuni* on the carcass

REFERENCES

1. Acuff GR, Vanderzant C, Hanna MO, et al., Prevalence of C. jejuni in turkey carcasses processing and further processing of turkey products. J of Food Protect 1987; 49:712-717.

2. Codex Alimentarius (1997) Proposed draft principles and guidelines for the conduct of microbiological risk assessment, Appendix, Report of the Thirtieth Session, Codex Committee on Food Hygiene, ALINORM 99/13, Codex Alimentarius Commission, FAO, Rome.

3. Deming MS, Tauxe RV, Blake PA, Dixon SE, Fowler BS, Jones TS, Lockamy EA, Patton CM, Sikes RO. Campylobacter enteritis at a university: transmission from eating chicken and from cats. Am J Epidemiol 1987; 126:526-34.

4. Finch M, Blake P. Foodborne outbreaks of campylobacteriosis. The United States experience 1990-1982. Am J of Epidemiol 19985; 122:262-268.

5. Graham, J.D. ed. (1995) *The role of epidemiology in regulatory risk assessment*. Elsevier, Amesterdam.

6. Gordis, L. ed. (1988) *Epidemiology and health Risk Assessment*. Oxford University Press, New York (New York, USA).

7. Harns NV, Weiss NS, Nolan CS. The role of poultry and meats in the etiology of Campylobacter jejuni/coli enteritis. Am J Pub Health 1986; 76:407-411.

8. Izat AL, Gardner FA, Denton JH, Golan FA. Incidence and level of Campylobacter jejuni in broiler processing. *Poult Sci* 1988 Nov; 67(11): 1568-72.

9. Haas CN, Rose JB, Gerba CP. Quantitative microbial risk assessment. John Wiley and Sons Inc., New York, 1999.

10. ICMSF (1996) *Micro-organisms in foods 5-Microbiological specifications of food pathogens*. International Commission on Microbiological Specifications for Foods of the International Union of Biological Societies. Black Academic & Professional: London (England, UK).

11. Kelsey JL, Thompson WD, Evans AS. Methods in observational epidemiology. Monograph in epidemioloy biostatistics. Oxford University Press, 1986.

12. Last, J.M. (1983) *A Dictionary of Epidemiology*. A Handbook Sponsored by the I.E.A. Oxford University Press: Oxford (England, UK).

13. Molak, V. (1997) Introduction and overview, in *Fundamentals of risk analysis and risk management* (V. Molak, ed.). CRC Lewis Publishers: Boca Raton (Florida, US), 1-12.

14. Rothman, K.J. (1996) *Modern epidemiology*. Little Brown and Company, Boston.

15. Vose, D. (1996). Quantitative risk analysis. *A guide to Monte Carlo simulation modelling*. John Wiley & Sons, Chichester (England, UK).

STRATEGIC ADMINISTRATION OF ENROFLOXACIN IN POULTRY TO ACHIEVE HIGHER SERUM CONCENTRATIONS

Héctor Sumano and Lilia Gutierrez.

Department of Physiology and Pharmacology, College of Veterinary Medicine,
National Autonomous University of Mexico. Mexico City 04510.

SUMMARY

In an attempt to achieve higher maximal serum concentrations (Cs_{max}) of enrofloxacin after oral administration, a two fold concentration of the drug (0.2%, total dose of 10 mg/kg) was administered to chicken. A set of control groups was dosed using a customary concentration (0.1%) of enrofloxacin. A quantitative/qualitative microbiological analytical method and a software program to obtain

pharmacokinetic variables revealed that time *vs* concentration relationships best fitted double hump shapes; therefore, Cs_{max1} and Cs_{max2} were obtained. Statistically significant (P> 0.01) increments were obtained when two-fold concentrated enrofloxacin oral solutions were administered. Cs_{max1} increments ranged from 175% to 338% and Cs_{max2} from 69% to 342%. Because optimal bactericidal concentration of enrofloxacin are twice the value of the minimal inhibitory concentration, doubling the concentration without increasing the dose can both reduce the rate of appearance of bacterial resistance and increase clinical efficacy without affecting the cost per treatment.

INTRODUCTON

Intensive and economically viable production of chicken meat often requires the administration of antibacterial drugs to treat specific outbreaks of bacterial diseases. In many countries, enrofloxacin has become the drug of choice for many of such cases. It has been stated that the antibacterial effects of enrofloxacin, at the clinical level, are more dependent upon a greater peak in serum concentration than upon an extended residence time (6,7). It is accepted for most drugs, including enrofloxacin, that bolus-like administration of the antibacterial will render higher maximum serum concentration (Cs_{max}) in the shortest possible time (T_{max}) (8). Considering the above, the aim of this study was to evaluate whether or not, the administration of a concentrated form of enrofloxacin (0.2%) in the drinking water could increase Cs_{max} and reduce T_{max} as compared with the administration of the customary concentration of enrofloxacin (0.1%), also in the drinking water, and setting the same dose in both cases (10 mg/kg).

MATERIALS AND METHODS

Pharmacokinetic analysis of a commercial preparation of enrofloxacin was carried out in triplicate, either at a 0.1% or 0.2% concentration, but using the same dose (10 mg/kg) in all cases. Two hundred and forty Arbor-Acres broilers, not previously medicated with any antibacterial, weighing approximately 750 g and clinically healthy were used. They were randomly divided into six groups of 40 animals each: odd number groups received the customary dilution of enrofloxacin, *i.e.*, 0.1% (A1, B3, C5), while even number groups received the two fold concentrated form of the drug *i.e.*, 0.2% (A2, B4, C6). To minimize variations in absorption patterns between birds, due to enrofloxacin-feed interactions, animals were fasted for 6 hours before dosing. Each chicken was individually weighed, and then received a single oral bolus dose of enrofloxacin by means of a semi-rigid tubing, placing the drug directly into the proventriculus. Then blood samples were taken from five birds per time at 25 and 45 min, 1.5, 3, 6, 9, 12, and 24 hours, with only one sample taken from each chicken. Blood samples were immediately centrifuged, serum recovered, identified, and frozen until analyzed. Close timing interval between administration of the drug and blood sampling was achieved. In this manner, an excess of three complete sets of samples for each repetition were obtained. Determination of enrofloxacin was carried out using the quantitative/qualitative microbiological agar diffusion analysis, described by Bennet *et al.* (1), using *Escherichia coli* ATCC 25922 as the test microorganism and chicken serum as vehicle to obtain a reference linear regression between 0.08 µg/ml and 10 µg/ml, with a per cent recovery of 94 ± 2.5 and an intra-assay error smaller than 5%. Standard enrofloxacin was donated by Bayer of Mexico. Serum activity/concentrations of enrofloxacin (and active metabolites) *vs.* time relationships were analyzed using the software from PKAnalyst[*]. Area under the curve (AUC), β half lives (T½β), maximal serum concentration (Cs_{max}), time to reach Cs_{max} (T_{max}), and absorption half-life (T½abs), values were recorded, utilizing standard formulas (5). Mean values of serum concentrations of enrofloxacin from the three groups (A, B and C), and the two concentrations tested (0.1% and 0.2%) were compared using a non-parametric Kruskall-Wallis analysis, through the software package JMP[†]. Considering that data showed a non normal distribution, individual pharmacokinetic values were compared using Dunn tests, after Kruskall-Wallis analysis.

RESULTS

Figure 1 shows the mean serum concentrations obtained for enrofloxacin dosed either as 0.1% or as 0.2% in the three replicates. Table 1 shows derived pharmacokinetic variables. Both Cs_{max} values were higher in all three even number groups (A2, B4 and C6) as compared with data in odd number groups, with increments as large as three fold increments for Cs_{max1} and 1.6 fold for Cs_{max2} ($P<0.01$). Also, AUC values were higher in the 0.2% enrofloxacin groups ($P<0.05$); T_{max} T½β and absorption half-lives, did not vary in any group.

DISCUSSION

The analytical method used has been regarded as reliable as HPLC (4). This study shows that higher

[*] MicroMath. Scientific Software. Salt Lake City, Utah.
[†] JMP Statistic Mode Visual 1989-1995 SAS Institute Inc. Version 3.1.6.2, SAS Campus Drive Cary, N.C. 27513

Cs_{max} in a shorter T_{max}, can be achieved by the simple maneuver of doubling the concentration of enrofloxacin (from 0.1 to 0.2%), without modifying the actual dose (10 mg/kg/day). Increments in serum concentrations of enrofloxacin averaged 338.46% for Cs_{max2}. However, neither T_{max} nor T½abs were altered as expected. This may be due to fast absorption of enrofloxacin and to insufficient number of blood samplings early after placing the drug in the proventriculus. In this study T½β remained almost unchanged, and therefore it is unlikely that withdrawal times need to be modified. However, biovailability based on AUC was higher in groups dosed with the 0.2% solution of enrofloxacin (P< 0-05). The double peak shape of the serum concentration/activities *vs*. time obtained in this experiment are considered to be the result of entero-hepatic recycling of the drug, a feature not described in other studies (2,3). Different blood sampling times and deliverance of the drug directly into the proventriculus may explain this discrepancy.

Usually, enrofloxacin is administered as the treatment of choice for outbreaks of various diseases such as chronic respiratory disease complicated with *Escherichia coli*, outbreaks caused by *Pasteurella* spp, *Haemophillus gallinarum*, etc. For these purposes a higher dose (10 mg/kg/day) is usually chosen and with it, a higher Cs_{max} value expected. Although the clinical significance of doubling the concentration of enrofloxacin still awaits further field investigations, the fact that the effect of enrofloxacin is more dependent upon reaching a higher peak concentration than upon a long residence time (7), allows the speculation that higher Cs_{max} values could result in better clinical outcomes. Because the daily total dose is unaltered, costs of medication would not be increased. To achieve 0.2% solutions of enrofloxacin half emptying the water tank or adjusting the dose-dispenser apparatus, will suffice. Considering that the optimal bactericidal concentration of enrofloxacin is usually twice the value of the minimal inhibitory concentration, a more bolus-like administration of enrofloxacin will tend to reduce the emergence of bacterial resistance.

REFERENCES

1. Bennet, J.B., Brodie, J. L., Benner, E. J. and Kirby, W.M. Simplified accurate method for antibiotic assay. Clinical specimens. American Society of Microbiology, 14: 170-177, 1966.

2. Garcia Ovando, H., Luders, C., Gorla, N., Errecalde, C. and Prieto G. Intravenous pharmacokinetics of enrofloxacin and ciprofloxacin in broiler chickens. Proceedings of the 7th European Assoc. Vet. Pharmacol. and Toxicol. Intl. Congress, Madrid Spain, 6 - 10 July. J. Vet. Pharmacol. Ther. 20 Suppl. pp. 203-204, 1997.

3. Knoll, U., Glünder, G. and Kietzmann, M. Comparative study of the plasma pharmacokinetics and tissue concentration of danofloxacin and enrofloxacin in broiler chickens J. Vet. Pharmacol. Ther. 22: 239-246, 1999.

4. Küng, K.J., Riond, L. and Wanner, M. Pharmacokinetics of enrofloxacin and its metabolite ciprofloxacine after intravenous and oral administration of enrofloxacin in dogs. J. Vet. Pharmacol. Ther. 16: 462-468, 1983.

5. Rowland, M., Tozer, T.N. (1989) Variability. In Clinical Pharmacokinetics: Concepts and Applications 2nd
Ed. pp. 197-211. Lea & Febiger, Philadelphia.

6. Sumano, L.H. Quinolonas y Fluoroquinolonas en medicina veterinaria. Vet. Mex. 2476-88, 1993.

7. Sumano, L.H. and Gutierrez, O.L. Problemática del uso de la enrofloxacina en la avicultura en México. Vet. Mex. 31: 137-145, 2000.

8. Wages, D.P. Proper medication procedures. Poultry Digest. 56: 18-19, 1997.

Table 1. Mean ± 1 SD pharmacokinetic variables obtained for enrofloxacin after a single bolus dose of a commercial preparations of the drug, at a dose of 10 mg/kg, delivered into the proventriculus by a semi-rigid tubing at a concentration of 0.1% (odd numbers) and at a concentration of 0.2% (even numbers).

Variable	Group A1		Group A2		Group B3		Group B4		Group C5		Group C6	
	X ± SD		X ± SD		X ± SD		X ± SD		X ± SD		X ± SD	
T½abs (min)	8.2	1.2	8.5	1.3	8.0	1.6	8.2	0.9	8.2	1.6	8.0	1.4
β_1	0.15	0.002	0.207	0.002	0.200	0.005	0.27	0.002	0.025	0.003	0.24	0.355
β_2	0.015	0.022	0.015	0.038	0.016	0.01	0.018	0.038	0.018	0.002	0.016	0.180
T½ β (hours)	12.15	0.02	11.58	0.02	12.25	0.03	10.55	0.02	11.05	1.58	11.55	0.23
Cs_{max1} (µg/ml)	1.3	0.04	4.4	0.02	1.0	0.06	4.3	0.02	1.6	0.03	4.3	0.23
Cs_{max2} (µg/ml)	2	0.05	2.9	0.07	1.8	0.52	3.2	0.07	2.1	0.08	3.3	0.09
AUC	14.57	0.03	23.59	0.02	12.75	0.04	26.59	0.02	10.61	0.02	24.65	0.45
T_{max1} (hours)	0.83	0.05	0.7	0.04	0.82	0.03	0.82	0.04	0.78	0.05	0.85	0.02
T_{max2} (hours)	2.8	0.03	3.2	0.03	2.7	0.01	3.1	0.07	2.9	0.07	3.0	0.08

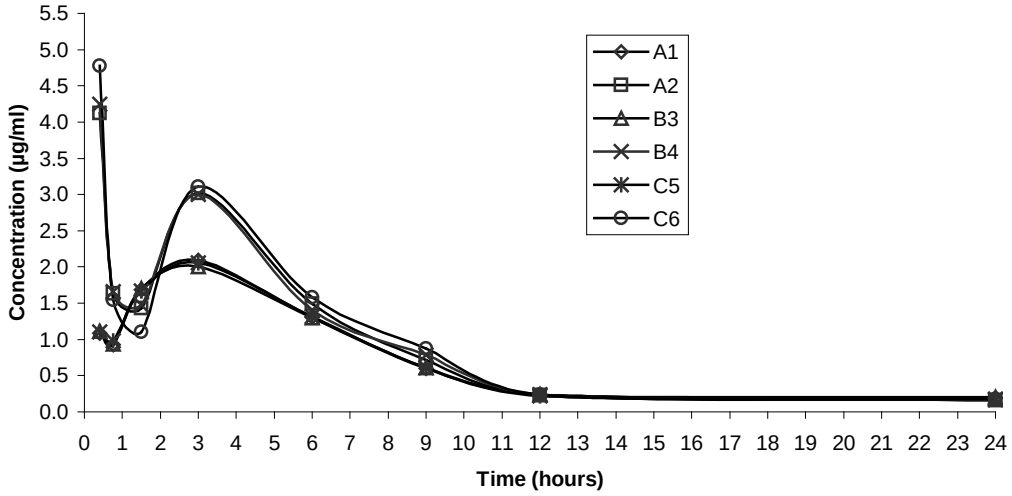


Figure 1. Double Cs_{max} shapes of the serum concentration of enrofloxacin *vs*. time relationships for enrofloxacin administered orally as a bolus to broilers, either as a 0.1% (odd numbers) or a 0.2% solution (even numbers).

ASSESSING THE EVIDENCE THAT USE OF ANTIBIOTIC GROWTH PROMOTERS IN ANIMALS INFLUENCES THE RESISTANCE PATTERNS IN HUMAN ENTEROCOCCAL INFECTIONS

Timothy S. Cummings, MS, DVM, Diplomate ACPV

College of Veterinary Medicine, Mississippi State University

The topic concerning antibiotic use in animals in farm animals and subsequent impact on the resistance patterns in human pathogens is not a new debate, beginning in the 1960s. The issue has been raised several times since then without any definitive conclusion being reached. As we're all aware, the latest flare-up came when the EU began the process to ban certain, long established growth promotant antibiotics from use in animal feeds. The EU invoked the "precautionary principle" to justify its position despite the lack of scientific data to support their concerns. In doing so, they essentially ignored the report of their own committee of experts (SCAN) which concluded that there was no justification to take action at the time. It should also be noted that this debate came at a time when food safety issues were at an all time high in Europe due to other unrelated concerns.

As a result, the debate became headlines in the US with much activity being initiated. There have been positions taken by individuals and agencies/groups on both sides of the issue with data being used to support each side's point of view, but there has been some sharp debate on the validity and conclusions of certain reports. In reality, it appears that the question is still very much unanswered, as the topic is a very complex matter. While research projects, risk assessments, and prudent use guidelines for antibiotic use in animals have been initiated worldwide and are to be commended, the simplistic belief that the banning of growth promotant antibiotics will halt or reverse the current trend towards antibiotic resistance patterns in human medicine is not rational and must be challenged.

The theoretical hazard to human public health related to antibiotic use in animals revolves around the transfer of resistant bacteria from animals to humans largely through the food chain. The best examples of these are the resistant, zoonotic organisms caused by non-typhoid salmonellae and campylobacters. Of these, the salmonellae are virtually unaffected by the growth promotants which have an overwhelmingly anti-gram-positive spectrum. The campylobacters can be affected by the macrolide growth promotants such as tylosin, but also by macrolides and quinolones used for the treatment of animal infections. It should be noted that very real differences exists when discussing this topic with regard to the therapeutic use of antibiotics via

injection or water versus those used for performance enhancement in the feed.

It seems much more difficult to assess the risk posed by commensal animal bacteria such as *E. coli* and enterococci, although resistance has been identified in the animal enterococci towards some of the growth promotant antibiotics. In order for resistant animal commensals to successfully transmit through the food chain, they must develop resistance and survive a myriad of obstacles throughout the production, processing, transportation, storage, and cooking of the food source. Once consumed, the animal enterococcus would have to contend with various enteric defenses to prevent their survival and then colonize if possible to optimize the potential for resistance transfer to human commensals.

The current debate evolves around *Enterococcal* infections in humans, which has become an important pathogen in immunosuppressed individuals and a potential nosocomial complication in post-operative patients. The initial concern involved Vancomycin resistance development by these organisms, which at the time was one of the few remaining efficacious antibiotics against *Enterococcal* infections approved for use in humans. Vancomycin is a glycopeptide class antibiotic. Avoparcin, a growth promotant antibiotic used in Europe at the time, was removed from the market in 1997 because of its relatedness to Vancomycin. On the other hand, Vancomycin resistance was much more prevalent in the US where Avoparcin had never been used. This would tend to suggest that human clinical use of Vancomycin was the primary instigator of glycopeptide resistance in the US.

Shortly afterwards, a new product, Synercid, was being reviewed for treatment of human *Enterococcal* infections in certain countries. It belonged to the streptogramin class of antibiotics. Virginiamycin, also a streptogramin antibiotic, had been in use in the worldwide animal market for over 25 years with little evidence of streptogramin resistance to date; yet it became the target of debate. It should be noted that enterococci resistance data is often reported as a group, but needs to be differentiated by species due to the natural resistance of certain species to different antibiotics. For instance, *E. fecalis* is naturally more resistant to virginiamycin, but is often included in relevancy data pertaining to this issue. This is of vital importance when attempting to draw accurate conclusions from the data pool. Unfortunately, most labs do not or cannot speciate *Enterococcal* infections due to the technical expertise required to do so.

In one very large, yet little cited clinical study by Jones et al (Diagnostic Microbiology and Infectious Disease, Volume 31, Issue 3, July 1998), which involved over 28,000 human clinical isolates from 200 different medical centers in the US and Canada, found streptogramin resistance to be 0.2% in the *E. faecium* isolates. This is a highly significant study in that it involved over 1,000 *E. faecium* isolates from a clinical setting. Similarly, pristinamycin, another human streptogramin approved for use in France since 1973, has been used along with virginiamycin for decades without any unusual rates of streptogramin resistance development in that country. Thus, there is circumstantial evidence that the contribution by virginiamycin to any streptogramin resistance problem in human *Enterococcal* infections is miniscule.

There are other findings which tend to suggest that the human health risk posed by the use of growth promotant antibiotics is negligible. For example, a number of studies suggest that enterococci strains have host preferences similar to the Group B streptococci and *Staphylococcus aureus* bacteria. Recent work by Dr Judith Johnson at the University of Maryland (2000 AVMA/AAAP meeting abstract) looked at fifty *E. faecium* field isolates from chickens and fifty *E. faecium* human isolates from hospitals in different geographical regions using ribotyping technology. Her findings suggested that enterococci from poultry and humans represent apparently distinct populations, suggesting that direct colonization from chickens to humans is rare.

Various governmental and independent organizations have weighed in on the matter, but most have agreed that there is simply not enough information to draw any conclusion. The 1998 National Research Council Report, The General Accounting Office Report of 1999, the SCAN Opinions, and the HAN Foundation Report of 1999 fall into this category. The consensus was that while use of drugs in food animals does have some concerns, the evidence to date does not constitute an immediate public health concern. Having said this, the WHO organization recently issued new guidelines to "slow the growth of microbes resistant to commonly used antimicrobials" which included calling for mandatory prescriptions for all antibiotics used to control diseases in animals and the cessation of the use of antimicrobials to promote animal growth.

In the US, a number if initiatives are underway or recently completed. A risk assessment model has been developed by CVM to estimate the human health impact of fluoroquinolone-resistant Campylobacter infections associated with the consumption of chicken. FDA-CVM subsequently proposed to withdraw approval for fluoroquinolone use in poultry. A second model is being developed by CVM to assess the association between the development of streptogramin-resistant *Enterococcus faecium* in humans and the use of virginiamycin in food producing animals. In addition, FDA has proposed a framework document in response to public concerns which would require

sponsors of new animal drugs to provide data on the risk of the antibiotic to human health. Various resistance surveillance projects have been funded and other research projects are underway to help fill in the data gaps, but it will take time to begin to get a reasonable chance for accurate answers.

In the meantime, the issue is far from over, but hopefully most involved parties have come to recognize that good scientific information is needed before taking any further regulatory action. In the US, there is a newly approved antibiotic called Zyvox, which is a new class of antibiotic available for the treatment of *Enterococcal* infections. This should alleviate some of the pressure for the need of Synercid, as Zyvox reportedly has a better efficacy and safety profile than Synercid. In addition, other products may be on the horizon. Hopefully, the true risk factors at stake will be elucidated, as this issue is much too important to human and animal health for wrong associations to be made.

FOWLPOX, A RE-EMERGING DISEASE OF CHICKENS: NEED FOR A NEW GENERATION OF VACCINES

Deoki N. Tripathy, William M. Schnitzlein, Pratik Singh and Viswanathan Srinivasan

Department of Veterinary Pathobiology, College of Veterinary Medicine
University of Illinois, Urbana, Illinois 61802

Fowl pox has a world wide distribution. This disease of chickens and turkeys is caused by a DNA virus of the genus Avipoxvirus of the family Poxviridae (14,15). Its incidence is variable in different areas because of variation in climate, management and hygiene, and the practice of regular vaccination. It can cause drops in egg production, or retarded growth in younger birds and significant mortality.

Fowlpox virus (FPV) infection is usually manifested in two forms: cutaneous and diphtheritic although an unrecognizable systemic form of infection also occurs. The cutaneous form (dry pox) is characterized by the development of proliferative lesions, ranging from small nodules to spherical wart-like masses on the skin of the comb, wattle and other unfeathered areas. In the diphtheritic form (wet pox), slightly elevated white opaque nodules develop on the mucous membranes. They rapidly increase in size to become yellowish diphtheritic lesions. When these proliferative lesions occur on the mucous membranes of the mouth, esophagus, larynx or trachea, difficulty in feeding and respiration ensues. The mortality rate due to the diphtheritic form associated with tracheal and mouth lesions is greater than that associated with the cutaneous form, and can sometimes near 50%, particularly in young birds. Both forms of the disease can occur in a single bird. Because of its economic importance, attempts are made towards prevention of the disease by regular immunization with live virus vaccines of fowlpox or pigeonpox virus origin.

In recent years, FPV has been isolated in several outbreaks of fowlpox in chickens previously vaccinated with commercial fowlpox or pigeonpox virus vaccines in Minnesota, Nebraska, Indiana, Arkansas, New York, California and Pennsylvania. In most of these occurrences, the diphtheritic form of the disease resulting in high mortality has been the common feature. Reduced egg production in affected layer flocks has also been observed. The continued persistence of fowlpox in poultry operations in spite of regular immunization with either fowlpox or pigeonpox virus vaccine strains alone or in combination has indicated a lack of adequate protection being provided by vaccination. Some possible reasons for this deficiency are: (a) passive immunity in young birds may interfere with the multiplication of the vaccine virus and thus prevent development of an adequate immune response; (b) a single vaccination of birds at a young age may not provide enough protection during the later part of their lives, especially in the case of layers; (c) inadequate concentration of virus in the vaccine dose; (d) improper vaccination practice in that some birds are missed and (e) lack of protection by the vaccines against re-emerging "variant" strains.

Experimental studies have shown differences in the protective ability of both vaccine and field strains of FPV. Because of its large size genome (300 kb), genetic and antigenic changes are difficult to detect by routine procedures. However, restriction fragment length polymorphism of the viral genomes and immunoblotting of antigens have demonstrated minor genetic and antigenic differences among FPV strains (5,7,9). It should be noted that in an environment where commercial poultry is raised in high density, the birds are not only immunized with live virus vaccines but may acquire pathogens through natural exposure. Thus, it is conceivable that some of the changes could occur as a result of interaction by

different viruses simultaneously infecting the respiratory system of birds resulting in the emergence of antigenically different viruses for which the immune response generated by vaccine viruses would not be adequate. For example, a dual viral infection by fowlpox virus and herpes virus has been reported previously (4,12). In one instance chickens had not been vaccinated against either type of virus and the FPV strain isolated from this outbreak was initially thought to be an avirulent strain but instead was highly pathogenic for susceptible day-old chicks (11). When 39 week old laying hens were infected with this virus, it caused a drop in egg production. Moreover, the lesions at the primary site of inoculation persisted for the 5 week duration of the experiment in contrast to vaccine strain-generated ones which usually resolve within 2 weeks. In addition, some birds developed secondary lesions (13). Recently**,** molecular genetic analysis of this virus revealed reticuloendotheliosis virus (REV) provirus integration in its genome. A similar presence of unrelated genetic material was reported when the homologues of FPV open reading frames were detected in the genome of Marek's disease virus, a herpes virus (2). These observations indicate the possibility of exchange of genetic material between distinct viruses. That such an event often occurs by "natural genetic engineering" has been further supported by the presence of REV sequences in the genome of Australian as well as US strains of FPV (3,6,8). While vaccine strains of FPV retain variable lengths of long terminal repeats (LTRs) of REV, all field isolates of FPV tested are mixed populations comprised primarily of viruses having intact REV provirus with a minority carrying partial LTR sequences. Since the site of REV integration has remained constant in all the strains so far examined, this event certainly has some advantage for the FPV. Further, most of these field strains show greater pathogenicity than vaccine viruses for susceptible chickens and an antibody response against both REV and FPV is detected in experimentally immunized birds. Since REV has been associated with immunosuppression, its presence in the genome of FPV seems to play an important role in the pathogenesis and prolonged persistence of FPV in the bird population**.** Susceptible young chickens immunized with field strains of FPV develop both primary and secondary lesions, which persist longer than those produced by the vaccine viruses (8). Moreover, in layers there is an appreciable drop in egg production, which is related to the severity of the disease (13).

FPV has one of the largest genomes containing more than 200 genes which encode for both essential and non-essential proteins. Currently, the entire sequence of the DNA of one vaccine-like strain of FPV has been determined (1). Although complete sequence information for other strains is not available at this time, differences can be discerned by the comparison of selected genomic fragments. It is apparent that FPV has acquired several genes which may not be essential for its multiplication but may prolong its survival in nature and persistence in the host. It is important to remember that poxviruses in dried scabs can survive in the environment for extended periods. Since fowlpox is a slow spreading disease, the virus can persist and circulate in a susceptible flock. In such cases, especially when only minor cutaneous lesions are present and a small percentage of birds are involved, the disease may remain unnoticed. In this regard, FPV persistence in some flocks for a long period as evidenced by the presence of minor cutaneous lesions has been observed. When the birds are subjected to various stresses e.g., molting, or concomitant microbial infections, the mild inapparent infection can be induced to become a serious outbreak.

While many viruses would not survive outside their host (s), FPV can be isolated from dried scabs that have been exposed to the environment for extended periods of time. Thus for outside survival, the virus must have developed strategies to prevent and/or resolve environmentally induced damages. In this regard, recently two genes encoding for photolyase and A-type inclusion protein were identified in the genome of FPV. Photolyase has been associated with the repair of UV-damaged DNA (10) while A-type inclusion body protein is believed to shield the virus within its matrix. Although the function of many FPV genes remains undetermined at this time, studies with photolyase and inclusion body protein deficient mutants as well as determination of the effect of the removal of REV sequences from the genome of FPV are important for the development of improved vaccines against fowlpox.

REFERENCES

1. Afonso, C.L. E.R. Tulman, Z. Lu, L. Zsak, G.FD. Kutish and D.L. Rock. The genome of fowlpox virus. J. Virol. 74: 3815-3831. 2000.

2. Brunovskis, P. and L. F. Velicer. The Marek's Disease virus (MDV) unique short region: alpha herpes virus-homologs, fowlpox virus-homologs, and MV-specific genes. Virology, 206: 324-338. 1995.

3. Diallo, I. S., M. A. MacKenzie, P. B. Spradbrow and W. F. Robinson. Field isolates of fowlpox virus contaminated with reticuloendotheliosis virus. Avian Pathology, 27: 60-66. 1998.

4. Fatunmbi, O. O., W. M Reed, D. L. Schwartz, and D. N. Tripathy. Dual infection of chickens with pox and infectious laryngotracheitis (ILT) confirmed

with specific pox and ILT DNA dot-blot hybridization assays. Avian Dis. 39: 925-930. 1995.

5. Ghildyal, N., Schnitzlein, W. M., and Tripathy, D. N. Genetic and antigenic differences between fowlpox and quailpox viruses. Arch. Virol. 106: 85-92. 1989.

6. Hertig, C. H., B. E. H. Coupar, A. R. Gould and D. B. Boyle. Field and vaccine strains of fowlpox virus carry integrated sequences from the avian retrovirus, reticuloendotheliosis virus. Virology, 235, 367-376. 1997.

7. Schnitzlein, W.M., N. Ghildyal, and D. N. Tripathy. Genomic and antigenic characterization of avianpox viruses. Virus Res., *10*: 65-76. 1988.

8. Singh, P., T. J. Kim and D. N. Tripathy. Re-emerging Fowlpox: Evaluation of isolates from vaccinated flocks. Avian Pathology, 29:449-455. 2000.

9 Singh, P. and Tripathy, D. N. Characterization of monoclonal antibodies against fowlpox virus. Avian Diseases, 44: 365-371. 2000.

10. Srinivasan, V., Schnitzlein W.M. and Tripathy D.N. Fowlpox virus encodes a novel DNA repair enzyme, CPD-Photolyase, that restores infectivity of UV light-damaged virus. Journal of Virology , 75:2001

11. Tripathy, D. N., L. E Hanson and A. H. Killinger. Atypical fowlpox in a poultry farm in Illinois. Avian Dis., *18*, 84-90. 1974.

12. Tripathy, D. N., D. M. Sells, and L. E. Hanson. Natural pox and herpes as a dual viral infection in chickens. Avian Dis.19: 75-81. 1975.

13. Tripathy, D. N. and L. E. Hanson. Pathoghenesis of fowlpox in laying hens. Avian Dis. 22: 259-265.1978.

14. Tripathy, D. N. and Reed, W. M. Pox. In: Diseases of Poultry. 10th ed., eds Calnek, B. W. et al. Iowa State University Press, Ames, IA, pp 643-659. 1997.

15. Tripathy, D. N. and Schnitzlein, W. M. Fowlpox Virus (Poxviridae). In Encyclopedia of Virology, Second Edition, eds. Granoff, A. and Webster, R. G., Academic Press, pp. 576-582. 1999.

AN UNUSUAL PRESENTATION OF POX IN PIGEONS

Victoria A. Bowes

Animal Health Center, 1767 Angus Campbell Road, Abbotsford, British Columbia, Canada V3G 2M3

The owner presented a young adult racing pigeon for necropsy to determine the cause of "black lumps" under the skin, originally thought to be pellet gun shot. Three of 150 birds were affected. Prior to submission, the owner had discussed this problem with fellow racers who recommended lowering the dietary protein. During the course of the diagnostic workup, the owner did this and reported that the lumps in the other affected birds had dried up and fallen off (thus confirming his diagnosis of low protein!).

At necropsy the bird was in good body condition and the gross lesions were localized to the skin. There were numerous, variably-sized (2–10 mm), solitary, raised, firm black skin nodules randomly distributed over the body. The nodules were loosely mobile and lay directly below the skin with occasional ulceration of the overlying epidermis. On cut section the nodules were densely black, moist and had thick rugal folds.

Bacterial culture of the nodules yielded heavy *Staphylococcus aureus*. Initial poxvirus culture and direct EM of a smeared nodule were negative. Subsequent retesting eventually did reveal a small number of poxvirus particles by direct EM.

Histologically, the skin masses consisted of discrete nonencapsulated lobules of solid nests of hyperchromatic basal cells containing abundant melanin pigment. These lobules are set against a background of proliferative fibrous connective tissue with a mild diffuse infiltration of mixed inflammatory cells and were typical of a basal cell tumor. Initially overlooked and considered debris, there were numerous degenerating eosinophilic intracytoplasmic inclusion bodies present in the central lumen of the lobules. The overlying epidermis was ulcerated in some sections.

Based on the initial histologic lesion and negative virology, a diagnosis of basal cell tumor was made. It was only through subsequent discussions on Birdmed, an Internet discussion group of avian veterinarians, that Dr Juergen Lohr, a German veterinarian, was able to provide the final pieces of information which allowed a diagnosis of pigeonpox (also known in Europe as “pox melanoma”) and encouraged the continued EM examination of the nodules until poxvirus particles were seen.

This form of pigeonpox has never been diagnosed in British Columbia so there was a lack of familiarity of this specific presentation, as well as no information in the available references. In Europe, pox melanoma is considered a cutaneous form of pigeonpox and is controlled by the annual use of vaccine. Associated mortality is low, young birds are most likely to develop

these nodules and the majority of nodules eventually dry up and fall off.

In this case I was unable to convince the owner of the diagnosis, since he believed he had solved the problem by lowering the dietary protein. Time will tell.

ARE THERE REALLY FOWL POX VARIANTS?

Ken Takeshita[A], Amy Stewart[B], Pat Waknell[C], Radivoje Spasojevic[D], Richard L. Dutton[E], M.G. Waldbaum[E]

[A]Lohmann Animal Health International, 4759 Ridgeview Ln, Vacaville, CA 95688
[B]School of Veterinary Medicine, University of California, Davis, CA 95616
[C]School of Veterinary Medicine, University of California, Davis, CA 95616
[D]Sparboe Companies, Litchfield, MN 320-693-2431
[E]105 Main St., Wakefield, NE 68784

Fowl Pox in combination with Pigeon Pox is being used on some layer complexes in the United States where Fowl Pox (CEO) vaccination alone has not provided adequate protection with outbreaks of upwards of 10% total mortality (Figure 1) due to the diptheritic or wet form of pox. In the following study, two field Pox isolates, P1 and P2 were obtained from two different companies with history of significant wet pox mortality despite vaccination with Fowl Pox. These isolates were then used in the challenge study described below for the purpose of trying to determine if there were truly field isolates that are not protected by Fowl Pox alone.

P2 isolate was received as frozen tracheas and P1 as liquid material. Both were injected into CAM of embryonated eggs to confirm presence of Pox virus and eliminate any bacterial contamination. After confirmation of presence of Pox viruses these isolates were eventually diluted for a wing web challenge titer of $10^{5.3}$/ml for P1 and $10^{5.5}$/ml for P2. Wing web challenge was done using double needle stick provided by manufacturer to deliver 0.01ml virus per stab. Tracheal challenge was also done using the P2 wing web challenge material further diluted to 0.01-ml virus/0.1 ml dose delivered directly into the trachea using a long dosage rounded tip needle.

SPF birds were vaccinated with either Fowl Pox ($10^{4.2}$/dose), Pigeon Pox ($10^{3.5}$/dose) or Fowl Pox/Pigeon Pox at 29 days of age. Vaccines were mixed as per label with the exception that for the Fowl Pox/Pigeon Pox combination; one vial of Fowl Pox was mixed together with one vial of Pigeon Pox using one diluent bottle. Vaccination takes were confirmed positive 10 days post vaccination. Birds were challenged 2 weeks post vaccination then euthanized and examined for lesions 10 days post challenge. Birds were considered positive for pox if they had pustules/pimples or scabs at the wing web challenge site or positive if diptheritic lesions occurred at trachea or larynx.

Results in Table 1 clearly show that P1 isolate is well protected by Fowl Pox alone. On the other hand P2 isolate was not well protected by Fowl Pox. Use of Pigeon Pox improved protection in combination or alone against P2, particularly against tracheal challenge. In the field, Fowl Pox/Pigeon Pox combination vaccination is providing good protection against P2 isolate at the company where it was isolated. One of the difference between the controlled challenge study vs. the field situation is birds in the field have been wing web stabbed, sometimes twice at one time, with multiple vaccinations. Other differences could include, lower challenge field dose or perhaps a different primary infection route by P2 isolate.

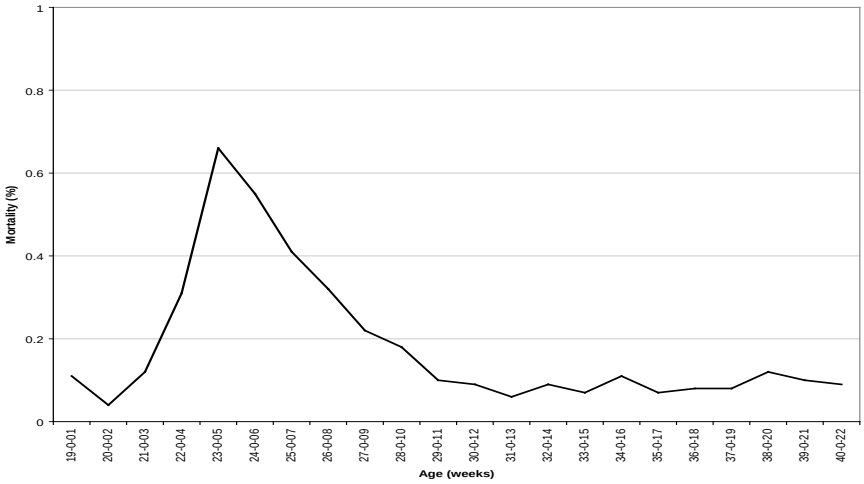


Figure 1. Wet Pox mortality on Farm P1

Table 1. Pox challenge results

Vaccines	% Protection					
	P1 isolate		P2 isolate			
	wing web		wing web		trachea	
Fowl Pox	100%	(21/21)	36.40%	(4/11)	72.10%	(8/11)
Fowl Pox/Pigeon Pox	90.90%	(20/22)	45.50%	(5/11)	81.80%	(9/11)
Pigeon Pox	95.50%	(21/22)	45.50%	(5/11)	91.90%	(10/11)
Positive Control	0%	(0/22)	0%	(0/11)	0%	(0/11)

OBSERVATIONS ON HATCH PROGRESSION AND CHICK QUALITY

Hector M. Cervantes

Phibro Animal Health, Watkinsville, GA 30677-2171

A method designed to evaluate hatch progression and its effect on chick quality was tested in several broiler hatcheries across the United States. The method consists of conducting chick counts from 6-9 predetermined hatcher trays per hatcher at various predetermined times prior to "pulling the hatch". More specifically, hatched chicks are counted in 2-3 hatcher trays from the top position, 2-3 hatcher trays from the middle position and 2-3 hatcher trays from the bottom position at approximately 24 and 12 hours before the time scheduled "to pull the hatch". Other records kept include the breed and age of the breeder flock when the eggs were set, the type of incubator and hatcher used, and the machine settings (wet and dry bulb temperatures and relative humidity). This evaluation is complemented with an analysis of causes of embryonic mortality and chick quality assessments on the day that the chicks are removed from the hatchers. Embryonic mortality is divided into 4 groups: early (0-4 d), middle I (5-10 d), middle II (11-17 d) and late (18-21 d), as described by R. Garza de la Fuente (1,2). Chick quality scores are derived from physical and microbiological scores as in the method described by H. Cervantes (3-5).

Applying this method to commercial evaluations of broiler hatcheries, the following practical observations have been made:

1. Incubators and hatchers from different manufacturers and different models produce significant variations in hatch progression even when set at the same temperature and humidity and used by the same hatchery. This emphasizes the importance of constantly monitoring hatch progression and chick

quality so that proper adjustments can be made to each machine in order to maximize uniformity of hatch progression and chick quality.

2. There is significant intra-hatcher and inter-hatcher variation within a hatchery even when the same breeder flock is compared in machines of the same type and of the same manufacturer. Ideally, one should see no more than 20 percentage points for intra-hatcher variation and 5 percentage points for inter-hatcher variation.
3. The type of breed and age of the breeder flock has a significant effect on the progression of the hatch.
4. Egg position during incubation has a significant influence on hatch progression in the hatcher. Due to the common use of the Texas transfer method in broiler hatcheries, frequently the top and bottom hatcher trays are hatching ahead of the middle trays.
5. Handling, storage and pretreatment of hatching eggs prior to setting often has a significant effect on hatch progression and final chick quality.
6. Examination of chicks during hatch progression monitoring is often a useful indicator of final chick quality.
7. In general, prematurely hatched chicks are a more common problem than delayed hatched chicks.

REFERENCES

1. Garza de la Fuente, R. Embriologia y Embrio-Diagnosis. Proc. Chick Master's Incubation Short Course. XII Latin American Poultry Congress, pp. 87-93, 1991.

2. Garza de la Fuente, R. Todo Sobre Incubacion. Tecnologia Avipecuaria en Latinoamerica. Vol. 1, No. 1, pp. 21-24, 1992.

3. Cervantes, H. A New Formula for Chick Quality. Broiler Industry, September, pp. 20-27, 1993.

4. Cervantes, H. Una Nueva Formula Para Definir La Calidad Del Pollito. Industria Avicola, May, pp. 10-16, 1994.

HEPATITIS-SPLENOMEGALY SYNDROME IN CHICKENS ASSOCIATED WITH 30-35 NM VIRUS PARTICLES

H. L. Shivaprasad[A], D. H. Read[B], P. R. Woolcock[A], J. Jeffrey[C], B. Daft[B], G. Haqshenas[D] and X. J. Meng[D]

[A]California Animal Health and Food Safety Laboratory System – Fresno Branch, University of California, Davis, 2789 S. Orange Ave., Fresno, CA 93725.

[B]California Animal Health and Food Safety Laboratory System – San Bernardino Branch, University of California, Davis, 105 W. Central Ave., San Bernardino, CA 92408.

[C]Department of Veterinary Extension and Population Health and Reproduction, University of California, Davis, VMTRC, Tulare, CA 93274

[D]Department of Biomedical Sciences and Pathobiology, Virginia Polytechnic Institute University, 1410 Price's Fork Rd., Blacksburg, VA 24061

Hepatitis-splenomegaly (HS) syndrome is a disease of both layer and broiler-type chickens characterized by increased mortality and decreased egg production. The syndrome is most common in laying hens between 30 and 72 weeks of age, with the highest incidence occurring between 40 and 50 weeks of age. The syndrome was first reported in western Canada in 1991, and since then has been recognized in the United States. There are no specific clinical signs associated with this syndrome. In some outbreaks, decreased egg production by as much as 20% has been reported. Weekly mortality can exceed 1.0% in some instances. Birds that die from this syndrome are generally in good body condition but may have pale combs and wattles. Reported gross lesions include clotted blood in the abdominal cavity and/or on the livers, as well as red fluid within the abdominal cavity. Livers can be enlarged, friable, and stippled with pale white, red or tan foci. Subcapsular hematomas can be seen occasionally in the liver. Spleens can be severely enlarged and pale. Ovaries are often regressing. Microscopically, liver lesions range from multifocal to extensive hepatic necrosis and hemorrhage, with infiltration of mononuclear inflammatory cells around portal triads. Infiltration of lymphocytes in and around the blood vessels in the liver is a characteristic lesion of this syndrome. Microscopic lesions in the spleen include lymphoid depletion, hyperplasia of mononuclear phagocytic system cells and the accumulation of eosinophilic material in and around small arteries and in the interstitium of the vascular sinuses. Similar eosinophilic material can also be

present in the interstitium of the liver. This material is usually positive for amyloid using Congo red stain.

No bacteria have been isolated consistently from affected livers and spleens of birds with HS. Serology has been unremarkable for various diseases. Toxicologic testing of the livers for heavy metals and feed for mycotoxins has been negative.

In the bile from some of the chickens with HS, we have observed viral particles ranging in size from 30 to 35 nm by negative stain electron microscopy. By RT-PCR and sequencing of a part of the RNA genome, the virus has been characterized as a hepatitis E-like virus. However, a definitive causal relationship between the hepatitis E-like virus identified in the bile of chickens and HS syndrome remains to be determined. Hepatitis E viruses have also been identified in humans, pigs and rodents.

The HS syndrome appears to be similar to Big Liver and Spleen (BLS) disease described in Australia in 1988. BLS is also characterized by decreased egg production, increased mortality and enlargement of the liver and spleen of mature broiler breeders and egg layers. A hepatitis E-like virus has been identified as the cause of BLS. Antibodies to BLS have been demonstrated in chickens in the US. We have determined that there is a 79 % nucleotide sequence homology between viruses associated with HS and BLS. Positive serology for BLS in the US and the genetic similarity between the viruses associated with HS and BLS suggests that these two viruses are closely related.

REFERENCES

1. Handlinger, J. H., and W. Williams. An egg drop associated with splenomegaly in broiler breeders. Avian Dis. 32:773-778. 1988.

2. Haqshenas, G., H. L. Shivaprasad, P. Woolcock, D. H. Read, and X. J. Meng. Genetic identification and characterization of a novel virus related to the human hepatitis E virus from chickens with Hepatitis-splenomegaly syndrome (In press).

3. Jeffrey, J. S. and H. L. Shivaprasad. Investigation of hemorrhagic hepatosplenomegaly syndrome in broiler breeder hens. Proc. 47th WPDC, Sacramento, CA, pp 46-48. 1998.

4. Payne, C. J., S. L. Plant, T. M. Ellis, P. W. Hillier and W. Hopkinson. The detection of the big liver and spleen agent in infected tissues via intravenous chick embryo inoculation. Avian Path. 22:245-246. 1993.

5. Payne, C. J., T. M. Ellis, S.L. Plant, A. R. Gregory and G.E. Wilcox. Sequence data suggests big liver and spleen disease virus (BLSV) is genetically related to hepatitis E virus. Vet. Micro. 68:119-125. 1999.

6. Read, D. H., B. M. Daft, J. T. Barton, P. R. Woolcock, G. Cutler, and F. Galey. Necrotic hemorrhagic hepatitis splenomegaly syndrome: An unsolved sudden death syndrome in layer Leghorn chickens. Proc. 43rd WPDC, Sacramento, CA, pp 8-9. 1993.

7. Riddell, C. Hepatitis-Splenomegaly syndrome. In: Diseases of Poultry, Ed by Calnek. B. W. et al., Iowa State University press, Ames, Iowa, pp 1041. 1997.

8. Shivaprasad, H. L., and P. R. Woolcock. Necrohemorrhagic hepatitis in broiler breeders. Proc. 45th WPDC, Sacramento, CA, pp 6. 1995.

9. Tablante, N. L., J-P. Vaillancourt and R. J. Julian. Necrotic, haemorrhagic, hepatomegalic hepatitis associated with vasculitis and amyloidosis in commercial laying hens. Avian Path. 23:725-732. 1994.

SALPINGITIS IN PEKIN DUCKS ASSOCIATED WITH CONCURRENT INFECTION OF TETRATRICHOMONAS SP. AND E. COLI

R. Crespo[A], R. L. Walker[B], R. Nordhausen[B], S. J. Sawyer[B], and R. B. Manalac[B]

California Animal Health and Food Safety Laboratory System, University of California, Davis
[A]Fresno Branch, 2789 South Orange Ave, Fresno, CA 93725
[B]Davis Branch, University of California, West Health Sciences Drive, Davis, CA 95616

A 5-10% decrease in egg production and sudden increase in mortality (up to 1.5% per week) in 30-32 weeks old female Pekin ducks were observed in a commercial breeding flock of 1,500 birds. Males from the same flock were clinically normal and did not have increased mortality. Mortality decreased to 0.8% after five days of treatment with tetracycline in the drinking water; but two weeks after treatment the mortality again rose to 1.5%. Although hatchability was

maintained within the expected values, egg production never reached normal levels.

At necropsy, the most striking lesions were in the oviduct, which was enlarged and distended, with a congested serosal surface, and accumulation of yellow-white caseous casts in the lumen of the magnum and part of the isthmus. Mucosal epithelium of the oviduct was necrotic and the ovary had regressing ova.

Histologically, moderate to large amounts of necrotic debris, bacteria, and degranulated heterophils were in the lumen of the oviduct, mainly vagina and shell gland. Scattered flagellated protozoa were observed within the necrotic debris and on the surface of the mucosa of the vagina and shell gland. Most of their mucosal epithelium was necrotic. In addition, numerous macrophages, lymphocytes, and plasma cells infiltrated their lamina propria.

Bacteriologic cultures revealed large numbers of *E. coli* from the oviduct. Trichomonads were recovered from the vagina and shell gland cultures. Examination by scanning electron microscopy revealed that the Trichomonads were oval (6-8μm long by 4.5-6μm wide), with an anterior nucleus and short axostyle, which protruded less than 1μm beyond the body. There were four anterior flagella, and an undulating membrane extending over the entire length of the body finishing in a long, posterior flagellum, consistent with the genus *Tetratrichomonas*.

A number of different trichomonads have been reported from birds and mainly affect the digestive tract. In severe cases of enteritis in ducks, *Tetratrichomonas anatis* has entered the oviduct from the cloaca causing salpingitis (2). The lesions in the ducks of this report were confined primarily to the oviduct, and enteritis was not observed.

In the present case, the protozoa were morphologically most similar to those of the genus *Tetratrichomonas* and differed in size and/or number of flagella from other trichomonads commonly recovered from ducks, including *T. anatis* and *Trichomonas gallinae*. The dimensions and number of flagella are similar to *T. anseris* (3). In addition, comparative sequence analysis of the 5.8S rRNA gene and the flanking internal transcribed space regions of the trichomonad isolate did not closely match with available sequences of the same region of other trichomonadid protozoa.

Both trichomonads and *E. coli* have been associated with salpingitis and vaginitis, causing mucosal inflammation and necrosis (1,3). Damage to epithelium of the reproductive tract then may allow secondary infections to develop. Since both trichomonads and *E. coli* were isolated from oviduct, it is difficult to determine whether either played a primary role in the disease.

(The full length article has been accepted for publication in the *Journal of Veterinary Diagnostic Investigation*)

REFERENCES

1. Barnes, H. J., and W. B. Gross. Colibacillosis. In: Diseases of Poultry, 10th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald and Y. M. Saif, eds. Iowa State University Press, Ames, Iowa. pp. 131-141. 1997.

2. Leibovitz, L. Necrotic enteritis of breeder ducks. Am. J. Vet. Res. 34:1053-1061. 1973.

3. Soulsby, E. J. L. Order: Trichomonadida Kirby, 1947. In: Helminths, Arthorpods and Protozoa of Domesticated Animals. 7th ed. Lea & Febiger, Philadelphia, PA. pp. 555-566. 1982.

ORAL LESIONS IN BROILER BREEDERS ASSOCIATED WITH FEEDING FINE MASHES

Chris Morrow, PhD BVSc

Ross Breeders Ltd, Midlothian Scotland EH28 8SZ

This investigation was prompted by the observation in Thailand (corn/soya based ration) of oral lesions in 20 week old broiler breeders associated with feeding fine mashes from 8 weeks of age. Other birds on diets compounded at the same feedmill from the same raw materials but pelleted were not affected. T-2 assays on feed could not demonstrate any detectable mycotoxin (detection limit 50 mg/kg) and associated production problems included inability to maintain uniformity. The incidence and severity of the lesions peaked at peak feed intake. Initially the lesions are seen on either side of the tongue with food particles adhering to the discrete areas (1 to 5 mm in diameter). Lesions then develop on the hard pallet rostral to the palatine cleft and/or in the top of the pharynx. No lesions in other tissues were observed.

Although these lesions have been described before on experimental diets (Gentle M., Aetiology of food-

related oral lesions in chickens. Res. Vet. Sci. 40: 219-224.1986.) I know of no recorded cases in the field. It is suggested that the pathogenesis of the lesion involves an impairment of the normal oral hygiene process with a lack of bigger feed particles leading to less efficient cleaning of oral mucosa. This condition appears to be common in Asia and other parts of the world where pellet quality is poor and may be being misdiagnosed at T-2 toxin ingestion. In contrast to reported T-2 associated lesions no lesions on the commisures of the beak were observed. In another case observed in Russia (wheat based ration) lesions healed rapidly after the pellet quality improved. Production effects of these lesions in lay appear to be mild unless confounded with hot environmental temperatures. In this latter case eating up time will extend and feed consumption will decrease with dramatic effects on egg production.

THE USE OF ANTICOCCIDIAL DRUGS IN BROILER CHICKENS IN THE USA FROM 1995 TO 1999

H. D. Chapman

Department of Poultry Science, University of Arkansas, Fayetteville, Arkansas 72701

INTRODUCTION

Anticoccidial drugs have been used for the control of coccidiosis for many years and today almost all commercial broilers are reared with an anticoccidial agent in their feed. Many compounds have been introduced but at the present time, in the USA, about a dozen are utilized. They can be categorized as either polyether ionophorous antibiotics (ionophores) that are produced by fermentation, or synthetic compounds (often referred to as "chemicals") that are produced by chemical synthesis. In some cases birds are given one drug, but two or more (so called "shuttle" programs) may be given during the life of a flock. The term "program" will be used to refer to the compounds used for the life of a single flock. By utilizing different permutations of drugs, about forty different programs have been employed in the USA in recent years.

A database has been developed that provides accurate, frequent information on a monthly basis from individual poultry plants (1). In addition to the drugs employed by each plant, the final bodyweight and measurements of performance such as calorie conversion (a measure of feed efficiency), the number of days taken to grow a flock to a specified average weight, and % mortality are included. Results for the years 1995 to 1999 were examined to provide information on the types of program and their frequency of use, and whether there was any correlation with performance characteristics of the birds.

RESULTS

Feeds. Most plants provided data for three feeds (starter, grower, and final withdrawal). Approximately 45% of plants used a fourth feed (first withdrawal) before the final withdrawal feed. The actual period for which each feed was given varied for different plants. The mean duration of feeding periods was 17 days (range 9-25) for the starter feed, 17 days (range 11-30) for the grower feed, 10 days (range 4-22) for the first withdrawal feed, and 8 days (range 1-28) for the final withdrawal feed.

Programs. An anticoccidial was used in the starter and grower feeds by 99 % of plants, and in the first and final withdrawal feeds by 41 % and 6 % of plants respectively.

The overall use of a single ionophore (ION), chemicals followed by an ionophore (CHE/ION) or two different ionophores (ION/ION) was 60.5 %, 27.5 % and 9.2 % respectively during the 5-year period. An ION program was used more frequently than an ION/ION program throughout the years, and more than a CHE/ION program except for the months of March, April, and May, when use was similar. Use of an ION program increased from June to September but subsequently declined. A CHE/ION program was used more frequently than an ION/ION program from December to July with a peak during March and April. No seasonal pattern in the use of an ION/ION program was apparent. A progressive increase in the use of an ION program was apparent during the 5-year period but use of a CHE/ION and ION/ION program gradually declined.

There was no significant difference in the use of an ION, or CHE/ION program, whether the final weight of birds was 1.5 to 2.0 kg, 2.0 to 2.5 kg, or > 2.5 kg.

The frequency with which different program types were employed during the 5-year period was extremely variable. The average number of changes made by 50 plants per year during the 5-year period was 2.1. There was a significant difference in the duration of ION, CHE/ION, and ION/ION programs. No consistent

pattern was evident in the sequence with which different types of programs were employed where birds were reared to different weights. Thus an ION program was equally likely to be followed by an ION/ION or a CHE/ION program.

No significant difference in calorie conversion or the number of days to 2.27 kg was found whether an ION, or CHE/ION program was used. Mortality was significantly higher where a CHE/ION program had been employed.

No significant differences in calorie conversion, days to 2.27 kg, or % mortality were observed for a 3 month period before and after a change from an ION to CHE/ION program and vice versa. No significant difference in the number of days to 2.27 kg was observed where use of a chemical had been less than 10%, or more than 40%, during the previous 4 years. Calorie conversion and % mortality were significantly higher in plants where use of a chemical had been more than 40%.

Individual drugs. Salinomycin was the most frequently used anticoccidial in a single drug program followed by narasin, monensin, and lasalocid. Semduramicin was first reported from November 1996 and was occasionally used in the starter, grower, or starter and grower feeds. Nicarbazin (followed by various ionophores) was the most frequently used chemical. Maxiban® and chemicals such as zoalene, robenidine, and halofuginone were occasionally utilized.

Percent mortality reported during the first week of age was significantly higher in plants where nicarbazin was used, compared with those where salinomycin had been employed. No significant differences in mortality were apparent at other times.

There was a significant difference in the use of salinomycin and nicarbazin in different regions of the USA during March and April. Nicarbazin was used more frequently in the north and south east whereas salinomycin was used more frequently in the south central region.

REFERENCE

1. Agri Stats. Inc., 6510 Mutual Drive, Fort Wayne, IN 46825

ANTICOCCIDIAL EFFICACY OF HISTOSTAT® (NITARSONE) IN TURKEYS

Steven Clark, Eugene Schildknecht, James Skinner and Lisa Rakebrand

Alpharma Animal Health Inc., P.O. Box 1399, Fort Lee, NJ 07024

SUMMARY

HISTOSTAT (nitarsone) is approved for use in the feeds of chickens and turkeys at 0.01875% as an aid in the prevention of blackhead. While used as a blackhead preventative, its anticoccidial efficacy is of interest. Mathis showed the efficacy of nitarsone against pathogenic turkey coccidia following experimental infection. This presentation will show the results and statistics of 12 anticoccidial sensitivity tests (AST) using different field isolates of coccidia. Nitarsone treatments, compared to the infected non-medicated controls, had varying effects on weight gain, feed conversion, lesion score and mortality against different strains of turkey *Eimeria*. Nitarsone does have activity against pathogenic turkey coccidia, as demonstrated under the conditions of these anticoccidial sensitivity tests.

INTRODUCTION

In recent years it appears that blackhead (histomoniasis) has resurfaced as a disease of concern for the commercial chicken (1) and turkey (2) industry. Blackhead is caused by the protozoan *Histomonas meleagridis*. HISTOSTAT® (3) (nitarsone, 4-nitrophenylarsonic acid) is approved for use in the feeds of chickens and turkeys at 0.01875% (170.3 grams/ton or 187.3 ppm nitarsone) as an aid in the prevention of blackhead (21 CFR §558.369). Nitarsone has been reported (4) to be effective against the causative agent of blackhead in turkeys, *H. meleagridis*. Early medication is essential to prevent spread of the disease. Blackhead is difficult to eliminate from farms with a history of the disease; preventative medication is the best way to control the disease. Nitarsone is currently the only medication marketed in the United States for prevention of blackhead. Some turkey producers that use nitarsone to prevent blackhead in their flocks have inquired if there is a risk for coccidiosis outbreaks. There are few indications that this does occur. Currently in the United States, the Food and Drug Administration has not approved the use of nitarsone in combination with any of the anticoccidials.

Boucher (5) showed that nitarsone decreased the *Cochlosoma* challenge and provided limited protection

against the effects (decreased weight gain, increased feed conversion) caused in turkeys experimentally infected with *Cochlosoma*. In the southeast and lower midwest, nitarsone is commonly used in turkey feeding programs during the brooding phase of the summer months to control *Cochlosoma* infections (6). Since it is most commonly used during the brooding phase, replacing approved anticoccidial feed medications, its anticoccidial efficacy is of interest. Mathis (7, 8) reported that roxarsone (3-NITRO®, 3-nitro-4-hydroxyphenylarsonic acid) (9) and nitarsone both have anticoccidial activity against pathogenic turkey coccidia.

MATERIALS AND METHODS

Twelve anticoccidial sensitivity tests were conducted to evaluate the efficacy of nitarsone against important species of coccidia of turkeys. Each of the 12-anticoccidial sensitivity tests was conducted separately; however, the procedures used were approximately the same. A different field isolate of coccidia was used in each test. Two-week old commercial type turkeys obtained from a commercial hatchery and kept in wire-floored, electrically heated, battery brooders were used in all tests. The turkeys were provided their experimental diets two days before infection and maintained on these treatments until the termination of the trial for a total test period of 8 days. There were four replicate pens of four or five birds each per treatment. Treatments included: controls (non-medicated and non-infected), infected controls (non-medicated) and nitarsone treated birds (infected). When nitarsone was included in the feed it was at a level of 0.01875%. This dietary level of nitarsone is approved as an aid in the prevention of blackhead in chickens and turkeys. In each trial sporulated oocysts, properly agitated and suspended in sterile distilled water, were given in amounts of 1.0 ml and inoculated directly into the turkey crop by means of a blunt needle attached to a calibrated syringe. The birds were challenged with oocysts of *Eimeria meleagrimitis*, *E. meleagrimitis* and *E. adenoeides* combined, *E. adenoeides*, and *E. dispersa*. The *Eimeria* used were isolated from samples submitted from turkey flocks in North Carolina (eleven samples) and Texas (one sample) and, represented four different commercial turkey companies. Some flocks had a history of nitarsone use. The species identification was based solely on oocyst size and location in the gastrointestinal tract.

At the termination of each trial, the surviving poults were sacrificed, necropsied and scored for gross lesions of the intestines. All birds that died during the experiments were subjected to necropsy; proper diagnoses made, and gross lesions scored. The intestinal lesions were scored at 0 = normal, 1 = slight, 2 = moderate, 3 = severe and 4 = dead. In addition, bird group weight, feed intake, feed conversion and mortality records were kept.

Each anticoccidial sensitivity test was conducted separately between 1996-1998. Data for the non-medicated non-infected control (UUC), non-medicated infected control (IUC) and nitarsone treatments were put into a file. The SAS® (10) program was used to analyze these data. The terms “test” and “trial” are used interchangeably in this text.

These tests were considered a completely randomized design. The pen was the experimental unit. The model included treatment. Treatment is a fixed effect, so the fixed effect model was used. The summary variables were weight gain, feed conversion ratio (feed to gain), average feed consumption (not included in summary), average lesion score, and mortality for each pen. Where no mortality occurred, no analysis was performed. An analysis was performed on the data using the PROC GLM procedure of SAS. All percentage data were transformed (11) (arc sin square root) prior to analysis. Back-transformed percentages were used for summary. Treatment effects were considered significant at $P < 0.05$. When treatment effects were determined to be significant ($P < 0.05$), means were separated using t-test probabilities generated by the LSMEANS option of SAS.

There were eight tests where the field isolate was *E. meleagrimitis*. In an attempt to pool these data, analyses were conducted where the model included treatment, trial and the treatment by trial interaction. There were two tests where the challenge contained both *E. meleagrimitis* and *E. adenoeides*. In an attempt to pool these data, analyses were also conducted using the data from these two tests where the model included treatment, trial and the treatment by trial interaction.

RESULTS

In most of the anticoccidial sensitivity tests where the challenge resulted in a significant ($P < 0.05$) depression in weight gain, the birds medicated with nitarsone gained weight at a rate that was generally less than the control but greater than the infected control birds. Feed conversion followed a similar pattern where those birds medicated with nitarsone had feed conversion ratios that were higher than the control but lower than the infected control birds. Intestinal lesion scores tended to be lower for those birds medicated with nitarsone compared to the infected controls. Mortality from coccidiosis occurred in only 2 of the 12 tests and no birds medicated with nitarsone died as a result of coccidiosis.

The data from those eight tests where the field isolate was *E. meleagrimitis* could not be pooled. There

were significant (P <0.05) trial by treatment interactions. The same is also true for the two tests where the challenge contained both *E. meleagrimitis* and *E. adenoeides* (Table 1).

Table 1. Anticoccidial sensitivity of nitarsone (*Eimeria meleagrimitis* and *E. adenoeides* challenge) for trial TU-8-96 and TU-28-96.

Trial and Treatments[1]	Weight gain (g)	Weight gain (%)[2]	Feed Conversion	Lesion Score	Mortality (%)[3]
TU-8-98					
UUC	295[a]	100	1.753[b]	0[c]	0[b]
IUC	187[c]	63	2.646[a]	2.2[a]	11.61[a]
Nitarsone	236[b]	80	1.888[b]	0.9[b]	0[b]
SEM[4]	12	--	0.115	0.2	3.83
TU-28-98					
UUC	373[a]	100	1.616[b]	0[c]	0
IUC	225[b]	60	2.343[a]	2.5[a]	0
Nitarsone	234[b]	63	2.279[a]	1.8[b]	0
SEM	12	--	0.172	0.1	--

[1]LSMEANS are provided for each treatment. Treatments included non-medicated non-infected control (UUC), non-medicated infected control (IUC) and infected nitarsone-medicated. When nitarsone was included in the feed it was at the level of 0.01875 %.

[2] Percentages are expressed as a proportion of the UUC treatment.

[3] Data for the percentages of mortality were transformed (arc sin square root) prior to analysis. The percentages were back-transformed for presentation.

[4] Standard error of the LSMEANS (SEM).

[a,b,c] Means within columns with different superscripts are significantly different (P < 0.05).

DISCUSSION

The degree of anticoccidial efficacy for nitarsone varied from isolate to isolate. The same thing can be observed with an anticoccidial after varied periods of use. It is reasonable that the different field isolates of *Eimeria* would differ, to some degree, in their sensitivity to nitarsone because some of them were previously exposed to the drug. Nitarsone has been used by the turkey industry for approximately fifty years.

REFERENCES

1. McDougald, L. R. Blackhead disease (histomoniasis) in chickens. Poultry Digest, September. Pp. 8-11. 1997.

2. Norton, R. A., and F. D. Clark. Histomoniasis - an unwelcome house guest returns. Turkey World, Jan - Feb. pp. 34-35. 1998.

3. HISTOSTAT is a trademark of Alpharma Inc.

4. Morehouse, N. F., and W. C. McGuire. Experiments in chemotherapy of blackhead in turkeys. Proc. Iowa. Acad. Sci. 57:475-482. 1950.

5. Boucher, M and A. Bermudez. Cochlosoma anatis infection in turkeys. Presented at AVMA/AAAP meeting. Salt Lake City, UT. July 23-26, 2000.

6. Clark, S. R. Industry methods to diagnose and control turkey coronavirus. Presented at National Turkey Federation Turkey Health Symposium: On Farm Diseases and Pathogen Control. Orlando, FL. January 7, 2000.

7. Mathis, G. F. Efficacy of arsenical feed additives against pathogenic turkey coccidia. Poultry Sci. 77 (Supplement 1): 156. 1998.

8. Mathis, G. F. Efficacy of arsenical feed additives against pathogenic turkey coccidia. Control of coccidiosis into the next millennium. Proc. Of the VII International Coccidiosis Conf. Sept. 1-5. p. 55. 1997.

9. 3-Nitro is a trademark of Alpharma Inc.

10. SAS Institute, Inc., Cary, NC, USA, 1996.

11. Steel, R. G. D., and J. H. Torrie. Principles and Procedures of Statistics. McGraw-Hill, Inc., New York, NY. 1980.

FLUORESCENCE-BASED PCR APPROACH FOR THE DIAGNOSIS OF AVIAN COCCIDIOSIS

Wayne Woods

School of Veterinary Science, The University of Melbourne, 250 Princes Highway, Werribee, Victoria, 3030. Australia

ABSTRACT

We have developed a fluorescence-based electrophoretic procedure for the identification of all seven currently recognized species of *Eimeria* which infect chickens. The second internal transcribed spacer (ITS-2) of ribosomal DNA was amplified by PCR using conserved primers labeled with fluorescent dyes. The amplicons were subjected to denaturing polyacrylamide gel electrophoresis using a 377 DNA sequencer (ABI) and analysed with GeneScan' software. Using control DNA samples extracted from multiple strains of each species, we identified peaks in the chromatograms that were diagnostic of each of the seven species, although some variation in the chromatograms was detected. Application of these results allowed us to determine the species composition of isolates from Australian and non-Australian poultry farms. The PCR step involved a single reaction for each sample, and electrophoretic reading and analysis was carried out automatically using a computer imaging system, thus making it a time- and cost-effective approach. It is well suited for high throughput screening of oocyst samples and should find applicability as a tool for prevalence studies, monitoring of coccidiosis outbreaks and the quality control of laboratory strains.

(The full manuscript of this paper is submitted to the *International Journal for Parasitology* for publication)

COMPARISON OF ORGANIC AND INORGANIC COPPER AS GROWTH STIMULANTS

Mathis, G. F.[A], A. E. (Ted) Sefton[B], and S. Collett[B]

[A]Southern Poultry Research, Inc., 2011 Brock Road, Athens, GA 30607
[B]Alltech, Inc, 3031 Catnip Hill Pike, Nicholasville, KY 40356

SUMMARY

Copper sulfate has been used as a growth stimulate in broiler production. For environmental reasons there is a desire to reduce use rates of copper and thus litter residual levels. In this trial, copper sulfate (0.75 kg/tonne) was compared to two levels of organic copper [Protide Copper (Alltech, Inc., Nicholasville KY) 0.25 and 0.5 kg/tonne] and a negative control. Both organic copper levels gave numerically improved male and female 42 day broiler body weights compared to both copper sulfate and the negative control; the higher organic copper level was statistically superior. There was a numerical trend for improved feed conversion with the organic copper although not significant. These data indicate that organic copper can replace copper sulfate as a growth stimulate while reducing litter copper levels.

INTRODUCTION

Copper sulfate has been used extensively in poultry and livestock production as a growth stimulant (1). The attraction to copper rests primarly in its low cost. However, the use of high copper levels has come under criticism and in some cases legislative pressure (2) due to the higher litter levels which in turn are reflected in higher soil copper levels when land spreading is the method of disposal. Organic trace minerals have been shown to have increased efficacy in many applications as compared to inorganic minerals (3,4,5,6).

The current study was to compare the efficacy of copper sulfate to organic copper (Protide Copper (Alltech, Inc. Nicholasville, KY) as growth stimulants.

MATERIALS AND METHODS

Broiler Chicks and Husbandry. Newly hatched straight run broiler chicks (Ross X Ross) were obtained from Seaboard Farms, Ltd., Athens, GA. The parent flock was 52 weeks of age. They were feather sexed

and placed (25 males, 25 females) at random within sex in 32 (4 treatments with 8 reps) floor pens. Pens (1.5 X 2.1 m) had 10 cm of reused litter (it had been mixed and redistributed to pens), 40 cm of fresh pine shavings was top dressed. This was done to simulate commercial conditions and to provide a challenge for the birds. Each pen had two tube-type feeders and one Plasson-type automatic waterer. Subtracting out the equipment, the bird density was 14 birds / m^2. Mortality was replaced during the first 3 days. The starter, grower, finisher feeds were formulated to be representative of commercial feeds used in the south east United States based on average calculated analysis (Agri Stats, Inc., Fort Wayne, IN). Nicarb (Nicarbazin, 125 g /tonne) was used in the starter ration, Bio-Cox (salinomycin, 60 g / tonne) in the grower and finisher rations. Again this is representative of anticoccidiosis programs used in the region. There were no growth promoting antibiotics added to the feed.

Treatments consisted of: negative control; copper sulfate 0.75 kg (185 ppm Cu) / tonne; Protide Cu (10%) 0.5 kg (25 ppm Cu)/ tonne; and Protide Cu (10%) 0.25 kg (50 ppm)/tonne.

Means and analysis of variance for weight gain and feed conversion were calculated using the General Linear Models procedure of SAS® (7).

RESULTS AND DISCUSSION

There were no sex by treatment interactions so male and female data were pooled for analysis. Copper sulfate fed birds did not differ from the control for either feed efficiency or 42-day body weight (Table 1). This would indicate that the challenge provided by the used litter was not sufficient to confer an advantage to the copper sulfate treated birds compared to the control. This was not totally unexpected. When 12,153 trials were reviewed (8) the addition of antibiotic growth promoters were found to enhance performance 72% of the time. The conditions of this trial were such the benefit of copper sulfate was not expressed.

Protide Cu at the higher level supported significantly greater growth than either the control treatment or copper sulfate. The lower Protide Cu level was intermediate. There were no statistical differences in FCR, although there was a numerical trend which may be worth further investigation with a design that would differentiate small differences in FCR as standardizing FCR by weight would give a numerical improvement which could have commercial interest.

Protide Cu gave an improvement in growth over copper sulfate, with less copper added. This in turn would mean less copper excreted, and thus less copper in the manure when it was land applied. Protide Cu thus can serve as an alternative to copper sulfate, with reduced environmental impact.

Table 1. Effect of Protide Cu and Copper Sulfate on 42-Day Broiler Performance

	Wt. Gain (kg)	FCR
Control	2.275^{b}	1.835^{a}
$CuSO_4$ 0.75 kg	2.266^{b}	1.835^{a}
Protide Cu O.25kg	2.307^{ab}	1.833^{a}
Protide Cu 0.5 kg	2.348^{a}	1.824^{a}

^{ab}Means within a column with different letters differ significantly ($P \leq 0.05$)

REFERENCES

1. Guenthner, E., and C. W. Carlson. Some effects of copper, high and low protein diets on aortic rupture and growth of turkeys. Poultry Sci. 50: 1582. 1971.

2. Spears, J. W. Review of trace mineral biological value: Environmental concerns. 24th Annual Carolina Poultry Conference. Charlotte, NC. Pg.68-77. 1971.

3. Ferket, P. R., L. Nicholson, K. D. Robertson, and C. K. Yoong. Effect of level of inorganic and organic zinc and manganese on the performance and leg abnormalities of turkey toms. Poultry Science 71 (Suppl. 1): 18. 1992.

4. Ferket, P. R., and M. A. Qureshi. Effect of level of inorganic and organic zinc and manganese on the immune function of turkey toms. Poultry Sci. 71(Suppl. 1): 60. 1992.

5. Kidd, M. T., N. B. Anthony, L. A. Newberry, and S. R. Lee. Effect of supplemental zinc in either a corn soybean or a milo and corn-soybean meal diet on the performance of young broiler breeders and their progeny. Poultry Sci. 72: 1492-1499. 1993.

6. Edens, F. W., T. A. Carter, and A. E. Sefton. Influence of dietary selenium sources on post mortem drip loss from breast meat of broilers grown on different litters. Poultry Sci. 75(Suppl.): 60. 1996.

7. SAS Institute. SAS/STATÒ User's Guide, Version 6, Fourth Edition. SAS Institute Inc., Cary, NC. 1989.

8. Rosen, G. D. The nutritional effects of tetracyclines in broiler feeds. Proceedings World's Poultry Congress. New Dehli vol II pg. 141-146. 1996.

INFLUENCE OF ORGANIC SELENIUM ON HSP70 RESPONSE OF HEAT-STRESSED AND ENTEROPATHOGENIC ESCHERICHIA COLI-CHALLENGED BROILER CHICKENS

F. W. Edens, P. E. N. Givisiez, K. Z. Mahmoud

North Carolina State University, Raleigh, North Carolina 27695-7635, and A. E. Sefton, Alltech Biotechnology Center, Nicholasville, KY40356

Heat shock protein 70 (HSP70) plays a major role in cellular adaptive responses to a number of stressors (1-3). Exposure of poultry species to mild stressors over a period of time enhances HSP70 expression, but eventually, the birds become acclimated and no further increase in cellular HSP70 can be demonstrated (3). However, one of the negative aspects of induction of HSP70 in response to a stressor is the fact that HSPs are synthesized preferentially to other cellular proteins (1-3).

In June 2000, organic selenium yeast (Sel Plex, Alltech, Inc., Nicholasville, KY 40356)) was approved by the US Food and Drug Administration as a source for feed-supplemented selenium for chickens. Selenium is an essential trace element that is involved in the regulation and control of the body's antioxidant glutathione and glutathione peroxidase system. This system plays a major role in the control of reactive oxygen metabolites (ROM; 4). It has been reported that selenium supplementation to chickens improved their resistance to the stress caused by *E. coli* infection and by exposure to cold (5). Exposure of animals to thermal extremes and bacterial infection are stressors that increase metabolic activity with a resultant increase in ROM production (4). HSP70 is also involved in mechanisms protecting the body from the deleterious effects of ROM (6,7). Furthermore, there are data that suggest a strong relationship between thiol oxidation and HSP70 synthesis in stressed cells (8).

Therefore, it was important to explore the possibility that selenium might be playing a role in the induction of heat shock proteins as well as playing a role in the glutathione system. Studies were conducted to determine the influence of Sel Plex supplementation to broiler chickens on the induction of HSP70 and glutathione response either to a bacterial challenge or to an acute heat distress.

MATERIALS AND METHODS

Broiler chickens and husbandry. Newly hatched male broiler chickens (Arbor Acres X Arbor Acres) were obtained from the North Carolina Agricultural Research Service Poultry Field Research Laboratory hatchery and reared in an environmentally-controlled isolation facility. The chicks were neck-tagged and placed in heated brooder-grower batteries with raised wire floors. Brooding temperature was 95 F (35 C), and this temperature was decreased incrementally (4 C at a time) to 70 F (20 C) by the time the birds were 21 days old. The photoperiod was 23 hours light and 1 hour darkness.

The experimental diets consisted of a Starter diet- 3177 kcal/kg ME, 22.5% CP (1-16 days of age), Grower diet- 3168 kcal/kg, 19.5% CP (16-35 days of age), and Finisher diet- 3160 kcal/kg, 17.5% CP (35-42 days of age). The diets were supplemented with organic selenium (Sel Plex, Alltech, Inc., Nicholasville, KY 40356) at a selenium level of 0.2 mg/kg of feed. The background levels of selenium were 0.28 mg/kg, 0.28 mg/kg, and 0.24 mg/kg, respectively, in the starter, grower and finisher diets. Body weights and feed conversions (FCR) were determined at 21 and 42 days of age (42 days data are presented here), and mortality was recorded on a daily basis.

Two different 2 x 2 factorially arranged studies were conducted and involved 60 chicks per treatment group in each study. The first involved a day one post-hatch intranasal challenge by an enteropathogenic *Escherichia coli*, and the second involved an acute heat challenge to chicks that had been raised in a thermoneutral environment.

Enteropathogenic *Escherichia coli*. The enteropathogenic *E. coli* (serotype O1, EPEC) was grown overnight at 37 C in trypticase soy broth. The EPEC challenge dose (10^6 CFU/mL) was administered in a volume of 100 μL into the external nares to 1d old chicks. There were four treatment groups: 1. No supplemental selenium, no EPEC (sterile trypticase broth); 2. Sel Plex, no EPEC (sterile trypticase soy broth); 3. No supplemental selenium + EPEC; 4. Sel Plex + EPEC. The EPEC challenge was boosted on days 2, 3, and 4 post hatch by adding 15 mL of the culture (10 X 10^6 CFU/ mL) to the drinking water.

Heat distress. The treatment groups are described as follows: 1. Control (no supplemental selenium, no heat distress); 2. Sel Plex, no heat; 3. Heat distress control (no supplemental selenium + heat distress); 4. Sel Plex + heat distress. At 42 days of age, 20 birds per

groups were subjected to heat distress by a one hour exposure to an elevated ambient temperature (40 C) in a temperature controlled chamber. Mortality was monitored, but there were no deaths during the short exposure period.

Tissue sampling and preparation. In each of the two separate studies, 20 birds per treatment group were killed by carbon dioxide asphyxiation. In the EPEC challenge study, a 1 g sample of the liver and two 1 g ileum samples were dissected and placed into ice-cold protein buffer. The tissues were washed three times to rinse away blood and were then homogenized. Supernatants were collected after a 1 hr 100,000 x G centrifugation. The total protein content was determined for each sample. Total, oxidized and reduced glutathione, and the Reduced:Oxidized glutathione ratios (R:O) were determined in the ileum (EPEC challenge study) and blood (heat distress study) colorimetric assays modified for a plate reader (OXIS International, Inc., Portland, OR 97217-3935). The heat shock protein 70 (HSP70) determinations were conducted with procedures using an enzyme-linked immunosorbent assay (9).

Statistical analysis. The HSP70 data were analyzed as a completely randomized design using the general linear models procedure of the statistical analysis system (10). When significant (P#0.05), main effect means were separated using least significant difference (LSD; 10).

RESULTS

There were significant effects due to Sel Plex and EPEC challenge (Table 1). Broilers given Sel Plex were significantly heavier, had improved FCR and lower mortality rates than birds not given a selenium supplement. Additionally, there was a Sel Plex X E. coli interaction in which, Sel Plex supplemented broilers with EPEC challenge were significantly heavier, had improved FCR, and lower mortality than EPEC challenged broilers without the selenium. In the heat distress study, Sel Plex-supplemented broilers had greater BW (2291g vs. 2004g), improved FCR, (1.84 vs. 1.98) and reduced mortality (5.7% vs. 18.3%) compared to those broilers with no selenium supplementation. There were no mortalities in broilers subjected to acute heat distress.

The HSP70 concentrations in the ileum from EPEC-challenged broilers were elevated significantly in both Sel Plex (Sel Plex, no *E. coli*- 0.305^{c} and Sel Plex + *E. coli*- 0.806^{b} in OD/ng total protein) and No Selenium (No selenium, no *E. coli*- 0.277^{c} and No selenium + *E. coli*- 1.229^{a} in OD/ng total protein) groups. Sel Plex supplementation allowed a smaller ileum HSP70 elevation when the broilers were challenged. Hepatic HSP70 was not affected significantly either by EPEC challenge or by selenium. With heat distress, Sel Plex compared with No Selenium supplementation was associated with significantly lower constitutive (0.90^{c} vs 1.04^{b} OD/ng total protein) and inducible (0.93^{c} vs. 1.12^{a} OD/ng total protein) hepatic HSP70 concentrations.

EPEC challenge increased ileum total glutathione content, but selenium did not influence that response (Table 2). Oxidized (GSSG) and reduced (GSH) glutathione in the ileum were elevated significantly by EPEC challenge, and Sel Plex treatment increased ileum GSSG in both challenged and nonchallenged birds. The concentration of GSH was increased significantly by EPEC challenge but was not affected by selenium. The ratios between GSH and GSSG (R:O) were elevated significantly in those birds given the diets without supplemental selenium in comparison to the ratios for birds in the Sel Plex groups.

Neither heat distress nor Sel Plex had any influence on either total blood glutathione or GSH concentrations (Table 3). However, blood GSSG was elevated by both heat distress and Sel Plex supplementation resulting in lower R:O ratios.

DISCUSSION

The National Research Council established 0.1 ppm of selenium as the minimum necessary to sustain growth and performance in broiler chickens (11), and the background selenium levels in the feeds used in these studies (0.24 to 0.28 ppm) ostensibly were adequate. However, those background levels were not sufficient to allow broilers to attain the same BW performance as those given Sel Plex. The FCR and mortality rates also were elevated significantly when the birds were given diets with less than adequate selenium, and those birds ultimately suffered from the development of a stress reaction characterized by elevated tissue concentrations of HSP70. Based upon the HSP70 response, one could conclude that consumption of inadequate levels of dietary selenium conferred the ability to resist some stressors, but that may be an inappropriate conclusion.

The expression of HSP70 is a classical sign of stress in animals because it is the physical manifestation of specific genes that are induced to combat stressors (1-3,7). However, there is a cost for this response, and it is paid in reduced growth due to a lower rate of synthesis of structural proteins in chronically stressed animals. In the studies reported herein, broilers given diets without supplemental selenium had higher constitutive levels of HSP70 than those given organic selenium as Sel Plex. Additionally, when those animals were subjected either to an EPEC challenge or to an acute heat distress, the inducible HSP70 concentrations were significantly greater in

those birds given diets without supplemental selenium. One could conclude that selenium inhibited development of the HSP70 response, making those birds receiving it more susceptible to the deleterious effects of stressors. One could also conclude that inadequate dietary selenium is a stressor in its own right and increases the level of stress proteins and reduced growth.

However, the glutathione redox cycle must be considered because HSP70 expression is responsive to increased thiol oxidation (GSSG production) and loss of cellular GSH as indicated by a lower R:O ratio (8). Normally, oxidative stress will induce an increase in blood and tissue concentrations of GSH (12) consistent with the observations reported here. The increased GSH can then react with ROM leading to the formation of GSSG. This was also found in treatments with EPEC and heat distress and would be consistent with increased ROM. The increase in GSSG would then induce increased levels of HSP70 as was apparent in those groups of animals given diets with inadequate selenium. However, with Sel Plex, the ileum HSP70 concentration in EPEC challenged birds and hepatic concentrations in heat-distressed birds were decreased significantly. This was contrary to the expected response, but may point to the mechanism whereby lower HSP70 indicated improved tolerance to stressors.

Although not measured in these studies, glutathione peroxidase activity is elevated significantly with the addition of organic selenium to poultry diets (12). Reduction of organic peroxides by glutathione is catalyzed by glutathione peroxidase and leads to increased oxidation of GSH to GSSG (13). Normally, within a few days after chickens begin to consume Sel Plex, maximal glutathione peroxidase activity can be achieved. Higher glutathione peroxidase activity in Sel Plex fed broilers will catalyze a more rapid oxidation of GSH to GSSH consistent with observations in these studies. Normally, GSSG is reduced back to GSH by GSH reductase. Reduction of GSSG back to GSH can be limited by the glutathione reductase activity if there is a rapid build-up of GSSG. In fact, there was an accumulation of GSSG in the Sel Plex fed broilers indicating a more efficient clearing of ROM in those birds. If it is true that accumulation of GSSG increases HSP70 expression (8), then tissues from stressed chickens should have shown an increased HSP70 concentrations, but that was not the condition in Sel Plex-fed broilers. It is speculated that early in the Sel Plex feeding regimen, HSP70 induction may have occurred. However, induction of HSP70 is an acute phase response that cannot be tolerated for long periods of time without resulting in the slowing of growth and performance in animals. Previous work from this laboratory (3) has shown that with chronic exposure to heat distress, HSP70 expression decreased as the birds become acclimated. It is hypothesized that the feeding of Sel Plex has conferred stress resistance by causing an early HSP70 response that later developed into an acclimation condition. In the long term of the six weeks growing period for those Sel Plex-fed broilers, their resistance to stressors was improved as indicated by superior performance traits and a lower HSP70 production.

REFERENCES

1. Edens, F. W., C. H. Hill, and S. Wang. Heat shock protein response in phosphorus-deficient heat-stressed broiler chickens. Comp. Biochem. Physiol. 103B: 827-831. 1992.

2. Wang, S. and F. W. Edens. Hsp70 mRNA expression in heat stressed chickens. Com. Biochem. Physiol. 107B: 33-37. 1994.

3. Wang, S, and F. W. Edens. Heat conditioning induces heat shock proteins in broiler chickens and turkey poults. Poultry Sci. 77: 1636-1645. 1998.

4. Palmer, H. J., and K. E. Paulson. Reactive oxygen species and antioxidants in signal transduction and gene expression. Nutri. Rev. 55: 353-361. 1997.

5. Larsen, C. T., F. W. Pierson, and W. B. Gross. Effect of dietary selenium on the response of stressed and unstressed chickens to Escherichia coli challenge and antigen. Biol. Trace Elem. Res. 58: 169-176. 1997.

6. Favatier, F, L. Bornman, L. E. Hightower, E. Günther, and B. S. Polla. Variation in hsp gene expression and Hsp polymorphism: do they contribute to differential disease susceptibility and stress tolerance? Cell Stress & Chaperones, 2: 141-155. 1997.

7. Polla, B. S. A role for heat shock protein in inflammation. Immunology Today, 9:134-137. 1998.

8. Sierra-Rivera, E., M. J. Meredith, G. J. Voorhees, L. W. Oberley, D. R. Eisert, and M. L. Freeman. Synthesis of heat shock proteins following oxidative challenge: role of glutathione. Int. J Hyperthermia 10: 573-586. 1988.

9. Anderson, R. L., C.-T. Wang, I. Van Kersen, K.-J. Lee, W. J. Welch, P. Lavagnini, and G. M. Hahn. An Immunoassay for heat shock protein 73/72: use of the assay to correlate HSP77/72 levels in mammalian cells with heat response. Int. J. Hyperthermia 9: 539-552. 1993.

10. SAS Institute. SAS/STAT User's Guide: Statistics. Version 6, 4th ed. SAS Institute, Cary, NC USA. 1996.

11. National Research Council. Nutrient Requirements of Poultry. 9th Revised Edition, National Academy Press, Washington, D.C. 1994.

12. Cantor, A. H., P. D. Moorhead, and M. S. Musser. Comparative effects of sodium selenite and selenomethionine upon nutritional muscular dystrophy, selenium-dependent glutathione peroxidase and tissue

selenium concentrations of turkey poults. Poultry Sci. 61: 478-484. 1982.

13. Burk, R. F., K. Nishiki, R. A. Lawrence, and B. Chance. Peroxide removal by selenium-dependent and selenium-independent peroxidases in hemoglobin-free perfused rat liver. J. Biol. Chem. 253:43-56. 1978.

Table 1. Performance at 42 days of age of male broiler chickens given a dietary supplement of organic selenium and challenged with an enteropathogenic *E. coli*.

TREATMENTS	BODY WEIGHT, g	FCR, g/g	MORTALITY, %
No Selenium, no E. coli	2047∀86[bc]	1.95[b]	16.7[b] (10/60)[1]
Sel Plex, no E. coli	2283∀67[a]	1.82[c]	5.0[c] (3/60)
No Selenium + E. coli	1896∀95[c]	2.09[a]	36.7[a] (22/60)
Sel Plex + E. coli	2098∀71[b]	1.93[b]	15.0[b] (9/60)

[a,b,c] In a column, means with unlike superscripts differ significantly (P#0.05).
[1]Numbers in parentheses represents total mortality per treatment group.

Table 2. Influence of Sel Plex on intestinal total glutathione (μM/g), oxidized glutathione (μM/g), reduced glutathione (μM/g), and the Reduced:Oxidized glutathione ratios in broiler chickens given a dietary supplement of organic selenium and challenged with an enteropathogenic *E. coli*.

Treatments	Total Glutathione	Oxidized (GSSG)	Reduced (GSH)	Ratio R:O
No Selenium, no E. coli	18954∀1904[ab]	1601∀196[c]	13349∀1835[b]	8.32∀1.05[a]
No Selenium + E. coli	21896∀1971[a]	3047∀180[a]	19099∀1688[a]	7.30∀1.44[ab]
Sel Plex, no E. coli	17045∀1843[b]	2238∀174[b]	13322∀1627[b]	5.95∀1.01[b]
Sel Plex + E. coli	22500∀1971[a]	3313∀188[a]	18911∀1757[a]	5.71∀1.09[b]

[a,b,c] In a column, means with unlike superscripts differ significantly (P#0.05).

Table 3. Influence of Sel Plex on blood total glutathione (μM/L), oxidized glutathione (μM/L), reduced glutathione (μM/L), and the Reduced:Oxidized glutathione ratios in broiler chickens given a dietary supplement of organic selenium and subjected to acute heat distress.

Treatments	Total Glutathione	Oxidized (GSSG)	Reduced (GSH)	Ratio R:O
Preheat, No Selenium	1715∀51[a]	49∀3[c]	1617∀52[a]	33∀3[a]
Heat, No Selenium	1817∀45[a]	69∀4[a]	1619∀47[a]	23∀3[bc]
Preheat, Sel Plex	1776∀46[a]	60∀4[b]	1646∀48[a]	27∀3[b]
Heat, Sel Plex	1821∀45[a]	91∀7[a]	1639∀47[a]	18∀3[c]

[a,b,c] In a column, means with unlike superscripts differ significantly (P#0.05).

LIVE COCCIDIOSIS VACCINATION: FIELD COCCIDIAL POPULATION CHANGES FOLLOWING VACCINATION

Linnea J. Newman[A] and Harry D. Danforth[B]

[A]Schering-Plough Animal Health, Union, NJ 07083
[B]USDA-ARS, Beltsville, MD 20705

INTRODUCTION

Dr. David Chapman (1) and Dr. Harry Danforth (2) have demonstrated via laboratory sensitivity studies that vaccination with a live sporulated coccidial oocyst vaccine (Coccivac-B) can modify the anticoccidial sensitivity of the field population of coccidial oocysts. Dr. Danforth's research has further demonstrated a reduction in the pathogenicity of field oocyst populations following vaccination.

These laboratory studies have never been corroborated by similar observations under field conditions. The following study details the field observations made at three large integrated broiler complexes over the course of 6 months of vaccination with Coccivac-B.

Change in oocyst population based upon sequential post-mortem sessions. Companies which initiate live coccidial oocyst vaccination are monitored with regular post-mortem sessions. Each session

involves 40 to 60 birds from flocks ranging in age from 14 to 42 days. Gross lesions are monitored using the Johnson and Reid scoring system for *Eimeria acervulina* and *Eimeria tenella*. *Eimeria maxima* scores are based upon microscopic observation of oocysts from a scraping of the midgut mucosa. The *E. maxima* microscopic scoring system is as follows:

+1 = 5 – 100 oocysts per 100X field
+2 = $\geq$ 100 oocysts per 100 X field
+3 = Too numerous to count (TNTC) oocysts per 100X field + gross lesions such as intestinal ballooning, mild enteritis or petechial hemorrhage.
+4 = TNTC + severe gross enteritis or hemorrhage.

This scoring system was developed by technical service veterinarians at Schering-Plough. The scoring system is designed to correlate microscopic scraping oocyst counts to actual gross pathology and to the probable impact of *E. maxima* on weight gain and feed conversion under field conditions.

It was determined that a *microscopic* scoring system was needed for *E. maxima* because oocyst counts greater than 100 could be observed without any gross lesions or enteritis in the affected birds.

The acceptable standard lesion scores for a Coccivac-B post-vaccination reaction are summarized in Table 1.

Broiler Integrators A and B were followed with field post-mortem exams from the beginning of the vaccination program in the spring of 2000 through the end of vaccination in the fall of 2000. Broiler Integrator A had a high percentage of *E. acervulina* lesions with scores of +1 to +3 at the beginning of the study. Broiler Integrator B had a high percentage of *E. maxima* lesions with scores of +1 to +3 at the beginning of the study. Both groups showed a marked decline in lesions from the first vaccinated growout cycle to the third vaccinated growout cycle. Results are summarized in table 2.

Broiler Integrator C had *E. tenella* lesion scores of +1 and +2 while using an ionophore program (salinomycin) in the fall of 1999. This complex was also followed with field post-mortem exams from the beginning of the vaccination program in the spring of 2000 through the end of vaccination in the fall of 2000. This group demonstrated a decline in *E. tenella* lesion scores from the first vaccinated growout cycle to the third vaccinated growout cycle, and in fact, demonstrated fewer lesions in vaccinated birds by the final growout cycle than had been observed on the ionophore program the prior fall. Results are summarized in table 3.

Change in oocyst population based upon laboratory sensitivity testing. Integrators B and C submitted litter samples to Dr. Harry Danforth (USDA-ARS laboratory, Beltsville, MD) for oocyst sensitivity testing before and after the vaccination program. Oocysts were isolated from the litter samples and a mixed population challenge dose was determined based upon a weight loss titration. SPF leghorns were fed 60 ppm salinomycin from day 1 through day 16. At day 10, chicks were weighed and then challenged with the field oocysts. At 6 days post-challenge, groups were weighed again and weights were compared to unchallenged salinomycin-fed controls. Poor weight gain in challenged birds compared to unchallenged salinomycin-fed controls indicates oocyst resistance to salinomycin.

Compared to unchallenged salinomycin-fed controls, the coccidial isolates from Integrator B showed moderate salinomycin resistance prior to vaccination. Following vaccination, the severity of salinomycin resistance was reduced, but resistance was still moderate, with weight gain at 77% of unchallenged controls. Results are summarized in table 4.

Compared to unchallenged salinomycin-fed controls, the coccidial isolates from Integrator C also showed moderate salinomycin resistance prior to vaccination. Following vaccination, the salinomycin sensitivity was greatly improved, as demonstrated by weight gain at 90% of unchallenged controls. Results are summarized in table 5.

SUMMARY

Field observation and laboratory sensitivity studies combined to demonstrate the ability of live oocyst vaccination with Coccivac-B to reduce salinomycin resistance and the observed lesion scores from field coccidial populations.

REFERENCES

1. Chapman, H.D. 1994. Sensitivity of Field Isolates of *Eimeria* to Monensin Following the Use of a Coccidiosis Vaccine in Broiler Chickens. Poultry Science 73:476-478.

2. Danforth, H.D., Shirley, M. 2000. Use of Polymerase Chain Reaction-Based Techniques to Identify Avian Coccidia. AAAP Symposium on Molecular Identification and Epidemiology of Avian Pathogens, 137th AVMA Convention, Salt Lake City, UT, pp 21-22.

Table 1. Acceptable Standard Lesion Scores For Coccivac-B Post-Vaccination Reaction

Coccidial species	+1	+2	+3	+4
E. acervulina	<30%	<10%	0	0
E. maxima[1]	<70%	<10%	0	0
E. tenella	Combined +1 and +2 < 10%		0	0

[1]Based upon microscopic oocyst scores

Table 2. Broiler Integrators A and B field post-mortem exam summary

Broiler Integrator	Coccidial species	Vaccinated Growout Cycle	Coccidial Lesion Score +1	+2	+3	+4
A	*E. acervulina*	1 (spring 2000)	64%	2%	2%	0
A	*E. acervulina*	3 (fall 2000)	16%	2%	0	0
B	*E. maxima*[1]	1 (spring 2000)	20%	6%	6%	1%
B	*E. maxima*[1]	3 (fall 2000)	23%	3%	0	0

[1]Based upon microscopic oocyst scores

Table 3. Broiler Integrator C field post-mortem exam summary

Broiler Integrator	Coccidial species	Anticoccidial Treatment/growout cycle	Coccidial Lesion Score +1	+2	+3	+4
C	*E. tenella*	salinomycin (fall 1999)	6%	4%	0	0
C	*E. tenella*	vaccination 1 (spring 2000)	10%	6%	0	0
C	*E. tenella*	vaccination 3 (fall 2000)	0	0	0	0

Table 4. Laboratory oocyst sensitivity study: Integrator B weight gain

Group	Challenge	salinomycin 60ppm	Average weight Gain (gm)	Weight gain as a % of unchallenged control weight
Integrator B (before vaccination)	+	-	123.1	51%
Integrator B (before vaccination)	+	+	155.6	64%
Control	-	+	242.0	100%
Integrator B (after vaccination)	+	-	153.5	51%
Integrator B (after vaccination)	+	+	231.0	77%
Control	-	+	301.0	100%

Table 5. Laboratory oocyst sensitivity study: Integrator C weight gain

Group	Challenge	salinomycin 60ppm	Average weight Gain (gm)	Weight gain as a % of unchallenged control weight
Integrator C (before vaccination)	+	-	127.74	53%
Integrator C (before vaccination)	+	+	163.2	67%
Control	-	+	242.0	100%
Integrator C (after vaccination)	+	-	177.0	59%
Integrator C (after vaccination)	+	+	271.0	90%
Control	-	+	301.0	100%

PASTEURELLA MULTOCIDA: TO SEROTYPE OR FINGERPRINT?

BR Charlton[A], MC Bland[A], GL Cooper[A], AA Bickford[A], GY Ghazikhanian[B] and MA Wilson[C]

[A]CAHFS Turlock, PO Box 1522, Turlock, CA 95381
[B]Nicholas Turkey Breeding Farm, PO Box Y, Sonoma, CA 95476
[C]National Veterinary Services Laboratories, 1800 Dayton Av, P.O. Box 844, Ames, IA 50010

Fowl Cholera is an infectious bacterial disease of poultry that can occur either as an acute septicemic form or a chronic localized form. The acute form is the predominant form which occurs in turkeys. Clinical signs of the acute form in turkeys are often are not observed except for sudden death. The most frequent lesions observed include those associated with septicemia, (hyperemic vessels, petechial and ecchymotic hemorrhages), pneumonia and in mature hens yolk peritonitis. Immediate control of fowl cholera involves prompt treatment with the appropriate antimicrobial agent. Long term control and prevention of fowl cholera is largely dependent on management. This includes a good biosecurity program of cleaning and disinfection, rodent control, traffic control, prompt and appropriate dead bird disposal; to mention only a few of the essential components. An appropriate vaccination program may also be utilized to provide some control of fowl cholera and may consist of either live vaccines or bacterins. To obtain full benefit of vaccination, an understanding of the antigenic variability of *P. multocida* is necessary.

P. multocida may be grouped either by capsular antigens (i.e. serogroups) or somatic antigens (i.e. serotypes). Somatic serotyping has been done most frequently with about 16 different serotypes recognized. Serotype 3,4 has historically been deemed the most important and this serotype is incorporated into commercial live *Pasteurella multocida* vaccines. Other serotypes may be incorporated into a vaccine if an autogenous bacterin is produced. Protection is primarily against autologous strains.

Determination of the serotype of *P. multocida* can be done by either the tube agglutination test or the gel diffusion precipitin test. There is a lack of correlation between the two tests (1). The gel diffusion precipitin test is more commonly performed due to its ease of performance and to its correlation with the immune response in chickens and turkeys (1). Additional tests have been developed to group *P. multocida* isolates; several of which have been reviewed by Dr. PJ Blackall (2). One of these tests is the restriction endonuclease (RE) analysis test of the genome of *Pasteurella multocida* offered by the National Veterinary Service Laboratory (NVSL) in Ames, Iowa (3). The following information is presented to demonstrate the benefits of the RE analysis method over the routine serotyping method.

A major turkey producer in the Central Valley of California had a series of fowl cholera outbreaks in both meat and breeder flocks. The primary focus of this report will be on the breeder flock outbreaks, which occurred from Dec 1998 to Sept 2000. The cholera vaccination program consisted of an autogenous bacterin containing *P. multocida* serotypes 3,4,7,10,12 was given at 18, 22 and 26 weeks of age. The M-9 strain was instituted to specific flocks after experiencing severe weekly mortality rates of 3-5%

and total flock mortality of 15 to 30%. Initially, after 12 isolations and serotyping, no consistent epidemiologic pattern could be discerned. Five isolates from four different ranches were sent to NVSL in Ames, Iowa for restriction endonuclease (RE) analysis. Two RE patterns were obtained and suggested cross-contamination between these ranches.

Currently, thirty-six isolations of *P. multocida* have been made from 24 different flocks. Serotyping results of these 36 isolates gave 10 different serotype combinations. Of the seven flocks that had multiple isolates of *P. multocida* (2 to 5 isolations/flock), six flocks had multiple serotypes present within the same flock. Again, no consistent epidemiologic pattern could be established based on serotype results.

RE analysis was performed on 35 of the 36 *P. multocida* isolates. Three RE patterns (Td45, 1001 and 1128) were detected. RE pattern 1128 was detected in two isolations from one flock at the beginning of the severe FC problem and, in retrospect examination, is similar to previous *P. multocida* isolates outside of the time frame of this study. RE pattern Td45 is the pattern associated with the live vaccine strains (CU strain, M9 strain and PM-1 strain). This pattern was obtained from 11 isolates (9 flocks) and had nine isolates with serotype 3, 4; one isolate with serotype 3; and one isolate with serotype 3,4,12. The predominant RE pattern was pattern 1001 obtained from 22 isolates (15 flocks). Six different serotyping patterns were associated with this RE pattern with serotype 3,4 being the most frequent (11 isolates).

Information of the RE analysis results strongly suggests that the FC outbreaks were associated with previous outbreaks within the company. This information could not have been obtained from the serotyping results. Even within one flock in which five *P. multocida* isolates were serotyped and four of these isolates were analyzed by RE, three serotype patterns were detected in this one flock as compared to one RE pattern. The information gained from the RE analysis of the *P. multocida* facilitated a critical evaluation of the companies management practices to bring this infectious disease under control. It must be remembered that the serotype test is dependent on the expression of antigenic determinants while the RE analysis is dependent on the DNA content of the organism.

REFERENCES

1. R.B. Rimler and J.R. Glisson. Fowl Cholera. In: Diseases of Poultry, 10th ed. B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald, and Y.M. Saif, eds. Iowa State University Press, Ames, Iowa. pp. 143-159. 1997.

2. P.J. Blackall and J.K. Miflin. Identification and typing of Pasteurella multocida: a review. Avian Path 29:
271-287. 2000.

3. Wilson, M.A., M.J. Morgan, and G.E. Barger. Comparison of DNA fingerprinting and serotyping for identification of avian Pasteurella multocida isolates. J Clin Microbiol. 31:255-9. 1993.

NECROTIC TRACHEITIS IN TURKEYS

Gabriel Senties-Cue, Rocio Crespo, and R.P. Chin

California Animal Health and Food Safety Laboratory System, Fresno Branch,
University of California, Davis, 2789 S. Orange Ave., Fresno, CA 93725

A sudden increase in mortality (up to 8.4% per week) was observed in 8- to 10-week-old turkeys from a commercial flock, kept on range. The turkeys had been treated with double the normal dose of copper sulfate in the water for a week (normal is 1:2000 dilution in drinking water). The birds were not vaccinated against any disease pathogen. Two weeks before submission to the California Animal Health and Food Safety Laboratory System, Fresno Branch, the owner lost about 10% of the turkeys on the farm due to heavy rains. At necropsy the most striking lesion was a necrotic band (1cm in length) in the upper third of the trachea, with destruction of the cartilage rings at that site. When the tracheas were opened, severe accumulation of necrotic exudate was observed in the tracheal lumen, blocking almost completely its lumen. Necrosis extended into the tracheal wall. Also, tracheas were moderately flattened throughout their length. Other necropsy findings included moderate airsacculitis and pneumonia.

Microscopically, there was a severe, extensive, transmural necrosis of the trachea; and accumulation of necrotic debris and fibrinopurulent exudate in the tracheal lumen. Bacterial colonies were seen within the necrotic debris of the tracheal lumen, as well as in the tracheal mucosa and lamina propria. Numerous inflammatory cells, mainly heterophils, macrophages, multinucleated giant cells, and occasional lymphocytes

infiltrated all layers of the trachea, from the mucosa to the serosa. Where the epithelium was still present, there was complete deciliation and flattening of epithelial cells. In the muscularis and serosa layer there was proliferation of fibrous tissue and neovascularization.

Mixed bacteria, including *Escherichia coli*, *Ornithobacterium rhinotracheale*, *Pseudomonas aeruginosa*, and *Bordetella hinzii* were isolated from the trachea. Turkeys were seropositive to *B. avium*. No viruses were identified by electron microscopy and virus isolation.

Since the birds had been exposed to high levels of copper sulfate, the hepatic levels of copper and other heavy metals were determined. High levels of copper in the diet may cause ulceration of the mucosa of the digestive tract (2). No significant lesions were seen in the digestive tract of these birds and the concentration of heavy metals in the liver, including copper, was within the normal range.

The pathology of the tracheas is suggestive of exposure to chemical aerosol. The bacteria isolated are probably opportunistic pathogens. The seropositive titers to *B. avium* and flattening of the trachea indicate previous exposure to this bacterium, which may have predisposed the tracheas to this severe necrosis. In 1995, we observed similar lesions in a flock of 3-week-old turkeys that was exposed to a cotton defoliant. In the present case, the turkeys were outdoors and 400 feet away from a cotton field that was probably just defoliated, based on the time of the year. The most common cotton defoliants used in the Fresno County include cacodylic acid (arsenic), dimethipin, endothall, paraquat, sodium chlorate, thidiazuron, tribufos and thidiazuron. Acute exposure to these chemicals may cause irritation and hemorrhages of the respiratory tract and other organs, and even death (1,3,4). If animals survive the chemical exposure, damage to the respiratory mucosa would be expected, which may predispose them to secondary bacteria infection. Unfortunately, paraquat was the only cotton defoliant that could be tested for from the respiratory tract, and it was not detected in a trachea/lung pool. In addition, the livers did not contain a detectable amount (1ppm or more) of arsenic. The exact cause of this severe necrotic tracheitis has not yet been determined.

(The full-length article will be submitted to *Avian Diseases*)

REFERENCES

1. Haschetz, W. M., and H. R. Witchi. Respiratory system. In: Handbook of Toxicology Pathology. W. M. Haschetz, and C. G. Rousseaux, eds. Academic Press, San Diego, California. pp. 761-827. 1991.

2. Julian, R. J., and T. P. Brown. Other toxins and poisons. In: Diseases of Poultry, 10th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Saif, eds. Iowa State University Press, Ames, Iowa. pp. 978-1005. 1997.

3. Osweiler, G. D., T. L. Carson, W. B. Buck, G. A. Van Gelder, eds. Arsenic I: Inorganic, aliphatic and trivalent organic arsenicals. In: Clinical and Diagnostic Veterinary Toxicology, 3rd ed. Kendall/Hunt Publishing Co., Dubuque, Iowa. pp. 72-79. 1985.

4. Weed Science Society of America, and Herbicide Handbook Committee, eds. Herbicide Handbook, 7th ed. Champaign, IL. pp. 10-59. 1994.

PRACTICAL SPF SENTINEL BIRD USE

Sharon Heins Miller

Merial Select, P.O. Drawer 2497, Gainesville, Georgia 30503

With the development and improvement of polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) technology, the ability to identify infectious bronchitis virus (IBV) serotypes from commercial production respiratory breaks has improved. One problem that occurs when identifying the causes of these breaks is obtaining bird samples while the virus is still present in the respiratory tissue. For this reason, the use of specific pathogen free (SPF) birds for the isolation and identification of IBV serotypes has increased over recent years. The purpose of this presentation is to describe the proper method for placement of these SPF sentinel birds and sample collections of tissues to obtain the best samples for IBV serotype identification.

There are two methods of using these birds: unvaccinated or vaccinated. The unvaccinated birds can be obtained quickly when a break occurs, but are susceptible to any and all viruses in the house including vaccine strains. The vaccinated birds require preparation time. They are vaccinated with the current vaccination program being used on the farm on which the birds are to be placed. The birds must be held for three weeks in isolation to allow the vaccine virus(es)

to clear so as to not isolate these same serotypes on testing. The advantage of using vaccinated birds is the ability to place these birds closer to field vaccination and to improve isolation of variant IBV serotypes.

Typically, the SPF bird placed for tissue collection is three to five weeks of age. Younger birds can be used, but the tissues are very small and difficult to handle. The sentinel birds are usually placed on a farm with a previous history of respiratory problems. The birds should be placed at the time the flock is expected to break. Though one should avoid placing sentinel birds on farms that have been vaccinated within three weeks of sentinel bird arrival. Again, the sentinels are very susceptible to all viruses including the common vaccine strains.

Normally, 10 to 14 birds are placed in a house. The birds should be placed in a cage with wire sides and access to litter to maximize bird exposure. The cage should have a top and wire spacing small enough to prevent escape. The sentinel birds are approximately the size of a one-week-old broiler. We have placed birds in a commercial layer facility. In this case, we placed the pen on top of one row of cages to allow aerosol exposure. In either situation, the birds should be placed near the center of the house with access to feed and water. The birds should have their own feed and water source (usually the nipple lines are difficult to reach). The feed should contain coccidiostat!

Tissue samples should be collected from half the sentinel birds at 5 days and the other half at 10 days after placement. Two collection periods offer the best chance at "catching" the IBV in the tissues. The two collections are later combined before virus isolation is performed. Combining the samples will give one the same results and help reduce the cost of testing, i.e., one cost per farm instead of two. The tracheas and lungs should be collected for IB virus isolation. The cecal tonsils will most likely not be helpful since the bird is exposed for a short amount of time.

Ideally, the birds should be taken to your local diagnostic laboratory for sample collection. However, if this is not available in your area, then the tissues should be removed from the bird as cleanly as possible and placed in a plastic bag. One should try to avoid allowing blood to get into the trachea for this could reduce the opportunity for virus isolation. If all the birds from several farms are brought to one location for tissue collection, be sure to clean your instruments between farms so that contamination does not occur. Once the tissues are removed from the bird, they should be placed immediately in dry ice. If dry ice is not available, cover the samples completely in regular ice and take them to a freezer immediately. IBV is very heat liable and must be kept cold at all times (frozen is best). The samples should be placed in bags labeled with the company name, farm name, age of the flock, and attach a note with a brief history of each farm.

The samples should be taken frozen to your local diagnostic laboratory. If a local laboratory is not available in your area and shipping of the samples is required, then ship the samples on dry ice for overnight delivery. The dry ice should keep the samples frozen for about 24 hours. If dry ice is not available, then place the frozen samples in a styrofoam cooler with several ice packs and again send for overnight delivery. Never allow samples to thaw or you risk losing the virus. Never ship samples on a Friday or before a holiday.

Most United States (US) state laboratories can perform IB virus isolation and serotyping. For samples collected outside the US, the Poultry Diagnostic Research Center (PDRC) at the University of Georgia has an import permit for foreign IBV isolates. The virus in allantoic fluid is inactivated in phenol at a 1:1 dilution and shipped to their laboratory.

PCR-RFLP results are only part of the puzzle in determining the cause of a respiratory problem. If the results show the isolation of a vaccine virus from unvaccinated sentinels, these results may not be significant or may suggest a rolling reaction. If the virus isolated is different from the vaccination strain, then it is probably a field virus. Isolation of virus in vaccinated sentinel birds suggests prevalent field virus. In either scenario, the IBV isolate should be used to challenge birds in a laboratory setting to determine its pathogenicity.

If properly used, SPF sentinel birds can be a valuable tool is evaluating field respiratory problems.

REFERENCES

1. Jackwood, M.W. Personal Communication. November 2000.

2. Veterinary Technical Services, Merial Select. Sentinel Bird Protocol. Building Blocks At Work bulletin.
January 2000.

AVIAN PNEUMOVIRUS INFECTIONS: CURRENT KNOWLEDGE, CURRENT IGNORANCE

R. C. Jones

Department of Veterinary Pathology, University of Liverpool, Leahurst, Neston, South Wirral CH64 7TE, United Kingdom

INTRODUCTION

Turkey rhinotracheitis (TRT) is an acute respiratory disease of turkeys that was first described in the late 1970s in South Africa and has subsequently been reported in Europe and many other parts of the world. It has been the major cause of economic loss to the turkey industry in recent years. Until very recently, the North American continent was free of infection. The disease is caused by a pneumovirus, the first to be described in avian species. Other than in the USA, what this article will refer to as 'conventional' avian pneumoviruses also cause respiratory infection and loss of production in chickens and are sometimes associated with 'swollen head syndrome'. Pheasants and guinea fowl are also susceptible. The disease in regions other than USA is controlled by live and killed vaccines. This paper reviews what is known about avian pneumoviruses and what areas of ignorance remain to be studied.

The causative virus. The avian pneumovirus is a pleomorphic RNA virus with an outer envelope bearing fusion (F) protein and glycoprotein (G) spikes. It has been classified as a metapneumovirus, to distinguish it from mammalian pneumoviruses. The virus does not hemagglutinate. The differentiation of conventional APVs into two subtypes has been made on the basis of the nucleotide and predicted amino acid sequences on the G glycoprotein. Originally, South African and UK strains were subtype A and other European strains were subtype B. However, now B seems to be the dominant type in the UK and A has been detected in Belgium and other European countries. The significance of the widespread existence of the two types of APV is a matter for argument. It has lead to complications in the interpretation of ELISA serological tests but may be of lesser importance in vaccinal immunity.

The conventional pneumovirus can be cultivated in tracheal organ cultures (TOC) where it is ciliostatic and it can also grow in fertile chicken eggs and be adapted to grow in several cell cultures. The American strains of virus have been cultivated in chick embryo fibroblasts, vero cells and fertile eggs.

The American virus. APV infection was not reported in the USA or Canada for many years, a situation which seemed surprising in view of its presence in Central and South America. The situation changed in 1996 with the reports of a TRT-like infection in turkeys in Colorado, from which an APV (designated the Colorado isolate, or 'type C') was isolated. While infection appeared to be eradicated from Colorado, turkeys in Minnesota have suffered from what is thought to be the same disease.

While 'conventional' strains of APV were thought to comprise a single serotype, the situation is now altered with the appearance of the Colorado isolate. The American virus is not neutralized by antisera to subtypes A or B, nor are subgroup A and B stains neutralized by monospecific antiserum raised against the Colorado virus. However, hyperimmune serum to a subgroup A isolate does neutralize the Colorado strain to some extent.

Recently, Etteradossi and co-workers in France have shown that a pneumovirus isolated from ducks is different again from A, B and C, so there are at least four distinct types and there may be more. However, it seems likely that for the time being, only A and B are common in Europe.

Host range. The turkey and the chicken seem to be natural hosts for conventional APVs but pheasants and guinea fowl are also susceptible. The American strains of APV do not appear to cause disease in the chicken naturally, although there is evidence of infection in wild birds. The origin of APVs is unknown, although wild birds have been suspected. Antibodies to APV have been reported in ostriches in Zimbabwe and more recently, German workers detected antibodies in the sera of herring gulls near the Baltic. It may be that APV can infect a wide range of wild avian species, in the way that Newcastle disease can, but little is known of this at present.

Epidemiology. Following infection of chickens or turkeys, conventional APV is shed from the nose and trachea but is not present in the feces. The virus can spread rapidly within a flock and aerosol transmission is the most important route. Experimental evidence suggests that APV persists in the chicken or the turkey for a relatively short period, perhaps no longer than 8-10 days after infection. Whether this applies in commercial conditions among thousands of birds, where there may be the opportunity for re-cycling of infection is unknown. Despite the frequent long-term persistence of antibodies, no evidence of long virus persistence could be demonstrated in the chicken or

turkey after T-cell immunosuppression. Although APV has been isolated from very young poults and there are abundant amounts of virus in the oviduct epithelium of the infected turkey hen, there are no reports of egg transmission.

Exacerbating factors for conventional APV infection are the usual ones for a respiratory infection: high ammonia and dust levels in the atmosphere, overcrowding and intercurrent infections including Mycoplasma gallisepticum and E. coli. However, when chicks are infected with virulent APV and infectious bronchitis virus (IBV), IBV inhibits the replication of APV. Studies in TOC also confirm this effect. Dual infections with other respiratory viruses and immunosuppressive viruses remain to be investigated.

Disease and pathogenesis. Clinical signs of conventional TRT in the turkey are those of an acute respiratory disease. Within 2-3 days of infection, birds appear depressed and show coughing, snicking and head shaking. There is turbid nasal exudate and later frothy ocular discharge. By 7-8 days post infection in uncomplicated cases, birds return to normal. Signs in the chicken resemble those in the turkey but may be less severe. When E. coli and other bacteria are involved, there may be significant mortalities due to colisepticemia and the disease is prolonged. In breeder turkeys, egg production falls significantly and there may be an effect on egg quality. Conventional APV infection causes falls in production in laying chicken flocks also and may be associated with the production of white eggs in brown egg layers.

The conventional virus can rarely be detected in the respiratory tissues after 7-8 days. Following initial replication, the virus reaches the oviduct of the mature female turkey, probably due to viremic virus, although a viremia has never been detected. Between 6-9 days after infection, virus can be detected by IF staining in the oviduct epithelium. This probably leads to a sequence of events which include the regression of the ovary and oviduct and loss of egg production.

Immune responses. Following infection with conventional APV, chickens and turkeys develop virus neutralizing and ELISA antibodies in the serum. These antibodies do not appear to be important in controlling the respiratory disease. Maternal antibodies have been shown to be ineffective in the face of early challenge of poults with virulent virus. In addition, when poults were B-cell immunosuppressed by cyclophosphamide and vaccinated at day-old, despite their inability to produce circulating antibodies, they were immune to challenge with virulent APV given at 21 days of age. Circulating antibodies are, however, likely to be of importance in protecting the oviduct after infection of breeders.

Local antibodies appear to be of importance in protection. Virus-specific IgA and IgG appear in the tears of both chicks and poults after infection with virulent APV and the tears have virus-neutralizing activity. T-cell immunosuppression of chicks and poults using cyclosporin has illustrated the importance of cell-mediated immunity. Immunosuppressed birds experienced clinical signs for up to six days longer than in normal birds and virus replication is prolonged in the tissues. This suggests that immunosuppressive infectious agents could prolong the disease.

Diagnosis. Isolation of conventional APV from turkeys is difficult and from chickens almost impossible. It can be best achieved by inoculation of material (tracheal or oropharyngeal swabs or macerated respiratory tissue) into TOC. The virus, if present, will cause ciliostasis after 6-8 days but up to three passages may be needed. Fertile eggs can also be used with yolk inoculation. The identity of the agent needs to be confirmed by virus neutralization or immunofluorescence. One problem of is that the optimal period for virus isolation may precede the peak of clinical signs, so early sampling is essential. Isolation is time-consuming. The reason for the difficulty in isolating virus from chickens is not understood. In contrast, American Colorado-type virus is not ciliostatic in TOC but strains have been isolated from clinical material in chick embryo fibroblasts or vero cells.

Polymerase chain reaction (PCR) technology has been used successfully for the diagnosis of APV infection, although its use so far has been confined to specialist laboratories. The method is very sensitive and quick, relative to isolation and can be tailored to distinguish between subtypes A and B, and the American strains.

Serum antibodies to APV may be detected by indirect immunofluorescence, by virus neutralization tests or by ELISAs. ELISAs are the preferred method, chiefly because many samples can be tested simultaneously. While detection of infections with the American virus requires the use of homologous antigen, a major problem with conventional APV ELISAs is the nature of the viral antigen used. Results with subtype A virus may differ from those with a subtype B virus when testing field sera from birds vaccinated with either or both and challenged with either or both. The use of inappropriate antigen may result in false negatives or apparent failure of vaccines to 'take'.

Control. There is no treatment available for APV infections so the main approach to control other than in the USA is vaccination. Commercial live vaccines have been developed by attenuation of virulent conventional virus after passage in vitro. In one study, it was found that almost 100 passages in TOC did not reduce the virulence of APV, but almost 39 passages in chick embryo fibroblasts removed both virulence and

immunogenicity. Passage in Vero cells, however, reduced virulence to zero but maintained immunogenicity sufficient to produce an acceptable vaccine.

Live vaccines are usually given to poults or chicks at or near to day old and usually by spray or eyedrop. Some manufacturers recommend one or more repeat live vaccinations. One company produces two live vaccines: one for turkeys and one for chicks. These vaccines are derived from strains isolated from the respective species and are attenuated to different degrees. Killed adjuvanted vaccines are used for breeder turkeys and laying chickens but only after live priming. They are sometimes given in combination with other killed vaccines.

The live vaccines are prepared from either subtype A or B and there is controversy as to whether one type will protect against both A and B. Experimental evidence suggests that they cross-protect well, but some veterinarians recommend using vaccines of both types either together or alternately. Generally, the vaccines are satisfactory, although it is very important that they are applied accurately so that every bird receives a full dose. A potential problem of interference is encountered in chicks, where it is known that IBV interferes with APV. Indeed, at least one vaccine company recommends that IBV vaccine is given one week before APV vaccine. However, despite the inhibition of APV by IBV in the bird, an immune response to APV develops which gives good protection.

When attempting to monitor the vaccine response to APV, it is important to appreciate that ELISA serology does not always indicate accurately if APV vaccine has taken and in the chick in particular, the ELISA response may be delayed. It is certainly not correct to equate ELISA antibody levels with immunity.

Questions still unanswered. There are still areas of ignorance about avian pneumoviruses which need further study. The origin of these viruses is unknown although it seems likely that wild birds are implicated. The global spread of the conventional APVs to almost everywhere except North America is also puzzling, although again, wild birds could be the main conveyers. We need to know more about the possibility of APVs being present in wild species.

The emergence of the American virus and the demonstration of a French strain from ducks which is different again suggests that still more variants may emerge. It is important that use all means available are used to recognize any new types. The lesson to be learned from the American experience is that we must not expect that all types can be cultivated in systems we use regularly for the viruses we are familiar with and serological recognition of infection may only be successful with homologous antigens.

WILD WATERFOWL AS A SOURCE OF AVIAN PNEUMOVIRUS (APV) INFECTION IN DOMESTIC POULTRY

Brian McComb, Richard Bennett, H.J. Shin, K.V. Nagaraja, F.J. Jirjis and David A. Halvorson

Department of Veterinary Pathobiology, University of Minnesota, St. Paul, Minnesota

Background. Avian pneumovirus (APV) has existed in the Minnesota turkey industry for the last four years. It causes significant financial losses to turkey growers, estimated to total approximately $15 million annually. Because other paramyxoviruses have been isolated from wild waterfowl, we wondered whether APV could also be found in wild waterfowl populations.

Objective. To determine if avian pneumovirus could be detected in wild waterfowl and their environment.

Methods. Three studies were conducted. In study one, day-old mallard ducklings were raised in isolation until 8 weeks old. At 8 weeks of age they were separated into three groups: two groups were placed as sentinels on ponds allowing contact with wild waterfowl in central Minnesota, the third group remained in isolation as controls. Each week choanal, tracheal, and cloacal swabs were collected from the sentinel ducks. Matrix gene based RT-PCR for avian pneumovirus and virus isolation were performed.

In study two, wild Canada geese, collected for an annual metropolitan goose round-up in Minneapolis and Saint Paul, were choanally swabbed on two different dates. Both juvenile and adult geese were swabbed. Blue winged teal were swabbed during a DNR banding exercise in North Dakota. RT-PCR and virus isolation were performed on the samples.

In study three, heads from hunter-killed snow geese from Saskatchewan were received and the choanal cleft was swabbed from each. RT-PCR and virus isolation were performed on the samples.

Results. In study one, pooled choanal and tracheal samples were found positive for APV viral RNA at both sites. Cloacal swabs were PCR negative. There was an isolation of APV from choanal-tracheal swabs from sentinel ducks at one site. Control ducks remained negative. The APV M genes isolated from sentinel ducks had over 96% predicted amino acid identity with APV turkey isolate MN/2A.

In study two, samples from both adult and juvenile metropolitan geese were positive for APV viral RNA. No virus was isolated. RT-PCR was also positive on choanal swab samples from blue winged teal. No virus was isolated.

In study 3, samples from choanal swab samples from snow geese were positive on RT-PCR. No virus was isolated.

Conclusion. Detection of APV viral RNA from sentinel ducks, Canada geese, blue winged teal and snow geese suggests that waterfowl maybe a reservoir of APV. Finding PCR evidence of APV in multiple species and at multiple sites indicates this virus may be widespread in nature.

SUBCUTANEOUS VACCINATION OF DAY OLD TURKEY POULTS WITH OIL EMULSIFIED KILLED AVIAN PNEUMOVIRUS AND NEWCASTLE DISEASE VIRUS AND THE SEROLOGICAL RESPONSE

Hugo A. Medina, M. A. Sheik[B], D. Patnayak[B] and S. M. Goyal[B]

[A]Jennie-O Foods, Inc. P.O. Box 778, Willmar, Minnesota 55201
[B]Veterinary Diagnostic Medicine, University of Minnesota1333 Gortner Ave, St Paul, MN 55108

Avian Pneumovirus (APV) causes a respiratory disease that has been reported from a number of countries sincethe late 1960's'. APV is the etiological agent of Turkey Rhinotracheitis (TRT) in turkeys and swollen head syndrome in chickens. TRT is an acute, rapidly spreading, and upper respiratory disease causing nasal and ocular discharge, rhinitis, swollen infra-orbital sinuses and impairment of tracheal cilia. A short incubation period of 1 - 3 days facilitates quick transmission of the disease, Turkeys of all ages are susceptible; however, poults younger than 6 weeks experience a more severe clinical disease. The infection is exacerbated by concurrent or secondary infections with bacteria or viruses; examples are *Bordetella avium*, *Escherichia coli*, and Newcastle Disease virus infections. Poor husbandry practices, ventilation and higher stocking densities can influence mortality and morbidity. APV was first isolated in the U.S.A. in 1997, after a respiratory disease outbreak in turkeys in Colorado.

APV, a non-segmented, single-stranded RNA virus, is a member of the family Paramyxoviridiae and genus *metapneumovirus.* Only one serotype of APV has been described with four subgroups (A, B, C & D). Cross neutralization has determined that the US Colorado is antigenically unique and has only limited cross-neutralization with subgroup A and B viruses. The US Colorado isolate has been designated as subgroup C.

It is believed that APV first appeared in Minnesota in 1996, but serologically diagnosed in 1997. This disease has had a considerable economic effect for the Turkey Industry in Minnesota. It is estimated that this disease and its complicated effects including mortality, medication, complicated factors, poor performance, condemnation and higher cost of production costs about $15,000,000.00 a year.

APV virus has been isolated and identified by several groups. The virus isolation and identification in Minnesota is considered part of the subgroup C. From the initiation of the APV respiratory problems in Minnesota, the turkey industry has worked very hard on farm isolation, implementation of all aspects of biosecurity measures and reduction of the factors that exacerbate secondary infections. APV infection is present year around with two main morbidity seasons in the spring and fall. During the early part of 1999 in the attempt to minimize the effects of APV in the field, the use of APV autogenous killed virus vaccine was initiated on birds 4 weeks of age. This autogenous APV oil emulsion virus vaccine was mixed with a commercially available oil base Newcastle Disease killed virus vaccine. A mix of both killed vaccines were subcutaneously injected at the middle of the neck on selected flocks – farms. The vaccinated flocks evaluation on with positive results by reducing mortality, weight loss, morbidity, therapeutic cost, more effective use of therapeutic practices, faster recuperation and reduction in condemnation.

At the end the year 1999 another strategy was used to control the effects of the field challenge and its effects by the "Control Exposure" using an APV isolated and tissue culture passage (P41). Some turkey growers have used this procedure to better control and treat this disease under this procedure.

Oil emulsion day old vaccination is currently used in chickens and turkeys. The subcutaneous injection in the middle of the neck vaccination has been used in parts of Latin America, Asia, Africa and other parts of the world for the immunization of Newcastle Disease Virus and Avian Influenza. Day old vaccination of a killed virus in an oil emulsion is not new in Minnesota; it was used with killed NDV oil emulsion vaccine in 1988 at the hatchery level.

On May 2000 we proposed a variation of the APV autogenous killed virus vaccination; from 4 weeks to day old vaccination at the hatchery level. The main objective of early immunization between the day of vaccination and the potential APV field exposure. Field exposure has been reported to occur as early as 2 weeks of age. Also day old vaccination increases the percent of immunized poults, reduces the potential of cross contamination from the vaccination crews to the flock being vaccinated and minimizes poults stress during vaccination.

Two trials were set.

A) First trial was to test the effectiveness of the APV killed oil emulsion day old vaccination in reducing respiratory symptoms on birds challenged with a virulent APV isolated on birds at two, four, six, eight, ten and twelve weeks post vaccination. The birds under this trial were challenged, in isolation units at the University of Minnesota, serologically tested prior and 10 days post challenged with an ELISA test. Also Polymerase Chain Reaction (PCR) testing was performed on to determent the presence of the APV post challenge.

B) Day old poults were APV (autogenous) + NDV (three different commercially available products) killed oil emulsion vaccinated placed under field conditions. Signs and symptoms from field APV challenge were determent, PCR testing on field birds, serological response to be evaluated by ELISA test before, during and after field exposure.

The following People and Institutions have helped in the development and implementation of these trials; Kyla Hallman; Dale Hess; Dr. Mahesh Kumar; Intervet Labs; Minnesota Turkey Growers Association; the University of Minnesota; Nova-Tech and The University of Minnesota Diagnostic Laboratory.

REFERENCES

1. Goyal, S. M., M. A. Sheikh, J. Webb, L. Liu and M.K. Njenga. Avian Pneumovirus ELISA: Determining Antibody Titers from a Single Serum Dilution.

2. Calnek, B.W., H.J. Barns, C.W. Beard, L.R. McDougald, and Y.M. Saif. Diseases of Poultry Tenth Edition 1997.

3. Pedersen, J.C., D.L. Reynolds and A. Ali. The sensitivity and Specificity of a Reverse Transcription-Polymerase Chain Reaction Assay for the Avian Pneumovirus (Colorado Strain). Avian Diseases Vol. 44 No. 3, Pages 681 to 685. July-September 2000.

4. Panigraphy, B., D.A. Senne, J.C. Pedersen & T. Gidlewski. Experimental and Serological Observations on Avian Pneumovirus (APV/turkey/Colorado/97), Infection in Turkeys. Avian Diseases Vol. 44 No.1, Pages 17 – 22 January – March 2000.

5. Samina, I., Y. Khinick, B. Gutter, A. Michael & B.A. Peleg. Day-old vaccination with live-in-oil vaccines: Newcastle disease (ND) and infectious bursal disease (IBD) in chick and ND in turkey poults. Avian Pathology (1999) 28, 73-78.

6. Kumar, M.C., Turkey World May-June 1988.

ENHANCEMENT OF SEVERITY OF AVIAN PNEUMOVIRUS INFECTION IN TURKEYS INOCULATED WITH PATHOGENIC BACTERIA

Daniel P. Shaw [A], Faris F. Jirjis [B], Sally L. Noll[C], David A. Halvorson[B], K.V. Nagaraja[B]

University of Minnesota, Departments of: [A]Veterinary Diagnostic Medicine, [B]Veterinary Pathobiology, and [C]Animal Science, St. Paul, MN 55108

The objective of this study was to evaluate the effects of bacterial co-infection on the severity of avian pneumovirus (APV) infection using *E. coli*, *Ornithobacterium rhinotracheale* (ORT), or *Bordetella*

avium (BA). In previous work it was found that inoculation of APV-infected poults with all three bacteria enhanced the clinical signs in comparison to poults that received APV only. The present study was undertaken in order to determine which of the three bacteria played the largest role in this result.

The poults were obtained from breeder stock that was negative for the presence of antibody to APV and reared in isolation until 4 or 9 weeks of age. They were then divided into four groups for each trial and each group was housed in a separate isolation room. Each trial was repeated in 4- and 9-week-old poults and set up as follows: 15 poults per group were placed into each of 4 different isolation rooms. On day 0, groups 2 and 4 were inoculated with APV infected cell culture fluid into each conjunctival space and nostril (total of 200 µl). The poults comprising groups 1 and 3 were inoculated with non-infected cell culture fluid and served as APV sham-inoculated controls. On day 3, groups 3 and 4 were inoculated with culture broth containing the appropriate bacteria. Clinical signs were observed and scored on days 3, 5, 7, 11, and 13. Two birds from each group were sacrificed for necropsy on days 2, 4, 6, 10, and 14 days post-inoculation (PI). At 14 days PI, serum samples were collected from all remaining poults and assayed for antibody against APV, *Bordetella avium*, and *Ornithobacterium rhinotracheale*.

The major clinical signs were nasal discharge, swollen infraorbital sinuses, and ocular discharge. Each of these 3 clinical signs were scored using the following scale: 0, no sign; 1, mild or slight; 2, moderate; 3, severe. The mean clinical score for each group was determined by adding clinical scores and dividing by the number of birds at each observation time. A maximum score of around 45 (severe) and a minimum score of 0 (no clinical signs) were attainable for each group.

It was found that, as a group, the poults that were inoculated with APV and either *E. coli* or ORT developed more severe clinical signs than those that were given APV or the bacteria alone (Tables 1, 2). The poults that were inoculated with BA alone or in combination with APV developed more severe signs than those that received APV alone (Table 3). More of the individual poults within the groups inoculated with APV + bacteria were affected than in the single agent inoculated groups. No clinical signs were seen in sham-inoculated controls.

In field outbreaks of APV infection, there has usually been high morbidity and variable mortality. In most of the outbreaks in which there have been high mortality rates, the death losses have been associated with secondary bacterial infections. In the isolated conditions of previous research trials, turkeys have developed only mild clinical signs when exposed to APV alone. The present work has attempted to reproduce the effects of exposure to opportunistic and potentially pathogenic bacterial infections and has shown a synergistic effect.

Table 1. Mean clinical scores in *E. coli* inoculated groups.

	4-week-old	9-week-old	Signs
APV	3.7	2.5	Nasal discharge and sinus swelling in 30%
E. coli	1.2	1.2	Nasal discharge in 36%
APV + *E. coli*	6.0	2.4	Nasal discharge and sinus in 70%

Table 2. Mean clinical scores in *Ornithobacterium rhinotracheale* inoculated groups.

	4-week-old	9-week-old	Signs
APV	3.8	2.2	Nasal discharge and sinus swelling in 30%
ORT	0.8	1.0	Nasal discharge in 27%
APV + ORT	6.2	2.7	Nasal discharge in 62% and sinus swelling in 70%

Table 3. Mean clinical scores in *Bordetella avium* inoculated groups.

	4-week-old	9-week-old	Signs
APV	3.8	2.2	Nasal discharge and sinus swelling in 33%
BA	7.7	8.7	Nasal discharge in 85%
APV + BA	15.4	11.8	Nasal discharge in 100% and sinus swelling in 39%

HOST RANGE AND EPIDEMIOLOGY OF AVIAN PNEUMOVIRUS INFECTION

K. V. Nagaraja, H.J. Shin, M. Njenga, D. Shaw and D. A. Halvorson

Departments of Veterinary PathoBiology and Veterinary Diagnostic Medicine
University of Minnesota, St. Paul, MN 55108

Avian Pneumovirus. Avian pneumovirus (APV) belongs to the genus Metapneumovirus in the subfamily pneumovirinae of the family Paramyxoviridae. APV is a highly infectious virus of the upper respiratory tract of turkeys and chickens. APV has been isolated from turkeys and/or chickens in South Africa, France, Israel, Spain, Italy, Japan, Netherlands, Germany, Mexico, Hungary, United Kingdom, Greece and Taiwan. In the United States, APV was first isolated in early 1997 by the National Veterinary Services Laboratory (NVSL) from Colorado turkeys with clinical signs of respiratory disease. The signs included coughing, sneezing and nasal discharge. This isolation was the first evidence of a pneumovirus in poultry in the USA. Subsequently, it has been isolated from turkeys in Minnesota.

Avian pneumovirus causes respiratory infection both in turkeys and chickens with a sudden onset and rapid spread through flocks. It causes rhinotracheitis in turkeys and swollen head syndrome in chickens. Turkey Rhinotracheitis is a disease caused by a pneumovirus which was first described in South Africa in 1978, and it is now recognized in many countries. The disease is associated with catarrhal infections (presence of mucus) of the upper respiratory tract, foamy eyes, swelling of the sinuses in young poults and it is exacerbated by secondary infections. In laying birds, a drop in egg production is seen. Morbidity in infected birds is often 100%. The mortality ranges from 1 to 90% and is highest in young poults. All ages of turkeys and chickens are susceptible to avian pneumovirus infection.

Avian Pneumovirus is transmitted by contact. Nasal discharge, movement of affected birds, contaminated water, contaminated equipment; contaminated feed trucks and movement of affected poults can contribute to the transmission of the virus. Air borne spread is speculated. Egg transmission and carrier birds also have been suggested. The virus is shown to infect the epithelium of the oviduct of laying turkeys.

Host Range. Most isolations of Avian pneumovirus have been made from turkeys and chickens implying that the host range is comprised of just these two species. When a similar but a different subtype of the virus from Europe (Turkey Rhinotracheitis virus- TRTV) was inoculated (1) into turkeys, chickens, ducks, geese, pheasants, guinea fowl, and pigeons, the virus was recovered only from chickens and turkeys. The investigators in this study suggested that the lack of more severe effects may have been related to the use of an attenuated TRTV in this case along with the absence of important environmental factors.

However, other studies show indirect evidence of involvement of other hosts. Antibodies to TRTV have been demonstrated in guinea fowl that died of swollen head syndrome. Sera from ostriches sampled in the country - Zimbabwe were found to be positive for antibodies to TRTV. Seagulls trapped near the shorelines in Europe were found to have high titers of antibodies to avian pneumovirus in their blood.

The researchers at the University of Minnesota with support from Minnesota Turkey Research and Promotion Council are performing a series of laboratory and field experiments to identify the potential carriers most commonly noted in the vicinity of turkey farm operations. The following sections describe the results as it pertains to the susceptibility of potential hosts to the Minnesota isolate of avian pneumovirus from turkeys.

A. Mice and Rats. We inoculated laboratory mice and rats (3, 5, and 7 weeks of age) with the avian pneumovirus isolate from Minnesota. At 4, 6, and 14 days post-exposure, a group of mice and rats were sacrificed. Samples from blood, lungs, trachea, swabs from mouth and rectum, fecal droppings, and litter were examined for evidence of APV. Blood was examined for any seroconversion to APV. A PCR test was used on all samples to detect APV. Contact spread of APV from infected mice and rats to contact controls also was examined.

All PCR test samples collected from mice at 4, 6, and 14 days post-exposure were positive for the presence of APV nucleic acid. Blood samples collected at 14 days post-exposure were positive for antibodies against APV. Droppings were positive for APV by PCR. There was a contact spread of the virus from APV exposed to non-exposed turkeys.

Rats in all three age groups (3, 5, and 7 weeks) were positive for APV infection mainly at 4 days post-inoculation. Their serum samples revealed the presence of antibodies to APV. APV exposed rats shed the virus in their droppings.

B. Waterfowl. Questions concerning the role of waterfowl in the transmission of avian pneumovirus were
examined by transmission experiments. Briefly, ducklings obtained from a commercial hatchery were obtained and experimentally inoculated at 10 days of age with the Minnesota isolate of APV. They were examined for their susceptibility to APV and their ability to transmit the virus. Ducks in one group were inoculated orally. Ducks in a second group were inoculated by the oculo-nasal route. An equal number of ducks were kept as uninfected controls. The development of any clinical manifestations of APV infection in ducks was monitored. At 3, 6, 9, 15, and 21 days post-exposure, two ducks from each group were sacrificed. Samples from blood, lungs, trachea, mouth and cloacal swabs, and intestinal samples were examined for the presence of APV infection. Blood samples were examined for seroconversion.

All ducks remained clinically normal. The PCR results revealed that APV viral RNA was present in all tissues taken from 3 to 21 days post-inoculation. There was no significant difference between birds infected by the oral or oculo-nasal routes. The virus was reisolated from the infected ducks.

C. Broiler chickens. Two- week-old broiler chicks free of antibodies to APV were exposed either by oculonasal or oral route with a cell cultured APV of turkey origin. Chickens from both APV-inoculated groups exhibited clinical signs of respiratory distress that included coughing, sneezing, nasal discharge and watery eyes during 2-8 days post-inoculation. APV was reisolated from samples taken from chickens in both groups inoculated orally and oculonasally.

D. Wild birds. The role of wild birds in transmitting the APV virus was examined in the vicinity of turkey barns. Table below shows the different species of birds examined for the presence of APV and their results. Nasal turbinates or swabs collected from different species of birds, and from sentinel ducks placed next to turkeys farms experiencing avian pneumovirus (APV) infections, were analyzed for APV genome and infectious particles. APV RNA was detected in samples examined from geese, sparrow, starlings, and sparrows. APV RNA and antibodies were also detected in two different groups of sentinel ducks. Infectious APV was recovered from sentinel ducks. The APV M gene isolated from the wild birds had over 96% predicted amino acid identity with APV/Minnesota 2A which was isolated earlier from domestic turkeys showing respiratory illness, suggesting that wild birds may be involved in spreading APV infection. Sparrows caught inside an APV outbreak building and sparrows trapped 20 yards away from a turkey building were found to be positive for APV by PCR. We feel this observation is very significant.

E. Frogs. On one occasion frogs from the perimeter of a turkey barn with APV positive birds were sampled. PCR results were negative for all frogs.

F. Other possible vectors (Humans, Management practices, etc.). A comprehensive questionnaire to assess APV risk factors in the day-to-day turkey farm operations was developed through several meetings with the APV Task Force of the MTGA and others. The survey questionnaire was distributed to turkey producers experiencing and to those NOT experiencing APV infections in their operations. The results of this survey will be presented.

PCR results from the different species of Wild bird

Species examined	Number examined	PCR Results
English Sparrow	67	Positive (5)
European Starling	16	Positive (2)
Barn Swallow	5	Positive (1)
Killdeer	2	Positive
Owl	2	Negative
Goose droppings	3	Negative
Canada Goose	8	Positive (3)
Mallard Duck	33	Positive (4)
Dark-eyed Junco	5	Negative
Yellow-bellied Sapsucker	12	Negative
Rock Dove	6	Negative
Wild Turkey	7	Negative
Robin	4	Negative
American Crow	1	Negative
Cedar Waxwing	1	Negative
Downy Woodpecker	3	Negative
Pheasants	2	Negative
Northern Flicker	2	Negative
Common Grackle	6	Negative
Pied-billed Grebe	1	Negative
American Coot	1	Negative
Great Blue Heron	1	Negative
Hairy Woodpecker	2	Suspicious

SEQUENCE COMPARISON OF 5 STRUCTURAL GENES OF 15 AVIAN PNEUMOVIRUS ISOLATES FROM UNITED STATES

Hyun-Jin Shin[A], Kjerstin T. Cameron[A], Janet A. Jacobs[B], Elizabeth A. Turpin[B], David A. Halvorson[A], Sagar M. Goyal[A], Kakambi.V. Nagaraja[A], Mahesh C. Kumar[C], Dale C. Lauer[D], Bruce S. Seal[B], and M. Kariuki Njenga[A]

[A]Department of Veterinary PathoBiology, College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108, USA
[B]Southeast Poultry Research Laboratory, Agricultural Research Service, USDA, Athens, GA 30605, USA
[C]EBO Farms Atwater Laboratory, Atwater, MN 56209, USA
[D]Minnesota Poultry Testing Laboratory, Minnesota Board of Animal Health, Willmar, MN 56201, USA

Avian pneumovirus (APV) is a member of the *Paramyxovirdae* family that causes acute rhinotracheitis in turkeys characterized by coughing, nasal discharge, tracheal rales, foamy conjunctivitis and sinusitis in turkeys of all ages. In laying birds, there is transient drop in egg production along with mild respiratory tract illness (4). Uncomplicated cases have low mortality (2 to 5%) but APV infections accompanied by secondary infections (bacterial and/or viral) can result in up to 25% mortality (4). APV was first detected in South Africa in 1978, but quickly spread to the United Kingdom, France, Spain, Germany, Italy, Netherlands, Israel, and Asia (4; Alexander, 1997). The United States was free of APV infection until 1996, when a 10-month outbreak of upper respiratory system infection among turkeys in Colorado resulted in isolation of APV (Kleven, 1997; 8). However, the APV outbreak in the western state of Colorado was controlled by intense biosecurity measures and the disease has not been reported there since early 1997 (Kleven, 1997). APV was detected in 1997 in the north-central state of Minnesota where the incidence of disease has increased over the past four years despite the establishment of biosecurity measures similar to those used in Colorado (1, 2, 3).

Mammalian pneumoviruses encode ten gene proteins that include non-structural (NS1 and NS2), nucleocapsid (N), phosphoprotein (P), matrix (M), small hydrophobic (SH), surface glycoprotein (G), fusion (F), second matrix (M2) and RNA-dependent RNA polymerase (L) genes. In contrast, APV lacks the NS1 and NS2 genes (6, Randhawa *et al*., 1997) and has a smaller L gene than mammalian pneumoviruses (6). The putative gene order of APV is 3'N-P-M-F-M2-SH-G-L5' (7). Based on these differences, the avian pneumoviruses were classified in the genus *Metapneumovirus*, whereas their mammalian counterparts belong to the genus *Pneumovirus* (6). Two subgroups (A and B) of APV were defined in Europe, initially based on the level of genetic variations in the attachment (G) glycoproteins of APV strains (5). Subsequent studies showed significant heterogeneity in M and F gene nucleotide sequences between the two subgroups so that the genetic composition of these genes could also be used to distinguish between the subgroups (11, 12, 6, 8, 9). However, there has been no comparative genetic analysis based on N, P, and M2 genes across the subgroups because these genes have never been sequenced from subgroup B viruses.

Sequence comparison of M and F genes from three 1997 APV isolates from the US revealed that these genes shared 98% nucleotide sequence identity among one another, but only 60% to 70% nucleotide identity with APV subgroups A and B viruses (8, 9). As a result of these differences, it was suggested that the US isolates should be classified as subgroup C (8,9).

Comparison of the nucleotide sequences of nucleoprotein (N), phosphoprotein (P), matrix (M), fusion (F), and second matrix (M2) genes revealed between 89% and 94% identity, and predicted amino acid sequence identity of 81% to 95% among the 15 US isolates. In contrast, genes from US viruses had 41% to 77% nucleotide identity and 52% to 78% predicted amino acid sequence identity with European subgroup A or B viruses, confirming that US viruses belonged to a separate subgroup. Of the five gene proteins analyzed in US viruses, P was the most variable (81% amino acid identity) and N the most conserved (95% amino acid identity). There was evidence of positive selection between the first APV isolate (APV/CO) from the western region and viruses later isolated from the north-central region of US (APV/MN 1A and APV/MN 2A), as demonstrated by high nonsynonymous versus synonymous nucleotide substitutions (between 1.8 and 2.0) in the F gene. Phylogenetic comparison of subgroups A, B, and C viruses indicated that subgroup A and B viruses were more closely related than either A or B viruses were to subgroup C viruses.

REFERENCES

1. Chiang, S. J., Dar, A. M., Goyal, S. M., Nagaraja, K. V., Halvorson, D. A. & Kapur, V. A modified enzyme-liked immunosorbent assay for the

detection of avian pneumovirus antibodies. J. Vet. Diag. Inv. 12:381-384. 2000.

2. Goyal S. M., Chiang, S., Dar, A. M. Nagaraja, K. V., Shaw, D. P., Halvorson, D. A. & Kapur, V. Isolation of avian pneumovirus from an outbreak of respiratory illness in Minnesota turkeys. J. Vet. Diag. Inv. 12:166-168. 2000.

3. Jirjis, F. E., Noll, S. L., Halvorson, D. A., Nagaraja, K. V., Townsend, E. L., Sheikh, A. M. & Shaw, D. P. Avian pneumovirus infection in Minnesota turkeys: experimental reproduction of the disease. Avi. Dis. 44:222-226. 2000.

4. Jones, R. C. Avian pneumovirus infection: questions still unanswered. Avi. Path. 25:639-648. 1996.

5. Juhasz, K. & Easton, A. J. Extensive sequence variation in the attachment (G) protein gene of avian pneumovirus: evidence for two distinct subgroups. J. Gen. Viro. 75:2873-80. 1994.

6. Li, J., Ling, R., Randhawa, J. S., Shaw, K., Davis, P. J., Juhasz, K., Pringle, C. R., Easton, A. J. & Cavanagh, D. Sequence of the nucleocapsid protein gene of subgroup A and B avian pneumoviruses. Vir. Res. 41:185-191. 1996.

7. Ling, R., Davis, P. J., Yu, Q., Wood, C. M., Pringle, C. R., Cavanagh, D. & Easton A. J. Sequence and in vitro expression of the phosphoprotein gene of avian pneumovirus. Avi. Res. 36:247-257. 1995.

8. Seal B. S. Matrix protein gene nucleotide and predicted amino acid sequence demonstrate that the first U.S. avian pneumovirus isolate is distinct from European strains. Vir. Res. 58:45-52. 1998.

9. Seal B. S., Sellers, H. S. & Meinersmann, R. J. Fusion protein predicted amino acid sequence of the first U.S. avian pneumovirus isolate and lack of heterogeneity among other U.S. isolates. Vir. Res. 66:139-147. 2000.

10. Shin H. J., Rajashekara, G., Jirjis, F. F., Shaw, D. P., Goyal, S. M., Halvorson, D. A. & Nagaraja, K. V. Specific detection of avian pneumovirus U.S.-isolates by RT-PCR. Arch. Viro. 145:1239-1246. 2000.

11. Yu, Q., Davis, P. J., Barret, T., Binns, M. M., Boursnell, M. E. G. & Cavanagh, D. Deduced amino acid sequence of the fusion glycoprotein of turkey rhinotracheitis virus has greater identity with that of human respiratory syncytial virus, a pneumovirus, that that of paramyxoviruses and morbilliviruses. J. Gen. Viro. 72:75-81. 1991.

12. Yu, Q., Davis, P. J., Li, J. & Cavanagh, D. Cloning and sequencing of the matrix protein (M) gene of turkey rhinotracheitis virus reveal a gene order different from that of respiratory syncytial virus. Viro. 186: 426-434. 1992.

13. Yu, Q., Davis. P. J., Brown, T. D. K. & Cavanagh D. Sequence and in vitro expression of the M2 gene of turkey rhinotracheitis pneumovirus. J. Gen. Viro. 73:1355-1363. 1992.

ECONOMIC IMPLICATIONS OF CONTROVERSIAL LAYER MANAGEMENT PROGRAMS

Donald Bell, Poultry Specialist

Cooperative Extension, University of California, Highlander Hall, Riverside, CA 92521

SUMMARY

Economics plays an essential role in the choice of management programs in the poultry industry as well as in most businesses. Economics drives the selection of systems, products, and procedures among a long list of alternative options. Costs, values, profit margins, competition, overhead, performance, efficiencies, etc. are all economic subjects and are of vital importance in their effect on the management of today's modern agricultural enterprises.

Management programs are chosen only following careful consideration of their relative worth compared to alternative programs. Managers are charged with choosing sound programs, enacting them in detail, monitoring their applications and continuing their evaluation when new alternatives come along or when price/cost conditions change.

This paper emphasizes some of the economic implications of program selection in the controversial areas of: caging systems for laying hens, beak trimming and induced molting. Analysis of relative biological performance is stressed with cost/price calculations emphasized to discover the economic impact on the operation. The impact of imposed regulations on systems is discussed.

INTRODUCTION

Commercial management practices for laying chickens are chosen on the basis of their ability to perform a basic task with a minimum of detrimental effects to the flock or to the environment in a cost-

effective manner (29). For example, feed must be delivered to a flock frequently and in an adequate quantity and quality to satisfy each chicken's basic needs for nutrients. The delivery system must be well designed, competitive in price, free of defects, and low in maintenance costs if it is to be selected. This same principle is followed for the selection of every management system in use today.

Obviously, there are many alternative systems which can do a comparable job and individual farmers have different needs which may require different systems. This is why we see a variety of systems and practices. Owners use different strains of chickens, different feeding programs, different poultry houses, and a wide range of other management techniques. Farmers strongly defend their choices and justify them on the basis of their own experience. They get good responses from their flocks, the help finds the systems easy to work with, and ownership believes they are cost-effective and yield the highest returns on their investments.

Some of the practices in use today by the commercial table-egg industry are being criticized by observers of the industry. These practices are perceived as being harmful to the flocks or ones which fail to address the specific behavioral needs of the chickens. They include:

1. The use of animals in any way.
2. The caging of chickens.
3. The use of beak trimming.
4. The use of induced molting.
5. Transportation and handling systems.
6. And others.

This list includes items which may require absolutely no change from current procedures, others which may need some modification to eliminate problem areas, and some which might justify major changes or even elimination from the list of choices. The industry, as well as individual producers, must take a hard look at their systems to determine whether on not adjustments should be made in areas of flock welfare and health without adversely affecting the economics of the operation.

This paper will address three areas from the list above, which have drawn the most criticism in recent years - caging, beak trimming and induced molting.

CAGES FOR TABLE EGG LAYING FLOCKS

The commercial application of cages for egg production began in the 1930's, became widespread in the 1940's and 1950's and is currently thought to represent 70-80% of the World's production. Today, we would estimate that 98% or more of the commercial production of the United States is in cage systems.

During this 50 or more years of use, cages and their associated equipment have been improved and modified, cage density has increased (more hens per cage and/or less space per hen), strains of birds have been developed to perform more efficiently in current management systems, and other programs (feeding, health, beak trimming, lighting, etc), have been adjusted to conform to the needs of birds in cage situations.

Concern has been expressed that chickens should not be caged. The argument is that birds are not able to express their "natural behavioral needs". They can't "nest" their eggs, dust their plumage, choose their feed, run around, or attempt to fly. In becoming domesticated and managed, the caretaker has either eliminated some of these practices or changed the way these needs are addressed. Originally, these concerns were not expressed as layers were housed in single-bird cages. Cages were applauded for removing chickens from their own feces and for eliminating the centuries-old problems of internal worms and parasites. Eggs were cleaner, working conditions for the farm laborers were better and general management was easier. But, most importantly, egg farmers made money with these new systems. Under these conditions, crowding was not a concern and single birds did not develop anti-social tendencies therefore beak trimming was not necessary when pen-mates were not present.

The original single-bird cages provided each layer with 968 to 1290 cm^2 (150 to 200 $in.^2$) of floor space and
12.8 to 25.4 cm (5 to 10 inches) of feeder space. As time passed, egg producers found they could add additional birds to their cages with little if any performance losses. As space allowances were reduced, performance was lost to the extent that further crowding could no longer be justified.

University of California research with the cage density issue dates back to 1961 when we studied the effects of adding a third bird to a standard 2-bird cage. The reduction from 697 to 464 cm^2 (108 to 72 $in.^2$) did not affect hen-day egg production, but mortality due to prolapse and pick-outs increased from 1.4% to 7.4%.

A second study in 1963 added a fourth bird to this same cage size and compared it to a 3-bird cage (464 vs. 348 cm^2) (72 vs. 54 in^2). In this case, hen-day egg production was reduced from 64.0% to 61.7% and prolapse/pick- out mortality was doubled from 3.4% to 7.8%. Obviously, this density was approaching an un-economic level.

During the 1960's and 1970's cage densities gradually increased until today, when 310 and 348 cm^2 (48 and 54 $in.^2$) per bird have become the standard space allowances for laying hens in the US (white-egg strains). This compares to the 450 cm^2 (70 in^2)

standard in Europe and other countries for predominately brown-egg birds. Current discussions in Europe center around the questions of increasing allowances to 800 cm^2 (124 $in.^2$) or complete re-designing or elimination of the cage altogether. Interestingly, government officials recognize the need to "block the import of eggs from countries with weaker animal welfare standards otherwise Economic Union egg farmers would be put out of business by cheap eggs from elsewhere in the world".

Also during this same time period, numerous research studies have demonstrated time and again that additional birds decrease hen-housed egg production and increase mortality. Our analysis of 45 different experiments conducted across the US and Europe show 14 fewer eggs and 3.9% higher mortality rates for each addition of one bird per cage. Even though performance is adversely affected by increasing cage densities, egg producers can often justify the more crowded cage densities at different cost/egg price relationships. With many producers, current levels of egg prices and feed prices will not justify the lower space allowances. On the other hand, some producers can justify crowding under almost any cost/price relationship because of their ability to manage such situations.

In the last 20 years, the laying cage has gone through many modifications. Whereas the original cages commonly held 1-4 birds, today's cages are designed for 6-10. As a result of University of California research relative to cage design and other factors, more emphasis is now placed on feeder space allowances with most systems allowing 7.6 to 10.2 cm (3 to 4 in) per bird (3). Cages have become more "square", thus allowing each chicken more feeder space. Multiple drinkers are recommended to avoid problems when an individual drinker becomes inoperative. Manure systems are designed to store wastes in a different level of the building or to be removed on a daily basis.

Today, we use larger cages than in the 1950-1960 period and the most popular cages are for 6 birds with space allowances of about 348 cm^2 (54 $in.^2$) per bird. In 1994 a large scale experiment was set-up on a commercial California farm to measure the performance and economic differences in placing 5, 6, and 7 birds per 40.6 cm wide by 50.8 cm deep cage (16 in by 20 in) (10). This experiment was conducted over a 38 week experiment (to 58 weeks of age) with 53 thousand DeKalb Delta White Leghorn hens. Data was based upon 24 rows of 2200 birds each. Results are listed in Table 1.

Table 1 illustrates that the highest returns per bird were obtained in the 6-bird cage. This was due primarily to a reduction in feed usage. The highest return on investment was also obtained in the 6-bird cage during low profit years, but with high profit years, the higher density (7 birds per cage) maximizes returns on investment. A fixed high density choice over time, might result in company failure during periods of extended low profit margins.

The choice of cages (design, size, shapes, etc.) and their management systems have many economic implications as discussed above, however, the proposed legislated elimination of cages in Europe will have even greater economic effects for egg producers throughout the region, to their suppliers and to the consuming public. The current proposal to eliminate cages within the next ten years is a major step backwards in the way flocks are managed. Flock health will be severely affected with major food safety implications. The current non-washing policy for eggs will likely have to be changed to adjust to the dirtier eggs produced by litter or free-range systems. Higher flock mortality rates are likely to occur thereby offsetting some of the claimed welfare advantages for non-cage systems. One European legislator was quoted as saying "Changing from battery to free-range eggs would cost the average consumer less than £2 a year". This would represent $850 million per year in the US - not a small amount of money!

Cages have many advantages that should not be discarded in exchange for the one presumed disadvantage of "the flocks' inability to express their natural behavior". The scientific community must communicate the net losses and gains which accrue when husbandry practices are abruptly and totally changed. Total effects are much broader and more complex than a mere £2 ($3.20) increase in costs to the consumer.

Caging is a pro-welfare system of housing laying hens. It results in **improved livability, healthier flocks and higher profitability.**

BEAK TRIMMING

Beak trimming is a management practice used to reduce cannibalism, feather pecking, and other anti-social behavior in chicken flocks. Its benefits are widely acknowledged in the commercial chicken industry. Benefits include:

1. Reduced mortality from pecking.
2. Reduced injuries and sub-normal performance.
3. A general calming of the flock.
4. Reduced feed wastage and feed usage.

Today's methods date back to the early 1940's when the University of California developed a technique using a sharp edged device capable of being heated to cauterize the beak (11). Dozens of experiments and field trials subsequently refined the practice as we know it today. Beak trimming involves a

complex set of decisions which describe in detail the process:

1. Age of birds to be trimmed.
2. Timing relative to other management practices.
3. Amount of beak to remove.
4. Shape of the cut.
5. Blade type and sharpness.
6. Blade temperature.
7. Time of cauterization.

Failure to monitor and control any of these can give less than desirable results. Even though, there are methods to reduce the severity of this problem, beak trimming still appears to be justified when one considers the advantages and disadvantages of this issue.

Lower light intensities in controlled environment houses will tend to reduce the problem of cannibalism and thus may eliminate the need to beak trim for cannibalism control per se. Some strains of birds have very low levels of anti-social behavior, but advantages can still be demonstrated for beak trimming. Reduced cage densities will lessen mortality problems associated with crowding, but economics may still dictate the use of beak trimming to control costs.

Commercial-scale experiments comparing beak trimming vs. non-trimmed controls are difficult to conduct as farmers are reluctant to risk the increase in mortality they expect by not trimming a large number of their birds. In addition, proper experiment design requires replication of treatments and large numbers of hens in each replicate are required to make meaningful assessments of mortality effects.

In 1994 an experiment was set up on a large commercial farm in California to measure the differences in performance between beak trimmed and non-trimmed birds. (Table 2) (14).

The California experiment included 71 thousand birds placed in 32 - 2200 bird rows. Cages were 40.6 cm wide by 50.8 cm deep (16 in by 20 in) and 6 birds were placed in each cage. The experiment was conducted for 40 weeks beginning at 18 wk. of age and ending at 58 wk. Because the birds were to be molted at 60 wk., the last 20 wk. of results were projected from performance levels during weeks 51-58. Economic differences at that time were due mainly to feed consumption savings for the beak-trimmed birds

Significantly higher egg production and egg weight was observed in the non-trimmed birds, but they also experienced more mortality and consumed more feed. Mortality in this experiment was exceedingly low in both treatments due to the strain of birds used. The 1.34% difference in mortality in favor of the beak-trimmed birds was highly significant ($P< 0.001$) and would have probably been missed in traditional smaller experiments. The 5.3 gram per day reduction in feed consumption in the beak trimmed birds was associated with lower body weights (105 grams/bird) and a slightly lower production of egg mass. Eighty percent of the differences in feed consumption were associated with these two factors. Waste did not appear to be a major contributor to the differences noted.

A similar experiment in 1997 by Anderson and Davis at North Carolina State University compared two beak- trimming methods with a non-trimmed control. This experiment included 3160 pullets for 64 weeks of production. This experiment was unique in that "fearfulness" and feathering were evaluated. Results are listed in Table 3 (16). Unlike the California study, higher hen-housed egg production was observed. This was due principally to high mortality and major differences in mortality between beak trimmed and non-rimmed treatments. Similar trends to the California research for feed consumption were seen with a marked reduction exhibited by the trimmed groups. The fearfulness score was significantly higher for the non-trimmed treatment indicating a further advantage for beak trimming. And finally, the feather coating was markedly superior in beak-trimmed birds. This may be a significant contributor to the lower feed consumption observed. Individual beak trimming methods also show dramatic differences in flock performance as seem in Table 3. Even though the 6 day precision and 11 wk severe method birds laid practically the same number of eggs, feed consumption, feather score and economics favored the 11 wk severe beak trimming method.

Performance differences between beak trimming methods have always been seen in University of California experiments dating back to 1972 (Table 4). Interestingly, similar to the North Carolina research, the more severe (apparent) methods commonly outperform the less severe methods. No economic analysis was made in this experiment. A significantly higher egg production rate was observed in the severely trimmed groups. The 18 eggs improvement was unexpected because of the apparent severity of the method.

A similar experiment was conducted in 1981 to verify the moderate/severe beak trimming comparison. A third method was added - a one cut technique for both beaks. All trimming was done at 12 weeks. This experiment was also designed to determine if results were different with different colony sizes. Results are shown in table 5. Results of this experiment verify the results of the previous experiment by demonstrating the superiority of the severe beak trimming method but primarily in the more crowded environment. Feed consumption was similar for all methods, but mortality differences were large. In summary, the more severe method was the method of choice, especially in the

more crowded condition. Mortality was reduced and profitability was higher.

Beak trimming is a practice that no one likes, but it does prevent higher levels of cannibalism and appears to be of major economic importance to the industry. The selection of the best method is also an important decision for poultry flock managers. But, of equal importance, the monitoring of the practice is essential to be sure that techniques are applied evenly across the entire flock.

Beak trimming is a pro-welfare management technique and is done to **reduce mortality** and to **improve profits in egg production.**

INDUCED MOLTING

Induced molting (forced molting) is a procedure used to rejuvenate laying flocks for a second cycle of egg production. Molting, as applied by the farmer, has been used off and on in the commercial egg industry for almost one hundred years. Early mention was made in Professor Rice's book in 1905. It was revived in the 1930's in the Pacific Northwest region and has been practiced at a high rate there ever since. Its second re-birth occurred in the late 1950's in Southern California and has been incorporated in a high percentage of replacement programs throughout the country.

Induced molting usually involves removal of feed for periods of 5 to 14 days followed by a low nutrient ration for the remaining days in a 28 day molt program. Molting, in nature or induced by the farmer, have the same effect - rejuvenation of the flock with resulting higher egg production, renewal of feathering, and improvements in egg quality.

Molting programs involve an estimated 75-80% of the commercial flocks in the US. At any point in time, 25-30% of the nation's layers are either in a molt or have been molted earlier - this represents some 75 million layers out of a total of 265 million. Molting is considered a part of the normal replacement policy on the majority of farms in the US today. Options for the farmer include 1, 2, or 3 cycle programs with disposal ages ranging from 75 to 140 weeks of age.

It's estimated that replacement programs that include molting result in at least 15% higher profit margins for the egg producer compared to all-pullet programs (1999). Model building computer software is available to construct typical 1,2, and 3 cycle flocks. Such models are based upon individual owner experiences or can be developed from breeder standards. Although developed to determine optimum replacement policies, they can also be used to determine "what if" situations for different cost/price situations or for conditions unique to a particular region of the world.

An example of performance, cost, and income for a typical molt and non-molt program is shown in table 6. In this example, after exclusion of other costs, the annual income per hen housed from the molt program is estimated to be $1.32 compared to $1.15 for the one cycle non-molted program - an increase of 15% in profits. With lower egg prices or higher feed prices, even greater differences would exist. Molting is more justified under low margin conditions (low egg prices or high feed prices).

As one can see, molting is an important tool for optimizing profits in the egg industry. Much of the controversy about molting is not about the practice itself, but is directed at the **methods** used to molt a flock. Practically all methods require some degree of feed or nutrient restriction and this is not acceptable to many. There are methods which limit specific nutrients (calcium, sodium and protein) which are used in countries that do not allow feed withdrawal**.** Most of the research with these methods has not proven them to be as satisfactory compared to traditional feed removal methods (19).

The elimination of induced molting in the egg industry would have far-reaching effects on egg producers, their suppliers and the general public. US egg industry's cost and egg price conditions result in very narrow profit margins and the choice of replacement programs has a major impact on a farm's profitability.

Technology is usually adopted slowly and the total effect is spread over the entire industry over a several year period. This prevents massive over-night changes in egg supplies and resulting disruption of the egg market. From time to time, different developments have come along that have dramatically change the performance characteristics of the nation's flock and major changes in the industry's profitability have occurred. Examples of this include: major disease epidemics, large changes in feed prices, and significant changes in the performance characteristics of different strains of chickens. Eliminating a primary management technique (molting) arbitrarily is an example of an extremely disruptive problem. It would result in:

1. The nation's laying flock would increase in size by about 3% as a result of higher house utilization.
2. All-pullet flocks would lay at a 4% higher rate than two-cycle flocks do today.

(Both of these would have a major negative effect on egg prices)

3. Higher costs of production.
4. Approximately 47% more:
    a. Additional chicks to hatch.
    b. More breeding farms and breeding flocks.
    c. More hatcheries.
    d. More male chicks to be destroyed.

e. More spent hens to market.

5. Higher percentages and numbers of medium and small eggs.

Induced molting is a vital component of the replacement programs used throughout the industry. Without molting, flocks would be kept beyond the optimum age for high egg quality, costs to the industry would be prohibitive and the age at disposal for flocks would be shortened from the current 105 to 110 weeks to 75 to 80 weeks.

Induced molting is a pro-welfare management technique and is done to **lengthen the productive life of flocks** and to **improve profits in egg production.**

CONCLUSIONS

The well-being of commercial laying flocks is the result of the systems chosen and the quality of management to make them work as intended. Oftentimes, simple changes can be made to improve these systems which result in both improvements in the well-being of the flock and the profitability of an operation. Careful monitoring of caging, beak trimming and induced molting procedures will minimize the risk of hurting our flocks and their performance. High reproductive performance is an excellent indicator of overall good management.

The choices the farmer makes are driven mostly by economics and economics cannot be arbitrarily dismissed from its important position. Most welfare issues are incremental ones:

* More birds per cage reduces performance.
* More days off feed increases mortality.
* The more beak removed, the greater the damage
* and so on.

Regulations either eliminate practices altogether (no cages) or place numeric restrictions (450 cm^2 per hen) (70 in ^{2}) on a practice. Such regulations are usually enacted to address the exceptional problems but are imposed upon all. If the regulatory route is chosen, it must be based upon scientific fact and not the expedient approach of totally disallowing a practice for political reasons.

SELECTED REFERENCES

(Listed by category and year of publication)

Caging

1. Bell, D.D., M. H. Swanson. Crowding Chickens in Cages Reduces Your Profits. University of California
leaflet 2273. 1975.

2. Bramhall, E.L., W.F. Rooney, and D.D. Bell. How Many Hens in a Cage. University of California leaflet 2652. 1976.

3. Hughes, B.O. Conventional and shallow cages: A summary of research from welfare and production aspects. World's Poultry Science Association, Vol. 39, No. 3, pages 218-228. 1983.

4. Craig, J.V., and A.W. Adams. Behaviour and well-being of hens (Gallus Domesticus) in alternative housing environments. World's Poultry Science Association, Vol. 40, No. 3, pages 221-240. 1984.

5. Nicol, C. Behaviour requirements within a cage environment. World's Poultry Science Association, Vol. 46, No. 1, pages 31-33. 1990.

6. Elson, H.A. Recent developments in laying cages designed to improve bird welfare. World's Poultry Science Association, Vol. 46, No. 1, pages 34-37. 1990.

7. Appleby, M.C., and B.O. Hughes. Cages modified with perches and nests for the improvement of bird welfare. World's Poultry Science Association, Vol.46, No. 1, pages 38-40. 1990

8. Wenger, R. Experience with the get-away cage system. World's Poultry Science Association, Vol. 46, No. 1, pages 41-47. 1990.

9. Appleby, M.C., and B.O. Hughes. Welfare of laying hens in cages and alternative systems: environmental, physical and behavioural aspects. World's Poultry Science Association, Vol. 47, No. 2, pages 107-128. 1991.

10. Bell, D.D. A case study with laying hens. In: Proceedings of Animal Behavior and the Design of Livestock and Poultry Systems Conference. Northeast Regional Agricultural Service, NRAES-84, pages 307-319. 1995.

11. Curtis, S.E., et al. Guide for the care and use of agricultural animals in agricultural research and teaching. First revised edition. Federation of Animal Science Societies. 1999

Beak Trimming

12. Newlon, W.E., and J.R. Tavernetti. Debeaking poultry. University of California, Agricultural Experiment Station Misc. Publication. 1944.

13. Gentle, M. J. Beak trimming in poultry. World's Poultry Science Association, Vol. 42, No.3, pages 268 275. 1986.

14. Hughes, B.O., and M. J. Gentle. Beak trimming of poultry: its implications for welfare. World's Poultry Science Association, Vol.51, No. 1, pages 51-61. 1995

15. Bell, D.D. Can egg producers afford to not beak trim their flocks? In Proceedings of the Forty-fifth Western Poultry Disease Conference, pages 92-93. 1996.

16. Bell, D.D. Beak trimming in caged layers. University of California Cooperative Extension misc. leaflet. 1998.

17. Bell, D.D., and R.A. Ernst. Performance and fearfulness during the production phase of Leghorn hens reared utilizing alternative beak trimming techniques. California Poultry Letter, January, pages 6-7. 1998.

Induced Molting

18. Wakeling, D.E. Induced moulting - A review of the literature, current practice and areas for further research. World's Poultry Science Association, Vol. 33, No. 1, pages 12-20. 1977.

19. Wolford, J.H. Induced moulting in laying fowls. World's Poultry Science Association, Vol. 40, No. 1, pages 66-73. 1984.

20. Bell, D.D. Moulting technologies - welfare issues. Speech given at: The Tenth Australian Poultry and Feed Convention, Melbourne, Australia, October. 1996.

21. Hussein, A.S. Induced moulting procedures in laying fowl. World's Poultry Science Association, Vol. 52, No. 2, pages 175-187. 1996.

General Welfare and Behavior

22. Freeman, B.M. Stress and the domestic fowl: A physiological re-appraisal. World's Poultry Science Association, Vol 32, No. 3, pages 249-256. 1976.

23. Bell, D.D. Stress research in California. Speech given at Colorado State University Poultry Symposium, June. 1986.

24. Freeman, B.M. The domestic fowl in biomedical research: physiological effects of the environment. World's Poultry Science Association, Vol. 44, No. 1, pages 41-60. 1988.

25. Wegner, R. Poultry welfare - problems and research to solve them. World's Poultry Science Association, Vol. 46, No. 1, pages 19-30. 1990.

Transportation and Handling

26. Freeman, B.M. Transportation of Poultry. World's Poultry Science Association, Vol. 40, No. 1, pages 19-30. 1984.

27. Broom, D.M. Effects of handling and transport on laying hens. World's Poultry Science Association, Vol. 46, No. 1, pages 48-50. 1990.

28. Scott, G.B. Poultry handling: a review of mechanical devices and their effect on bird welfare. World's Poultry Science Association, Vol. 49, No. 1, pages 44-57. 1993.

29. Sparrey, J.M., and P.J. Kettlewell. Shackling of poultry: is it a welfare problem? World's Poultry Science Association, Vol. 50, No. 2, pages 167-176. 1994.

Other

30. Bell, D., D. McMartin, F. Bradley, R. Ernst. Egg-type Layer Flock Care Practices. California Poultry Workgroup, University of California, Cooperative Extension. 1998.

31. United Egg Producers. Animal husbandry guidelines for U.S. egg laying flocks. 2000.

Table 1. Performance results - Univ. of California cage density experiment - 1994.

Trait	5/cage *	6/cage *	7/cage *
Hen-housed eggs	198.0	194.3	185.2
Av. Egg weight (g/egg)	59.8	60.1	60.3
Total weight of egg mass/hen housed (kg)	11.84	11.65	11.16
Mortality (%)	6.5	8.4	9.4
Daily feed intake (g)	105.6	101.4	99.4
Profit index/hen-housed ($)	3.97	4.08	3.79
Profit/cage (high costs) ($/cage) **	4.68	6.18	5.32
Profit/cage (low costs) ($/cage) ***	11.98	14.66	15.06

*Cage size = 16 in. (40.6 cm) wide x 20 in (50.8 cm) deep.
** High costs = $2.50 per pullet, $7.50/100 pounds of feed, $.50/dozen eggs.
******* Low costs = $2.00 per pullet, $6.00/100 pounds of feed, $.50/dozen eggs.

Table 2. Performance results - University of California beak trimming study - 1993/94[1]
(40 weeks of results with projection of economic results to 78 wk.).

Trait	Beak trimmed	Not trimmed	Statistical Significance[2]
Hen-housed eggs	191.5	195.7	***
Av. egg weight (g/egg)	58.9	59.7	***
Total weight of egg mass/hen housed (kg)	11.27	11.68	***
Mortality (%)	3.39	4.73	***
Daily feed intake (g)	96.0	101.3	***
Profit index/hen-housed ($)	3.99	4.00	not significant
Profit (projected to 78 weeks of age) ($)	+ $.24/hen housed [3]		

[1] 71 thousand Hy-Line W-36 White Leghorn hens (18-58 weeks of age)
Non-trimmed versus 7-week trimmed.
[2] * (P < 0.05), ** (P < 0.01), *** (P < 0.001)
[3] Projected profits to 78 wk of age is based upon 1.25 ¢/wk profits during the 51-58 wk period.

Table 3. Performance results - North Carolina State University beak trimming study - 1996/97.

Trait	Non - trimmed	6 day precision method	11 wk severe method
Hen-housed eggs	316	335*	333*
Hen-day egg production (%)	79.8	81.2*	80.9
Av. egg weight (g/egg)	61.1	61.5	60.5
Fearfulness score[1]	2.95	2.50*	2.20*
Feather score[2]	3.00	4.80*	5.75*
Mortality (%)	26.3	18.7*	17.1*
Daily feed intake (g)	122	114*	107*
Egg income minus feed cost ($/hen-housed)	8.38	9.87*	10.23*

[1,2] The higher the number the greater fearfulness and greater feather cover.
* Significantly different than the non-trimmed birds.

Table 4. Beak trimming methods and performance - University of California - 1972[1]

Trait	7 day precision	12 wk moderate[2]	12 wk severe[3]
Hen-day egg production (%)	69.7	69.4	72.8
Hen-housed eggs	216	213	231
Mortality (%)	13.9	16.5	12.0
Egg weight (g/egg)	55.5	56.0	55.9
Daily feed intake (g)	116	113	114

[1] 22 to 70 wk. of age.
[2] Top beak to 1/4 inch of nostril, bottom beak 1/3 trimmed.
[3] Top beak to 1/4 inch of nostril, bottom beak 2/3 trimmed.

Table 5. Performance results - University of California beak trimming study - 1981[1].

Trait	Moderate 3/cage	Moderate 4/cage	Severe 3/cage	Severe 4/cage	One cut 3/cage	One cut 4/cage
Hen-day egg production (%)	77.1	71.5	78.0	76.0	74.8	74.9
Hen-housed eggs	246	217	243	244	232	216
Daily feed intake (g)	104	105	103	103	103	105
Mortality (%)	7.3	18.0	11.5	8.6	15.6	24.2
Egg income minus feed cost ($/hen-housed)	3.24	2.35	3.18	3.11	2.84	2.63

[1] 20 to 68 wk of age.

Table 6. Comparison of a single cycle program with a two cycle program - 1999

Trait	Single cycle (80 wk sale)	Two cycle (110 wk sale)[1]
Av. hens (%)	95.6	93.4
Av. Wkly mortality (%)	.150	.154
Hen-day egg production (%)	77.9	72.9
Eggs per hen housed	312.9	428.7*
Large & above eggs (%)	76.9	81.1
Total egg mass (lbs/hen housed)	41.7	58.1*
Undergrade eggs (%)	5.5	5.6
Av. egg value (¢/dozen)	52.7	53.4
Daily feed consumption (g)	101.6	98.9
Feed per dozen (lbs)	3.45	3.60
Feed cost (¢/dozen)	25.0	26.0
Pullet cost (¢/dozen)	9.6	7.0
Feed + pullet (¢/dozen)	34.6	33.0

[1] Molted at 65 weeks of age
* Longer period of time.

FIFTY YEARS OF POULTRY DISEASE RESEARCH AND CONTROL IN THE AUSTRALIAN POULTRY INDUSTRY

Clive A W Jackson

Biological Technology Transfer Pty. Ltd., Camden, NSW, 2570, Australia

ABSTRACT

This paper reviews the main developments in research and control of disease in the Australian poultry industry against a background of major contributions by Drs Hart and Hungerford prior to 1950. The disease status of poultry was well defined by the poultry veterinarians of the time with only five viral diseases and about 12 bacterial diseases causing significant loss in poultry. However, since 1950, the changes in the industry in the form of integration and intensification have been accompanied by the recognition of over 11 new disease agents or antigenic variation that have caused either significant chronic or acute loss to the poultry industry. Major initiatives have been undertaken by poultry companies underpinned by government/industry research funding. Governments have contributed through the development of exotic disease control plans, vaccine standards and codes of practice. A large number of company veterinarians developed biosecurity and quality assurance programs that have reduced disease loss. Research has contributed a range of vaccines and

pharmaceutical products that allowed effective preventative programs to be implemented.

INTRODUCTION

Fifty years ago, the Australian poultry industry consisted of an egg industry based on family farms with chicken meat being supplied through live bird markets derived from spent hens and off-sex cockerels. The broiler industry did not commence until the 1960s.The disease status of the Australian poultry industry at the time was well defined by a limited number of poultry practitioners and thoroughly documented in the publications of Hungerford (10,11,12). The infectious viral diseases that were identified in Australia in 1949 (when a quarantine ban was placed on all poultry imports) included Marek's disease (MD), infectious laryngotracheitis (ILT), Fowl Pox, avian leucosis, and avian encephalomyelitis. Ornithosis had been recognised. Bacterial disease that were present included pullorum disease, paratyphoid, infectious coryza, fowl cholera, spirochaetosis, avian tuberculosis, *Mycoplasma gallisepticum* infection, listeriosis, erysipelas and a variety of infections due to *Staphylococci*, *Streptococci* and *Escherichia coli*. Over the next 50 years, new infectious poultry disease agents emerged with some showing evidence of evolutionary change in respect to virulence.

THE DISEASE ENVIRONMENT

The rapid development of the broiler industry from 1960 and the accompanying intensification of poultry production adjacent to large towns alongside the family egg farms provided an ideal environment for disease transmission through air-borne or vicinity spread. The introduction of cages for egg production in the 1960s increased egg farm size. New broiler genotypes (some of which were alleged to have been derived from smuggled eggs) varied in susceptibility to infectious agents. Vertical integration of the industry facilitated egg transmission of agents. Biosecurity was limited. Quality assurance had not yet been considered. Growth and profit were the driving forces.

THE DISEASE CONTROL ENVIRONMENT

Family egg farms of the 1950s relied heavily on vaccination and medication. The government role was limited to mandatory pullorum testing and ILT vaccination. The expanding poultry industry did not have a poultry improvement plan like the USPIP (U.S. Poultry Improvement Program). The chicken meat industry developed several politically and scientifically strong organizations whereas the egg industry was disparate but maintained its strength through state controlled marketing organizations that limited production to aid returns to the producer. The poultry industry wanted limited government interference and set about establishing company-based disease control plans. In the late 1960s, both the egg and chicken meat industries established compulsory levies on producers to contribute to funds for research. The government matched these funds $ for $. Whilst the primary aim of these funds was for research, many of the discoveries have been instrumental in establishing disease control measures within companies.

Whilst pharmaceutical products were and continue to be a major disease control measure adopted by the poultry industry, there has been a gradual replacement by vaccines and other control measures over time. Eradication programs for pullorum disease were in place over 50 years ago but were only seriously approached when the disease became a problem in the broiler breeding industry in the 1960s (14). Vertically integrated broiler companies followed their USA counterparts in eradicating *M.gallisepticum* and *M.synoviae* from genetic stock in the 1970s.

The dangers of producing vaccines on a non-SPF substrate for parentally administered vaccines became apparent in 1974 when a herpesvirus of turkeys (HVT) vaccine was contaminated with reticuloendotheliosis virus. Standards for avian viral vaccines were improved and have been a major contributor to high quality vaccine production in Australia. The importation of vaccines has been resisted by the poultry industry and it has only been since 1997 that limited quantities of inactivated poultry vaccines have been imported.

VETERINARY RESOURCES

Veterinary services to the poultry industry were provided by a few private veterinarians and state diagnostic laboratories up until about 1970, when integrated poultry companies employed veterinarians who developed diagnostic laboratories to undertake disease surveillance and product quality control. More recently, there has been a reduction in state veterinary services and many of the smaller farmers rely on technical services being provided through feed, vaccine and pharmaceutical companies. In 1961, poultry veterinarians formed an association, the Australian Veterinary Poultry Association (AVPA), which has continued to contribute to government policy and scientific exchange. The AVPA celebrated its 40th year this February past.

THE RECOGNITION OF NEW DISEASE AGENTS

Infectious laryngotracheitis virus (ILTV): This viral disease could be considered synonymous with the development of avian virology in Australia. Despite its discovery in 1934 (20) and control using attenuated vaccines (22), additional research on its pathogenesis and immunity (5) and proposals for eradication (1) have not limited its ability to cause sporadic epidemics in Australia.

Infectious bronchitis virus (IBV): Evidence for a transmissible agent as the cause of a nephropathogenic syndrome observed in the 1950s had been proposed by others but it was not until the work of Cumming (3) that IBV was proven to be the cause. Vaccines became available in 1966 (4) but optimisation of vaccination has proven difficult in some situations. Recent studies (13) have demonstrated that antigenic variation may explain some of the vaccine failures.

Newcastle disease virus (NDV): NDV was found to be widespread in Australia following the isolation of a lentogenic strain (21). The endemic strain proved to be valuable vaccine seed for production of a vaccine for use in developing countries (23). Evidence of evolving virulence was found in the 1990s in association with late respiratory disease (9). When virulent clones of NDV were isolated from clinical outbreaks of ND in 1998 and 2000 and were shown to be antigenically similar (in gene sequences in the fusion protein) to the original endemic viruses, a further evolution in virulence was apparent.

Infectious bursal disease virus (IBDV): Although antibody to IBDV had been recognised in 1968, the virus was not isolated until 1974 (6). Viruses that were isolated have been identified as classical serotype 1 mainly associated with immunosuppression. However, more recent studies (18) have identified a range of classical and of variant strains different to those in the USA. However, no evidence of very virulent strains of IBDV has been found. An internationally successful vaccine virus stain, V877 (16), was developed from a local isolate.

Virulent Marek's disease viruses (vMDV): The evolution of MDV in terms of virulence in Australia was evident when the cause and the pathology were distinguished from avian leucosis in 1967. From the 1960s onwards, there were clearly MDVs present that were capable of causing predominantly lymphoma in visceral organs. In the mid 1980s, virulent strains were identified and from 1992 there was evidence of the presence very virulent strains of MDV (26). Failure to control these more virulent viruses in imported genotypes required the local manufacture of HVT and Rispens MDV vaccines from imported masterseeds (15).

Avian Influenza virus (AIV): Australia has experienced five outbreaks of highly pathogenic avian influenza in 1976, 1985, 1992, 1994 and 1997. All outbreaks were due to the H7 strains of AIV and were eradicated by slaughter of infected farms. Serological surveillance did not detect any other infected farms. No relationship was demonstrated between the outbreaks although in all cases contact with domestic ducks or wild waterfowl was suspected. Testing of Australian waterfowl has found a wide range of haemagglutinin types including H7 and H5 representing a risk to commercial poultry farms (24).

Other viral diseases: Egg drop syndrome virus was identified as the cause of two major epidemics in 1980 and 1996. Antibody to chick anaemia virus (CAV) was found in 1983 and the virus was subsequently isolated. Inclusion body hepatitis was a major cause of mortality in broilers until vaccination of breeding flocks was practiced (8). Avian leucosis virus subgroup J has caused significant losses in a poultry breeding company since 1998.

Bacterial diseases: Control of pullorum disease was achieved in the 1960s and this was followed by prolonged attempts by breeding companies from 1970 to minimise the vertical spread of a range of salmonella serotypes (17). A marked reduction in the level of salmonella on poultry carcasses has been achieved but eradication remains an elusive goal. Australia also gained from early studies on vaccination against infectious coryza. *Mycoplasma synoviae* was associated with respiratory disease (7). More recent advances have been achieved in the development of methods of identification of bacteria, principally *Haemophilus* and *Pasteurella* spp. (2) as aids to improvements in vaccination and disease control. International recognition of advances in the control of mycoplasmosis has been achieved through the development of temperature sensitive *Mycoplasma gallisepticum* TS-11 and *Mycoplasma synoviae* MS-H vaccines (25). Genetically modified live bacterial vaccines for control of fowl cholera have recently been described (19).

DEVELOPMENTS IN DISEASE CONTROL

Disease control in the poultry industry is now firmly based within poultry companies. Quality assurance programs based on HACCP principles are in place for food safety and are developing for welfare, biosecurity and food labeling. There is little government intervention apart from exotic disease control and development of codes of practice. Exotic disease strategy documents exist that direct the way in which an exotic disease should be eradicated and funded. Attempts are being made to tie compensation

for the cost of eradication to the level of biosecurity being practiced by the farmer.

The Australian poultry industry will face new challenges as global markets place pressure on local producers. Imported genotypes and vaccines will continue to test quarantine laws but research and development in molecular science offer new aspirations that disease control will keep pace with new threats to poultry health and consumer demands.

REFERENCES

1. Bagust, T J. and M.A, Johnson. Avian infectious laryngotracheitis: Virus-host interactions in relation to prospects for eradication. Avian Path. 24:373-391. 1986.

2. Blackall, P.J. and J.K. Miflin. Identification and typing of Pasteurella multocida: a review. Avian Path. 29:271-287. 2000.

3. Cumming, R.B. Infectious avian nephrosis (uraemia) in Australia. Aust. Vet. J. 39:145-147. 1963.

4. Cumming, R.B. The control of avian infectious bronchitis/nephrosis in Australia. Aust. Vet. J. 45:200-203. 1969.

5. Fahey, K J and J.J. York. The role of mucosal antibody in immunity to infectious laryngotracheitis virus in chickens. J. Gen. Virol. 71:2401-2405. 1990.

6. Firth, G.A. Occurrence of an infectious bursal syndrome within an Australian poultry flock. Aust. Vet. J. 50:128-130. 1974.

7. Gilchrist, P.T. and G.S. Cottew. Isolation of Mycoplasma synoviae from respiratory disease in chickens. Aust. Vet. J. 50:81

8. Grimes, T.G. Cause and control of a peracute form of inclusion body hepatitis. Proc. 41st West. Poult. Conf., Davis, California. pp 42-44. 1992.

9. Hansson, E., J.G.Young, P.T. Hooper and A.J. Della-Porta. Virulence and transmissibility of some Australian and exotic strains of Newcastle disease virus used in some vaccines. Aust. Vet. J. 77:51-53. 1999.

10. Hungerford, T.G. Diseases of poultry in New South Wales. First Edition. Ed. Department of Agriculture, New South Wales. 1939.

11. Hungerford, T.G. Diseases of poultry. Second Edition. Ed. Angus and Robertson Ltd. Sydney. 1951.

12. Hungerford, T.G. Diseases of poultry. Third Edition. Ed. Angus and Robertson Ltd. Sydney. 1962.

13. Ignjatovic, J., S.I.Sapats, and F. Ashton. A long-term study of Australian infectious bronchitis viruses indicates a major antigenic change in recently isolated strains. Avian Path. 26:535-552. 1997.

14. Jackson, C.A.W. Progress in Pullorum disease control in Australia. Aust. Vet. J. 44:195-201. 1968.

15. Jackson, C.A.W. Multiple causes of Marek's disease vaccination failures in Australian poultry flocks. Proc. 47th West. Poult. Conf., Sacramento, California. pp 49-51. 1998.

16. Jackson, C.A.W. and J.D. Madeley. Characteristics of an infectious bursal disease vaccine effective against very virulent bursal disease in Europe and Asia. Proc. 44th West. Poult. Conf., Sacramento, California. pp 105-107. 1995.

17. Jackson, C.A.W., M.J. Lindsay, and F. Shiel. A Study of the epizootiology and control of Salmonella typhimurium infection in a commercial poultry organisation. Aust. Vet. J. 47:458-462. 1971

18. Sapats, S. and J. Ignjatovic. Antigenic variation and sequence heterogeneity of infectious bursal disease virus strains isolated in Australia. Arch. Virol. 145:773-785. 2000.

19. Scott, P.C., and K.G. Whithear. A new approach to Fowl Cholera control using AroA mutants of Pasteurella multocida as live vaccines. Proc. 135th Ann. Conv. AVMA. Baltimore , Maryland. Pp 186 (Abstract). 1998.

20. Seddon, H.R. and L Hart. The occurrence of infectious laryngotracheitis in fowls in New South Wales. Aust. Vet. J. 11:212-222. 1935.

21. Simmons, G.C. The isolation of Newcastle disease virus in Queensland. Aust. Vet. J. 43:29-30. 1967.

22. Sinkovic, B. and S. Hunt. Vaccination of day old chickens against infectious laryngotracheitis by conjunctival installation. Aust. Vet. J. 44:55-58. 1968.

23. Spradbrow, P.B. Newcastle disease in village chickens. Poultry Science Rev. 5:57-96. 1993/4.

24. Westbury, H.A. History of highly pathogenic Avian Influenza in Australia. Proc. 4th Int. Symp. on Avian Influenza. US Animal Health Association. Athens, Georgia, pp 23-30. 1997.

25. Whithear, K.G. Control of avian mycoplasmosis by vaccination Rev-Sci-Tech.15 (4): 1527-1553. 1996.

26. Zerbes, M., G.A.Tannock, R.J., Jenner, and P.L. Young. Some characteristics of a recent virulent isolate of Marek's disease virus. Aust. Vet. J. 71:21-22. 1994.

A DECADE OF EXPERIENCE WITH FREE-RANGE POULTRY FARMING IN AUSTRIA. IS THIS THE FUTURE?

Franz Sommer

CAHFS, Turlock Branch, 1550, N. Soderquist Road, Turlock, Ca, 95380

Prior to the late 1980's, commercial free-range poultry farming in Austria was predominately restricted to small units that had an opportunity for direct marketing, or to backyard flocks. Since that time consumer demand for free range and organic products has increased, and production has risen to meet this demand. Currently roughly 60 % of Austria's in-shell marketed eggs are produced conventionally in cage/battery houses. 25 % of eggs are produced in floor and volary system houses and 15 % in free-range houses. In 1997 the percentage of free-range eggs sold in grocery stores was 19.1 %, in 1999 it had reached 19.9 % (1, 2). Similarly in Switzerland more than one third of all produced eggs are from free-range farms (3).

In Austria, free-range requires the same in-house standards (feeder space, water supply system, laying nests) as for floor production. Six to 7 hens are allowed per square meter indoors with 10 square meters per hen outdoor space. Hens must be allowed to move outside during periods of daylight, usually between 7 a.m. and sundown. Organic production has the same space requirements but different requirements in feed usage. Poultry houses also need to meet additional criteria, which are summarized in the so-called "Tiergerechtsheitsindex TGI". This is a "poultry welfare index" and includes specific criteria such as distance to gates, number of hens per gate, size of gates, but provides little information on animal health. The monitoring or assessment of this index is done by animal welfare organizations, and the points assessed should be directly related to the degree of animal welfare on the farm.

In 1997, a health status informational survey was conducted on free-range layer flocks. The survey obtained information on management, egg-production and state of health. Fifty free-range poultry flocks from 40 farms were examined during springtime 1997 (4). The farm size varied from 500 to 3000 hens, with individual flocks of 500 to 700 hens. The eggs were collected manually or, on a small number of farms also mechanically, sorted by weight and packed into half-dozen or dozen-sized cartons at the farm. A sheet with general information on how these eggs were produced along with the producers address was added to each carton. The boxed cartons were picked up at the farms two times per week and transported to a marketing organization. The boxed cartons were then brought to the different grocery store distribution centers. This guarantees the same per-egg-price to each producer, irrespective of the distance from the consumer. In Austria, most of free-range operations are members of marketing organizations. This particular organization consists of about 300 farms and produces about 75 million free range or organic eggs per year.

The health status survey covered 50 flocks on 40 farms. Individual farm visits were conducted. The poultry houses were either newly constructed or were adapted for hens, and they had pens with forecourts of concrete, asphalt or gravel and paddocks for rotation. A 10 m bare-ground border was around each house, and usually had deep holes for the hens to use for dust baths.

The hygienic conditions in 34 out of 40 surveyed farms were acceptable, with basic biosecurity rules fulfilled (disinfections mats, separate clothes for the poultry houses). Seven flocks had IBV titers suggestive of a field challenge. Seven non-vaccinated flocks had Elisa titers to Salmonella enteritidis and bacteriological examination of fecal droppings showed that Salmonella group D was present in one flock. The presence of endoparasites was relatively low with 19 flocks testing free. Ectoparasites were not detected. Cannibalism was present in 14 flocks; feather pecking was present in 28 flocks.

Production data were obtained from 18 flocks. During a 12 months period, the average hen laid between 228 and 357 (mean 290) eggs. The rate of mortality ranged from 0-32 % (mean 7.2 %). The 32 % mortality rate seen in one flock was due to cannibalism and raptor attacks. The average benefit a producer gets for free range produced eggs is about 25 % higher than for floor eggs, which are also about 30 % higher than cage eggs.

A decade ago, very few farmers were looking at alternative methods of egg production. But with the higher egg demand the supply had to be improved. In addition, consumers, especially in Austria, began to pay more and more attention to how their food is produced. Due to the higher demand from customers and a their willingness to pay a higher price for "alternative" produced food, more farmers have changed to those methods. In the consumer's mind, the product or the price does not only determine quality, but also by the method of production of farming. Consequently some of the main grocery stores or

chains use this knowledge of the consumers' preferences to ban cage-produced eggs and suggesting in their advertisements their strong desire to support animal welfare and provide a healthy food product.

Austrian operations appeared to do better under the free-range program than other European countries. In Germany, for example, there are reports of mortality rates of 50 % (5, 6). One possible reason could be the smaller flock and farm size in Austria, where members of the family usually perform free-range poultry farming, and hired assistance is not involved. Successful free-range farming requires a high degree of emphasis on management and hygiene.

REFERENCES

1. ACNielsen. Eier im Lebensmittelhandel 1997. In: ACNielsen, Frischeindex (Kundenbericht). ACNielsen, Moeringgasse 20, A-1150 Wien. 1998

2. ACNielsen. Eier im Lebensmittelhandel 1999. In: ACNielsen, Frischeindex (Kundenbericht). ACNielsen, Moeringgasse 20, A-1150 Wien. 2000

3. Meierhans, D., Menzi, H. Phosphatbelastung ist problematisch. Schweiz. Gefluegelzeitung 8: pp. 6-13. 1995.

4. Sommer, F., Vasicek, L. Haltungsbedingungen und Gesundheitsstatus von Legehennen in Freilandhaltung. Wien. Tztl. Mschr. 87: pp. 202-212. 2000.

5. Boehland, K. Aktuelle Gefluegelkrankheiten im Zusammenhang mit alternativen Haltungssystemen: Praxiserfahrungen. Proc. 57. Fachgespraech Gefluegelkrankheiten, German Veterinary Medical Society, Hannover, Germany. pp. 50-67. 1999.

6. Hafez, H.M. Re-emerge of some infectious diseases in layer flocks kept under alternative rearing systems. Proc. 49th West. Poult. Dis. Conf., Sacramento, California. pp. 68-69. 2000.

A GLIMPSE AT POULTRY HEALTH AND MANAGEMENT IN ASIA

C. R. Gustafson

Embrex, Inc., 1040 Swabia Court, P.O. Box 13989, Research Triangle Park, NC 27709-3989

Despite the economic crisis the region experienced in 1997, poultry production in Asia continues to increase. Chickenmeat production rose from 8.17 million ton in 1989 to 16.62 million ton in 1999 in Asia. Number of layers in the region jumped from 1480 million in 1989 to 2935 million in 1999 (*source:* FAO). The popularity of poultry in Asia is no surprise since the stock for most commercial birds originated in Asia. Farmers were producing chicken in China, India and SE Asia long before Europe and America. Although the per capita consumption of animal protein is still low in these areas (lower values than recommended by FAO and WHO) the trend to consume more is increasing.

Poultry health and management in Asia is as varied as the region itself. There are extremes of conditions both in bird health and management from computerized tunnel ventilated state-of-the-art facilities to simple open housed management. Both situations being capable of resulting in very profitable flocks or disastrous losses. Even with the potential for poultry industrial growth in the region, the variety of conditions existing make development of the industry a particular challenge. Variety begins with the weather where temperate conditions are found in the North, i.e. China, Japan, Korea and than the hotter humid conditions being more prevalent in SE Asia, such as, Thailand, Philippines, Malaysia, and Indonesia. If one considers housing to be one of the basics of poultry production, along with feed and water, this region is quite diverse and all types of poultry housing can be found. With all types of housing, the same factors must be considered, such as, temperature, moisture, air movement, and light. Efficient methods of feeding, watering and waste removal are still considerations no matter what type of house is used. When controlling or working with the basic elements at hand, the health of the bird is maintained and the potential production parameters are realized.

The disease challenges in Asia are very real and must be considered when evaluating health programs and management schemes. Still present today in certain Asian countries are vvIBD, vvNDV, AI, and other poultry diseases that cause serious economic losses. Consideration of the variations in Asia, both in the management and field challenge situation, is important for the overall success of the industry and control of poultry health as it relates to the region and worldwide.

SOUTHEASTERN UNITED STATES INFECTIOUS BRONCHITIS VIRUS UPDATE

Frederic J. Hoerr, Lanqing Li, Susan B. Lockaby, Tami Kelly, Francene Van Sambeek, and Joel Cline

Alabama Department of Agriculture and Industries, Veterinary Diagnostic Laboratories
Auburn, Alabama 36831-2209

ABSTRACT

In our laboratory system, infectious bronchitis virus (IBV) is isolated in embryonated eggs and virus is detected in allantoic fluid by RT-PCR with the IBV serotype deduced from restriction fragment length polymorphism (RFLP) analysis (Jackwood, M.W., H.M. Kwon, and D.A. Hilt. Avian Dis. 36:403-409. 1992; Kwon, H.M., M.W. Jackwood, and J. Gelb, Jr. Avian Dis. 37:194-202. 1993). Although isolates are obtained each month of the year; IBV is isolated from broilers with highest frequency from December through April. Most of the isolates are from broilers with clinical illness, however, SPF sentinel Leghorns are also placed in broiler houses and then submitted to the laboratory for virus isolation. The isolation patterns of routine IBV serotypes are similar between sentinels and broilers with respiratory disease. Sentinels provide a higher yield of virus isolates but RT/PCR-RFLP variant IBVs are typically isolated first from sick broilers. The Arkansas serotype is the most prevalent IBV serotype isolated in Alabama with Ark DPI predominant. Ark 99 was the most common in 1997 but has rarely occurred since 1998. Mass and Conn are less common, presumably representing the re-isolation of vaccine viruses. In 1999, an IBV with an RFLP pattern similar to DE 072 was first isolated in north Alabama from broiler breeders, and then from broilers. Ninety-six isolates of DE 072-like IBV, called GA 98, were obtained from broilers from March 1999 until December 2000. Six isolates of GA 98 were from broiler breeders, including the index Alabama isolate in February 1999. Clinical disease in broilers was characterized by few indications of respiratory disease within the flock, however, high condemnations occurred at processing from pneumonia and air sacculitis. Because of slight differences in the RFLP with DE 072, the virus was examined using primers specific for DE 072 S1 spike protein (Keeler, C.L., K.L. Reed, W.A. Nix, and J. Gelb, Jr. Avian Dis. 42:275-284, 1998). An amplicon of 475 bp was obtained that had 96.2% nucleotide homology and 90.5% deduced amino acid homology with DE 072. Similar results were obtained by sequencing studies of nine Alabama isolates of GA 98 by Dr. Jack Gelb of the University of Delaware. Concurrent with the Alabama experience, this virus was being isolated from broilers in Georgia in the laboratory of Dr. Mark Jackwood at the University of Georgia. His studies indicated that significant serological differences existed between this virus and DE 072, and among other IBV serotypes (M. Jackwood, personal communication). In consideration of these challenge study results and the nomenclature confusion among poultry producers, the DE 072-like virus was named GA 98, based on isolation of the index case from broilers in Georgia in late 1998. Even with the antigenic differences, DE 072 live IBV vaccine has been the primary means of control with the rationale that the partial protection offered by DE 072 vaccine offers better protection than having immunologically naïve birds. The isolation of GA 98 from Alabama broilers dropped sharply after widespread use of DE 072 vaccine was incorporated into vaccination programs. Another virus has recently emerged in Alabama, AL/97/9021, first isolated from broilers in June 1997. Only a single isolate was obtained in 1997, and the virus was not observed again until April 2000. Sixteen isolates were subsequently obtained from broilers in 2000, and two additional isolates from broiler breeders. Twelve IBVs have been isolated from broiler breeders ($\geq$ 12 weeks) in Alabama, coinciding with variable respiratory disease, egg production decreases, and mortality from secondary causes. GA 98 is the most frequent isolate, followed by Conn, then Mass, Ark 99, and Ark DPI. Ten IBVs have been isolated from noncommercial chickens. RT/PCR-RFLP variants are the most common, followed by Ark DPI, and then GA 98 and Mass. Broiler breeders and noncommercial poultry could be reservoirs of IBV and a source of new IBV variants, reinforced by the fact that GA 98 was first isolated in Alabama from broiler breeders.

ACKNOWLEDGEMENTS

The authors thank Dr. Sara Rowe, A. C. Ellis, Jeanette Caesar, Michael Luther, Sara Brown, Melissa Roseman, Ed Lackey, Scott Helton, Bruce McMurtrey, Julia Bright, Billy Parker, Tammy Watson, Taneaka Rowe, and Cathy Fincher for technical support. The support of the Alabama Department of Agriculture and Industries is gratefully acknowledged.

(Publication intentions: *Avian Diseases*)

SEROTYPE DIFFERENCES, SHOWN BY VIRUS NEUTRALIZATION, AMONG INFECTIOUS BRONCHITIS VIRUS ISOLATES FROM CALIFORNIA

P. R. Woolcock[A], M. D. McFarland[A], S. Hietala[B] and L. M. Shih[B]

California Animal Health and Food Safety Laboratory System, University of California, Davis
[A]Fresno Branch, 2789 South Orange Avenue, Fresno, CA 93725
[B]Davis Branch, PO Box 1770, Davis, CA 95617

Isolates of infectious bronchitis virus (IBV) can be characterized by a variety of methods. The typing methods used are dependent upon different properties of the virus, but in terms of nomenclature the identification usually relates back to a serotype name, e.g., Massachusetts 41, Arkansas 99. Serotyping of a virus isolate can be performed by either virus neutralization or hemagglutinin inhibition using serotype specific antisera. Both methods of serotyping take a considerable time to complete as well as being labor and material intensive; they are therefore not practical as routine tests in a diagnostic virology laboratory.

Alternative, faster methods of typing IBV isolates that are not based upon serotyping do not always give a conclusive result. A weakness of these methods is that they are only able to identify previously characterized serotypes. Also, we have found that the correlation between results obtained by different methods is not always 100%. These methods include immunoblotting, typing with serotype specific monoclonal antibodies, reverse transcription polymerase chain reaction (RT-PCR) and restriction fragment length polymorphism (RFLP), and RT-PCR and direct automated cycle sequencing (DACS).

Serotype specific antisera to eight Californian IBV isolates were prepared in chickens using the method described by Gelb *et al*. (1). Each antiserum and each isolate were titrated so that a standardized virus neutralization assay could be performed consisting of 10^2-10^3 $EID_{50\ of}$ virus and 10-20 neutralizing units of antiserum. Each antiserum was reacted with each of the IBV isolates. For each test, 5 embryonating chicken eggs were inoculated with the virus-serum mixtures, results were scored as positive or negative based on an indirect inmunofluorescence test using a monoclonal antibody cocktail to group IBV antigens on frozen sections of chorioallantoic membranes from the inoculated eggs.

Little cross-neutralizing activity was detected among the eight IBV isolates investigated. Two of the antisera neutralized viruses of the Arkansas, Connecticut and Massachusetts serotypes, one antiserum neutralized viruses of the Arkansas and Connecticut serotypes, and two antisera neutralized virus of the Cal var. serotype. These serotyping results will be further discussed and related to results obtained by other methods of virus "typing".

REFERENCE

1. Gelb. J. Jr., J. B. Wolff, and C. A. Moran. Variant serotypes of infectious bronchitis virus isolated from commercial layer and broiler chickens. Avian Dis. 35:82-87. 1991.

FIELD EXPERIENCES WITH VARIANT STRAINS OF IBV IN MEXICO

Miguel A. Márquez

Boehringer Ingelheim Vetmedica, S.A. de C.V. Calle 30 No. 2614, Zona Industrial. 44940. Guadalajara, Jalisco. México

INTRODUCTION

Infectious Bronchitis (IB) is an acute, highly contagious viral respiratory disease, characterized by

tracheal rales, coughing and sneezing. Besides, this malady may affect the urogenital tract of chickens. In laying flocks there is usually a drop in egg production and egg quality. Mortality may occur in young chickens due to respiratory or kidney manifestations of the infection.

The causative microorganism is a coronavirus member of the family *Coronaviridae*. The disease is quite common throughout the poultry industries worldwide where *Gallus gallus domesticus* is produced commercially. Mixed infections involving IB virus (IBV) with Newcastle disease, Avian Influenza and Laryngotracheitis viruses, in addition with Mycoplasma and *Eschericha coli*, are common and may confuse the diagnosis.

Many serotypes of IB viruses have been recognized, since the disease was first reported in 1931 (1) and have practical significance in the control and prevention of IB, because immunity following infection or vaccination with one serotype often is not protective against infections with unrelated serotypes.

The most often isolated IB serotypes in commercial poultry have been Massachusetts, Connecticut, Georgia, Arkansas, Delaware (DE/072/92), California, UK/7/93=4/91, etc.

NEW IBV VARIANT SEROTYPES AND SUBTYPES

IB viruses are well known because the presence of various antigenic types. Although antigenic variation of IBV has been recognized for years, it is only within recent years that the scientific community has had the capability to indeed appreciate the genetic diversity of the virus.

IBV is uniquely suited to undergo mutation during its replication/life cycle. Replication of the virus RNA genome is error-prone and the resulting mutations may alter important antigenic components of the virus and result in new variants. The major target for mutation is the spike (S) envelope protein gene that the virus uses to attach to the host cell. Mutations especially in S1, result in antigenic changes and the emergence of variant serotypes as well as subtypes of recognized serotypes. Furthermore, S protein gene is able to tolerate numerous mutations without compromising the virus ability to replicate and cause disease.

Newly mutated variant serotypes are subject to immunological selection, so that, only the most antigenically novel variants persist in a poultry population. A new variant that is not antigenically novel, e.g. one similar to a vaccine strain used on a farm, will not persist in the flock because the vaccine induced immunity will eliminate it from the population. Conversely, newly mutated variants that are antigenically distinct from vaccine strains will, in essence, have a far greater potential to escape vaccine – induced immunity, persist and potentially cause disease.

It is not a coincidence then, that new variant serotypes arise in commercial layers, breeders or broilers raised on farms with multiple-age flocks. These farms provide the adequate condition which favor the emergence of new mutant IBV variants. Layer and broiler flocks of different ages frequently numbering millions of birds, are housed in close proximity. Periodic introduction of new pullets and baby broiler chicks, and the continual reinfection favor the recycling of IBV, resulting in a greater opportunity and spread than occurs on farms using a single age, “all in – all out” management system. Novel IBV variants build up in the poultry house environment, despite the cleaning, disinfection and biosecurity actions Importantly, vaccine immunological mechanisms provide a major selective pressure for the most antigenically novel variants since new variants are continually arising. Gelb et al., (2)

EMERGENCE OF INFECTIOUS BRONCHITIS VIRUS VARIANTS IN MEXICO

During the last four winters and very particularly during the cold season of 1999- 2000, in Central and Western Mexico, it was observed with increasing frequency and severity the presence of respiratory problems with high mortalities in broiler flocks.

Clinically, the birds have a good performance up to four weeks of age, with mortality according to the normal parameters. However, at 28 days of age, broilers start presenting respiratory symptoms. One week later this condition gets worse, in such a manner that by 42 days of age mortality can reach one percent per day, or more. When flocks are sent to the slaughter house mortality levels can be higher than 20% to 35%.

POST-MORTEM EXAM

The typical gross lesions observed during necropsy work are: Conjunctivitis, catarrhal rhinitis, congestive-hemorrhagic tracheitis, caseous airsacculitis, pneumonitis with pulmonar hepatization, pericarditis, perihepatitis and yellow caseous peritonitis. In some cases, a caseous plug may be found in the lower trachea or primary bronchi.

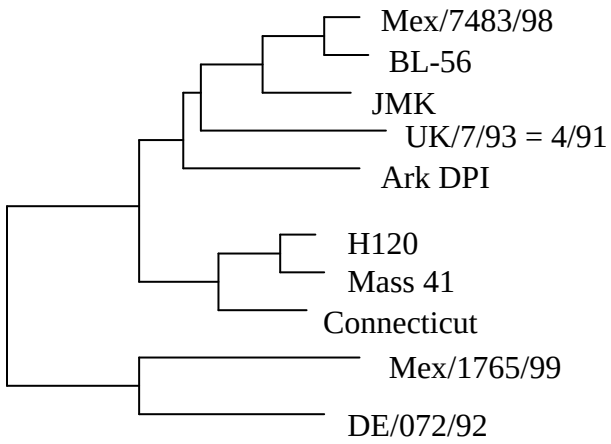
In 1996, Escorcia and coll., reported the isolation from 5 week old broilers of a different agent after 4 blind passages in SPF chicken embryos. When it was further passaged they found the particular formation of a serous bursa full of urates next to the yolk sac (3). This isolate was identified a year later by RT-PCR/ RFLP by M. Jackwood at the University of Georgia and named UNAM-97, (4). In 1998 a second virus was

isolated after 28 blind passages in SPF embryonated eggs. This isolate was named BL-56 and is capable to produce respiratory and renal lesions. Both agents have been officially recognized by the Mexican veterinary authorities.

IDENTIFICATION OF IBV MEXICAN VARIANTS

Gelb and coll., received 17 IBV isolates from commercial chickens in Central Mexico for identification during late fall 1999 and early spring 2000. The isolates were from broiler flocks vaccinated for IB using live Mass and Conn strains at one day of age and again at 9-14 days of age. They were identified by "Direct Automated Cycle Sequencing", (DACS) for identifying new, previously unrecognized variant strains of IBV (5). The procedure uses universal PCR primers, CK4 and CK2, to amplify a diagnostically relevant portion of the S1 gene of an IBV variant. Then the amplified S-1 gene product of the unknown variant was sequence by direct automated cycle sequencing, analyzed and compared to S1 sequences for previously recognized field serotypes.

Two different strains of IBV were identified. One strain MX/1765/99, the most common, represented 82% (14/17) of the isolates tested. This virus clearly represents a new strain and is unrelated to other known serotypes of the virus. The second strain MX/7483/98 was less common and represented 18% (3/17) of the isolates tested.

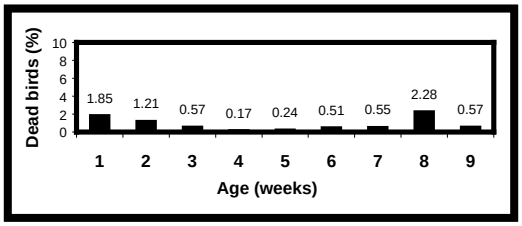


Infectious Bronchitis virus dendogram depicting S-1 protein similarities (Gelb, 2000)

Due to the heavy economical losses reported by the broiler producers, we prepared an experimental oil emulsified inactivated vaccine was prepared containing the IBV variant strains, and was given to broiler flocks according to the following vaccination program:

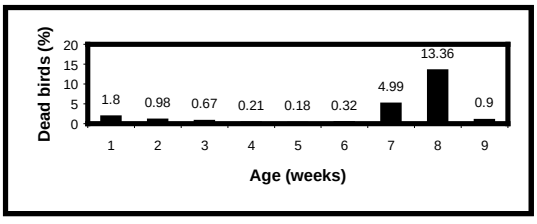
1 day of age Marek disease (HVT)
6 days of age Infectious Bursal Disease, (Lukert strain), Modified Live Virus by drinking water
10 days of age Newcastle + Avian Influenza + Infectious Bronchitis, variant, killed oil vaccines, 0.5 ml, S/Q in the back of the neck and Newcastle disease, (LaSota strain), Modified Live virus by eyedrop
14 days of age Infectious Bursal Disease, (Lukert strain), Modified Live Virus by drinking water
28 days of age NewcastleDisease (LaSota strain), Modified Live Virus by drinking water.

Figure 1. Mortality of experimental IBV variant killed virus. Vaccinated birds (Flock A).



52,000 birds; total mortality: 7.95%; slaughter age: 54.6 days; body weight: 2.380 kg; feed conversion rate : 2.0

Figure 2. Mortality of experimental IB variant killed vaccine. Non-vaccinated birds (Flock B).



55,000 birds; total mortality: 23.41 %; slaughter age: 50.8 days; body weight: 2.275 k.; feed conversion rate: 2.3

DISCUSSION

Up to now the only option in this country, to prevent and control the IBV Mexican variant strains, is the use of inactivated oil vaccines prepared with the mentioned viruses. The development of an attenuated live vaccine is to risky due to the possibility of introducing new and different variant subtypes into the already over heated population of field and vaccinal IB viruses in the farms. On the other hand, officially, the only IBV vaccine strains recognized and registered in Mexico are Massachusetts and Connecticut serotypes.

Recommendations. The strategy and actions to be taken are the following:

1. Establish a differential diagnosis of the clinical condition and rule-out other causes other than Infectious Bronchitis such as Avian Influenza, Newcastle, Laryngotracheitis, etc. If an IB outbreak is confirmed by the isolation of an IB isolate proceed to carry on with the serology, virology and molecular biology identification of the virus.
2. Provide, *a priori* a solid early immunity of respiratory tract, by vaccinating with modified live vaccines prepared with Mass and Conn serotypes, in order to prevent challenges by the dominant native IB viruses.
3. Build a high immune wall of circulating antibodies by 28 or 30 days of age, vaccinating between 8 to 10 days, or even at one day of age with an inactivated oil vaccine prepared with the IBV variants.
4. Investigate in the short term, the option of an integral prevention and control of this condition by vaccinating the broiler breeders at 18 and 40 weeks of age with an oil polyvalent inactivated vaccine (ND+IB+IBD+Reo), that in the IB fraction contains M41 and IBV variants antigens, in order, to transfer maternal antibodies to the progeny.
5. Reduce predisposing stress factors, such as over crowding (12-18 chickens per square meter) by handling only 9 to 10 birds per square meter. Diminish and unload density by slaughtering heavy birds at 42 days of age.
6. Improve good producing farm practices: cleanness, disinfection, real and effective biosecurity, two weeks resting period for the farm facilities before receiving new flock, good ventilation (Reduce CO, CO_2, ammonia, dust levels). Avoid wide internal house temperatures variations during a 24 hours period, e.g. 7 to 26°C. The key issues are: provide adequate internal temperatures without huge variations and excellent ventilation, which are critical during wintertime.
7. We all know that vaccines by themselves cannot be the only integral solution.

CONCLUSION

The experimental IBV variant oil emulsified vaccine conferred a satisfactory protection when given subcutaneously in 10 day old broilers, by reducing mortality under field conditions at the beginning of the current high challenge season. Further field studies must be carried out, in order to have the final assessment of this vaccine through the present winter.

REFERENCES

1. Schalk, A.F. and M.C. Hawn. 1931. An apparently new respiratory disease in baby chicks. J Am Vet Med
Assoc 78: 413-422.

2. Gelb Jack, Jr., Brian S. Ladman, W. Allan Nix, Bruce F. Kingham, David S. Troeber and Calvin L. Keeler. Emerging IBV vaccine - derived field strains and new serotypes. Proceedings of 49 Western Poultry Disease Conference. March 5-7, 2000. Sacramento, California. p. 6-8.

3. Escorcia, Magdalena. Molecular characterization of an Infectious Bronchitis virus isolated in Mexico. Master of Sciences thesis. Faculty of veterinary Medicine. National Autonomous University of Mexico. October, 1999.

4. Jackwood, M.N., N. M. Youssef, and D.A. Hilt. Further development and use of a molecular serotype identification test for infectious bronchitis virus. Avian Dis. 41.105-110,1997.

5. Kwon, H.M., M.W. Jackwood, and J. Gelb Jr. Differentiation of Infectious Bronchitis virus serotypes using the polymerase chain reaction and restriction fragment length polymorphism analysis. Avian Dis. 37: 194 - 202, 1993.

AN OVERVIEW OF INFECTIOUS BRONCHITIS IN MEXICO

Gay, G. M., Soto, P.E., Suarez, A. Garcia-Garcia, J., Sarfati, D. and Lozano, D.B.

Laboratorio Avi-Mex S. A. de C. V. Research and Development Division.
Maiz 18, Col Granjas Esmeralda, Del. Iztapalapa, México D. F.

INTRODUCTION

Avian Infectious Bronchitis (IB) is an economic important, highly contagious disease for poultry industry (6). The disease was reported first on 1931 (14), thereafter in 1937 the etiological agent, the infectious bronchitis virus (IBV), was isolated (2). The pathogen virus is the prototype genus of the *Coronaviridae* family, now located in the taxa *Nidovirales* order (13).

Five structural proteins, the S1 and S2 glycoproteins, which form the peplomer (S) projection, seen at the surface of the virion; the membrane (M), the small membrane (sM) glycoproteins and the nucleocapsid (N) protein are responsibles of the antibody induce response in chickens, following the first contact with the virus (5).

Virus neutralizing epitopes have been identified on the S1 glycoprotein at the N-terminal which also is responsible for viral attachment. Serotyping differences in IBV are associated with changes in the sequence of the S glycoprotein (11).

In the literature have been reported the molecular basis of IBV antigenic heterogeneity due to point mutation in the virus genome focused on S1 subunit protein. Cavanagh et al (3) reported differences from 2 to 3% in four different strains accounted for extreme antigenic differences among them. Meanwhile, Adzhar et al (1) reported differences between 21 to 25 % between 793/B with respect to other 17 strains analyzed, those variations resulted in antigenic variation too.

Numerous IBV strains have been isolated from vaccinated flocks in many countries where predominantly the Massachusetts strains have been used for vaccination. (6)

The antigenic variation observed in different strains of IBV clearly accounts for lack protection induced by vaccination with standard strains. (7)

Mexico background. The disease and the isolation of the virus were first reported in Mexico by Moreno-Chan in 1962 (11). Lucio-Martinez in 1969 (10) recognizes the presence in Mexico of Massachusetts and Connecticut strains by imunofluorescence studies. Poultry Department of the Faculty of Veterinary Medicine, belonging to the National Autonomous University of Mexico (UNAM) reported from 2 to 9% of virology diagnosis cases, worked there from 1963 to 1996, corresponded to IBV (14).

Epidemiological studies done in 1993 shown that IB is country wide spread. Ministry of Agriculture in Mexico, based on this information, authorized the use of live vaccines prepared with Mass and Conn strains to support the control.

Disease processes observed. Generally in Mexico, the infectious process has been described in young chickens as respiratory distress, with low mortality; but associated to other complicate infectious agents could be severe with high mortality rates. In egg layers can also be detected by an increase in the antibody titers with and without reduction of egg production. Frequently, due to, no laboratory follow cases are considered as bacterial processes. Clinical evidences have been described with high morbidity after one week of the episode. (14).

Evidences of variants presence in Mexico. In Mexico different reports were done in the past by practitioners, where the main subject was IB problems in the field due to lack of protection conferred by vaccines produced.

Diagnostic laboratories encourage the necessity to improve diagnostic tools for accurately service. Serotyping IBV isolates by virus neutralization (VN) and ELISA for serology were established as well the use of monoclonal antibodies provided by Dr. Naqui for serotyping of isolates were also conducted.

Arkansas virus was reported by Quiroz et al, in 1993 (12) using indirect immunofluorescence with monoclonal antibodies. Serological evidence for 793/B strain was done by Cook et al in 1996, probably due to cross reaction, since no isolation of this strain has been reported.

Different isolates collected from various regions in Mexico could not be identified by this procedures. Available molecular characterization studies for these isolates were done at University of Georgia and University of Delaware. Results of those molecular typing studies have been presented in Mexico conferences where the information is that two different groups of genotypic variants, no reported elsewhere, were identified. One of them denominated BL-56 genotypic group variant and the other one UNAM-97 genotypic group variant (7, 8).

According with the available information samples were inactivated with phenol and shipped to the

Universities under US veterinary permit. The sequence alignment between Delaware 072 to UNAM-97 shows 65% degree of S1 protein sequence similarity, these do not represent that UNAM-97 and Delaware 072 would be the same strain. BL56 shows 82% of S1 protein similarity to JMK However, the 18% difference seem to be enough to be considered different strain (7).

No Delaware 072 nor JMK were identified in the samples submitted for molecular typing up present. Other studies reveal that UNAM-97 genotypic group variant seems to be more distributed than BL-56. However, It is necessary to conduct a more scientific epidemiological study to achieve an accurately information. Moreover, in one molecular typing study was detected the possible existence of other two variants that may account to establish further studies for IBV evolution to cause emerging diseases and spread.

Macroscopic lesions. BL-56 and UNAM-97 genotypic group variants do not show pathogenic differences in experimentally infected chickens to those observed with other IB cases. Mildly respiratory symptoms were observed associated with sneezing and nasal discharge, feed and water consumption is decreased, Post-mortem observation shown serous exudates in trachea and opalescence in air sacs. Desqumation of respiratory epithelium and mononuclear cell infiltration in trachea were observed on microscopic. Blind passages are necessary for virus isolation before any lesions in the embryo can be detected. Uric acids crystals were observed (9).

Recently the Ministry of Agriculture in Mexico officially declared the existence of the BL-56 and UNAM-97 variants and allowed the production of inactivated vaccines to be licensed. Advances on the epidemiological survey if available will be presented.

REFERENCES

1. Adzhaar, A., Goough, R.E., Haydon, D., Shaw, K., Britton, P. and Cavanaagh, D. Molecular analisis of the
793/B serotype of infectious bronchitis virus in Great Britain. Avian Pathology (1997) 26, 625 - 640.

2. Beaudette, F. R., and Hudson C. B. Cultivation of the virus of infectious bronchitis. J. Am Vet. Med. Assoc.
90, 51, 1937

3. Cavanagh, D., Davis, P.J., Cook, J. K. A., Li, D. Kant, A and Koch, G.Loacation of the aminoacid differences in the S1 spike glycoprotein subunit of closely related serotypes of infectious bronchitis virus. Avian Path 21 33-43, 1992.

4. Cook, J.K.A., Orbell, S.J., Woods, M.A. and Huggins, . B. A survey of the precense of a new infectious bronchitis virus designated 4/91 (793/B) Vet. Rec. 138:178-180. 1996.

5. Collison, W. E., Parr, L. R.,, Li, W. and Williams, K.A. An overview of the molecular Characteristics of Avian Infectious Bronchitis virus. Poultry Science Rev. 4: 41-55, 1992.

6. Gelb, J. Jr. and Jackwood W. M. Infectious bronchitis In: A Laboratory manual for the isolation and identification of avian pathogens 4th ed. The American Association of Avian Pathologists. University of Pennsylvania pp. 169-173. 1998

7. Gelb, J. Jr Molecular studie of IBV isolated in México. Proc. 25Th convención annual de ANECA. México pp 124-127, 2000.

8. Jackwood W. M. personal communication.

9. Lozano D.B. Gay G. M., Suarez A. Soto P.E. Sarfati D. And García-Garcia J. Aislamiento y caracterización de un virus de bronquitis infecciosa designado BL-56 Proc. X Curso de Actualización Avimex, pp. 18-24, 1998.

10. Lucio, M. B. Differentiation and detection of Infectious Bronchitis virus subtypes by Immunofluorescence. B. D. Thesis, Universidad Nacional Autónoma de México. 1969.

11. Moreno Ch. R. Presencia del virus (Terpeia pulli) de la bronquitis infecciosa en las aves de México. In: Ciencia Veterinaria 5. UNAM ed. Pp. 143-147. 1991.

12. Quiroz M. A. y col. Determinación del serotipo Arkansas a partir de aislamientos del VBI aviar en México. Proc. 18th Convención Nacional ANECA, México pp 235-241, 1993.

13. Murphy A. F., Gibbs J.E.P., Horzinek C. M. and Studdert J. M. Veterinary Virology pp. 27, 1999.

14. Shalk, A.F. and Hawn, M.C. An apparently new respiratory disease of baby chicks J. Am. Vet. Med. Assoc. 78, 413, 1931.

15. Soto P. E. Situación actual de la bronquitis infecciosa en México. Proc. 8th curso de actualización Avi.Mex. pp. 6-13 1996.

PROTECTIVE EFFICACY OF HYPERIMMUNIZATION AGAINST INFECTIOUS BRONCHITIS

Van Dao[A], Dennis Wages[A], Andrea Miles[A], and Jim S. Guy[B]

[A]Department of Farm Animal Health and Resource Management
[B]Department of Microbiology, Pathology, and Parasitology
North Carolina State University, Raleigh, NC 27606

Infectious bronchitis virus (IBV) continues to be a challenge and threat to the poultry industry despite numerous and intensive efforts to protect chickens from infection. Since current IBV vaccination strategies provide no solid assurance against infection and disease, the objective of this research project was to evaluate a new vaccination strategy for broilers. Typically commercial broilers receive two IBV vaccinations, one at hatch and one at 14-18 days of age. In this experiment, one group received additional vaccinations, one at 21 days and one at 28 days of age, and in effect, were hyperimmunized. This multiple IBV vaccination (MIBV) group was compared to a sham-vaccinated control and typical IBV vaccination (TIBV) group. The study was concluded at 42 days. Vaccinations were applied by coarse spray using the Connecticut strain of IBV. Groups of 10 birds in each experimental group were challenged with a cell culture adapted Arkansas IBV strain at 31 days of age (3 days post-vaccination) and a subsequent group at 38 days of age (10 days post-vaccination). Virus isolation was used to determine the protective effects of the MIBV schedule versus the TIBV schedule. Mortality, differences in body weight, clinical signs and necropsy lesions were also used to evaluate the efficacy of IBV hyperimmunization. No differences were observed among the groups. However, there were differences in virus isolation rates among the experimental groups. Virus isolation for the control, SIV, and MIV groups were 60%, 20%, and 0% respectively for the first challenge and 60%, 0%, and 0% respectively for the second challenge. Results of this experiment suggest there is merit to this novel vaccination strategy, such that improved protection may be afforded against IBV by hyperimmunization.

(These findings will be submitted for publication in *Avian Diseases*)

CHARACTERIZATION OF MEXICAN STRAINS OF AVIAN INFECTIOUS BRONCHITIS ISOLATED DURING 1997

M. Escorcia[A], M. W. Jackwood[B], V. M. Petrone[A], C. López[A], T. Fehervari[A], and G. Téllez [A]

[A] Departamento de Producción Animal Aves, FMVZ, UNAM, Circuito Exterior de C.U., Coyoacán, D. F., México. CP 04510
[B] Poultry Diagnostic and Research Center, University of Georgia, 953 College Station Road, Athens, GA. 30602

Infectious bronchitis (IB) is a disease caused by a virus with ribonucleic acid (RNA) genome that belongs to the order *Nidovirales* (13) family *Coronaviridae*, genus *Coronavirus*. It is a highly transmissible virus. Morbidity can reach up to 100% in only 36 hours. Infectious bronchitis virus (IBV) causes basically three pathologic entities. In young birds, a respiratory infection results, often complicated with secondary bacterial infections. In adult hens IBV causes drops in egg production, and internal and external egg quality. Finally, IBV causes renal damage in both young and adult birds (3). A characteristic of IBV is that frequent errors occur while genetic information is being copied, resulting in antigenic and pathogenic variations (9). In the face of an outbreak, and given that different serotypes of the virus do not cross protect, accurate serotype identification is essential in order to prevent future outbreaks (2).

The advent of molecular biology techniques such as reverse transcriptase-polymerase chain reaction/ restriction fragment length polymorphism (RT-PCR/RFLP) has resulted in new opportunities for the rapid diagnosis and identification of IBV serotypes (6,10).

For many years, poultry producers have used biosecurity and vaccination for the prevention of IB. During the last decade the presence of the disease in IB-vaccinated flocks has been reported with increasing frequency in different parts of the world (4, 8, 11).

In Mexico, IBV it is one of the most important respiratory diseases; however, only the Massachusetts

(Mass) and Connecticut (Conn) vaccine strains are officially authorized. Despite the use of these IBV vaccines, it is common to find IB problems in vaccinated chickens, causing a tremendous economic impact. This gives a strong indication that IBV variant strains antigenically different from Mass and Conn may be present in Mexico. The purpose of this research was to determine if IBV variant strains are present in Mexico.

MATERIALS AND METHODS

Clinical Samples. A total of ten farms with respiratory problems from the same broiler company were evaluated. Six farms from the State of Queretaro, and four farms from the State of Guanajuato. These two States are adjacent to each other. In these farms, five-to-seven-week-old broiler flocks, vaccinated with Mass and Conn strains of IBV at 8 days in drinking water were selected for virus isolation. Farm veterinarians reported that these birds had recently shown clinical signs consistent with IB. Trachea, lung, kidney and cecal tonsil samples were obtained from 8 to 10 birds from each of the flocks.

Virus isolation in chicken embryos. According to the technique described previously (7).

Virus titration and neutralization. Virus titration was performed on the isolates that caused lesions in chicken embryos. The neutralizing titer was calculated by the Reed and Muench method (1,14). The VN test (7) was conducted using monoclonal antibodies against Mass and Conn strains (monoclonal antibodies were kindly supplied by S.A. Naqi, Unit of Avian Medicine, Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, New York, 14853, USA.) (1).

RT-PCR/RFLP

The inactivated IBV samples were submitted to the University of Georgia under the authorization of the Mexican Secretariat of Agriculture and Rural Development *(Secretaría de Agricultura y Desarrollo Rural, SAGDR),* for RT-PCR/RFLP analysis of the S1 gene (10,12). The RT-PCR/RFLP test was performed using viral RNA extracted from phenol-inactivated virus in allantoic fluid as previously described (10).

RESULTS AND DISCUSSION

Ten IBV were obtained from trachea, lung, kidney, and cecal tonsil of broiler chickens. These four isolates were further characterized using the reverse transcriptase-polymerase chain reaction (RT-PCR) and restriction fragment length polymorphism (RFLP) techniques. The electrophoretic patterns for the four isolates were identical, but were different from other known IBV isolates.

In this study we have identified a variant strain of IBV in Mexico. It appears that this isolate is unique. The significance of this study lies in this fact that it shows for the first time that there are variant IBV strains in Mexico that may not be controlled by Mass and Conn strains. However, more research is needed with these isolates in order to determine their antigenic characteristics and their pathogenicity in birds.

It is important to emphasize that the novel IBV strain is unrelated to the European 4/91 vaccine strain, because its pattern did not match any of the viruses thus far identified by that technique including the 4/91 strain of IBV (5)

Currently it is not known how widespread is the new IBV variant in Mexico. Epidemiological studies are needed to identify the variant in other poultry producing areas in Mexico, as well as to look for other variant viruses that may be present.

REFERENCES

1. Beard, Ch. W. Serologic Procedures. In: A Laboratory Manual for the Isolation and Identification of Avian Pathogens. 3th edition. Purchase H. G., Ch. Lawrence, H. Arp, Ch. H. Domermuth, and J. E. Pearson eds. American Association of Avian Pathologists, Kennett Square, PA. pp. 129-135. 1980.

2. Cargill, P. Contemporary European infectious bronchitis variants. World Poultry. 14: 52-53. 1998.

3. Cavanagh, D. and S. A. Naqi. Infectious Bronchitis. In: Diseases of Poultry. 10th edition. Calnek, B. W., H. J. Barnes, Ch. W. Beard, L. R. McDougald, and Y. M. Saif, eds. Ames, Iowa: Iowa State University Press. pp. 511-526. 1997.

4. Cook, J. Novel infectious bronchitis virus causes higher mortality. World Poultry. 12:78-79. 1996.

5. Cook, J., S. Orbell, M. Woods, and M. A Huggins. Survey of the presence of a new infectious bronchitis virus designated 4/91 (793B). Vet Rec. 138: 178-180. 1996.

6. Falcone, E. Rapid diagnosis of avian infectious bronchitis virus by the polymerase chain reaction. J. Med.Virol. 64: 125-130. 1997.

7. Gelb, J. Infectious Bronchitis. In: A Laboratory Manual for the Isolation and Identification of Avian Pathogens. 3th edition. Purchase et al. eds. American Association of Avian Pathologists, Kennett Square, PA. pp 124-127. 1989.

8. Gelb J., J. B. Wolff, and C. A. Moran. Variant serotypes of infectious bronchitis virus isolated from commercial layer and broiler chickens. Avian Dis. 35: 82-87. 1991.

9. Gough R. E., C. J. Randall, M. Dagless, D. J. Alexander, W. J. Cox, and D. Pearson. A new strain of infectious bronchitis virus infecting domestic fowl in Great Britain. Vet. Rec. pp 493. 1992.

10. Jackwood, M. W., N. M. Yousef, and D. A. Hilt. Further development and use of a molecular serotype identification test for infectious bronchitis virus. Avian Dis. 41: 105-110. 1997.

11. Kusters J. G., G. M. Niesters, J. A., Lenstra, M. C., Horzinedk, and B: A. M. Van Der Zeust. Phylogeny of antigenic variants of avian Coronavirus IBV. Virology. 169: 217-221. 1989.

12. 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 Dis. 37:194-202, 1993.

13. Mayo, M. A., and C. R. Pringle. Virus taxonomy - 1997. J Gen Virol 79: 649-657. 1998.

14. Reed L. J., and H. Muench. A simple method of estimating fifty percent end points. J. Hyg. 27: 493-497. 1938.

CONTROL STRATEGIES OF INFECTIOUS BRONCHITIS VARIANTS IN MEXICO

G. Torres[A], C. González[A], V. Sivanandan[A], R. Campogarrido[A], and J. Gelb, Jr.[B]

[A]Boehringer Ingelheim Vetmedica, S.A. de C.V., Research and Development Area, Calle 30 No. 2614, Zona Industrial, Guadalajara, Jalisco 44940, Mexico

[B]Department of Animal and Food Sciences, College of Agriculture and National Resources, University of Delaware, Newark, DE 19717-1303, USA

INTRODUCTION

Infectious Bronchitis (IB) is a highly contagious respiratory disease of chickens, causing significant economic losses to the poultry industry, affecting broilers, layers as well as breeders. It causes poor weight gain and feed efficiency in broilers and reduction of egg production and egg quality in layers and breeders. However, high mortality some times occurs in young chickens (1). Although IB is primarily a respiratory disease, some strains are nephropathogenic (kidney involvement), and associated with mortality up to 30% in affected flocks (2).

Although vaccines are available on outbreaks of the disease still occurs in vaccinated flocks; because of antigenic variation between serotypes and the inability of different IBV serotypes to cross protect. Hence the diagnostic and serotype identification is critical for development of vaccine for control of the disease.

To date there are more than 30 recognized serotypes of IBV.

IDENTIFICATION OF NEW VARIANT SEROTYPES

The first report on a new IBV typing method using reverse transcriptase-polymerase chain reaction (RT-PCR) and restriction enzymes was developed to differentiate serotypes of IBV (3).

These tests are designed to amplify the S1 gene which contains serotype specific sequences.

Two types of molecular based tests have been developed.

(1) RT-PCR

(2) RT-PCR / Restriction fragment length polymorphism (RFLP)

RT-PCR method. Uses PCR primers specific for Ark, Conn, Mass, DEO72, JMK and California serotypes (4).

Those primes where designed to anneal to hypervariable regions in the S1 gene that are unique to each serotype of the virus.

In addition, a set of "general" degenerate primers complementary to conserved regions on the S1 gene are used to amplify all IBV strains.

RT-PCR / RFLP method. Uses a set of primer designed to amplify the entire S1 gene of all IBV strains (5).

Then they are digested with three restriction enzymes: BstYI, Hae III and Xcm1 in separate reactions and the restriction fragments are electrophoresed on an agarose gel. The resulting band is unique to each serotype. This technique will not only distinguish between all currently known serotypes but will also identify variants.

With all advanced technologies there are also some disadvantages. The major disadvantage is based on the ability of IBV to rapidly change by point mutations (6), insertions, deletions and recombination between 2 different vaccines virus replicating in the same cell (7).

It could affect PCR prime annealing, restriction enzyme digestion or both. Hence continuos development of new PCR primers is necessary to identify newer strains.

S1 Sequence Analysis. When new strains of IBV are identified, it is always helpful to obtain the sequence of the S1 glycoprotein gene, so that it can be compared to S1 sequences of other IBV serotypes. The data obtained by this method will reveal the relative closeness between the serotypes.

Very often in farms with flocks of multiple ages, would provide all the conditions that would favor the emergence of new mutant IBV variants. Periodic introduction of new pullets, continual reinfection and recycling of IBV will favor this, as opposed to "all in – all out" management system. Negligence in effective cleaning and disinfection of farms will further add to all of this. More importantly, vaccine induced immunological mechanisms provide a major selective pressure for the most antigenicaly novel variants since new variants are continuously appearing all over the world.

Recently developed new procedure, direct automated cycle sequencing (DACS) is used for identifying previously unrecognized variant strains of IBV (8). The procedure uses universal PCR primes CK4 and CK2 to amplify a diagnostically relevant portion of the S1 gene of an IB variant. Thee amplified S1 gene product is then compared to S1 sequences of previously recognized serotypes.

IB Variants in Mexico. Seventeen IBV isolates from commercial broiler flocks in Mexico that were submitted during the last one year were by DACS of the S1 IBV gene. Two different strains were identified. One of them, was most common representing 82% (14/17) of the isolates tested, while the other was less common representing 18% (3/17). Both the Mexican variant strains were unique, not related to other common reference strains of IBV such as Mass, Conn, Ark, DEO72 or the 4/91.

The purpose of this study was to evaluate the protection conferred with the use of experimental vaccine prepared containing one of this variant strain (predominant prevalence strain) of Infectious Bronchitis Virus.

IB challenge experiment of broilers vaccinated with inactivated (KV) IB variant experimental vaccine

Table 1. Experimental design.

Step	Age	Route	Comments
Vaccination	1 day	Subcutaneously	Marek + IBDV + FP ND KV
	7 days	Drinking water	IBDV
	9 days	Eye drop Subcutaneously	ND "La Sota" + IB Mass-Conn ND KV
	19 days	Subcutaneously	IB KV Variant
Challenge	7 weeks	Eye drop	Using a $10^{4.0}EID_{50}$/bird, Homologous and Heterologous Virus Strains
Tracheal swabs for Virus Reisolation	7/5	SPF chicken embryos	Tryptose Phosphate Broth supplemented with antibiotics was used for virus reisolation. Embryos of 10-12 days of age using 0.1-0.2 ml/embryo

Characteristics of the KV emulsion: Water/oil emulsion, dose 0.5 ml / bird, inoculation route: subcutaneously, 8 vaccinated birds were used per isolation unit.

Table 2. Results of IBV challenge experiment.

Group	Challenge virus	Tracheal virus reisolation	Protection (%)
IB KV. Vac.	Heterologous #1	4/7	43
Challenge control			+
IB KV. Vac.	Heterologous #2	5/6	17
Challenge control			+
IB KV. Vac.	Homologous	0/8	100
Challenge control			+

CONCLUSIONS

Experimental inactivated (KV) IB variant vaccine conferred 100% protection to homologous IB variant virus challenge, with 17% and 43% to a heterologous IB variant challenge strains. Based on the above results the use of (KV) inactivated vaccine containing IB variants, together with Mass/Conn MLV as primer, could be used as a control strategy against IBV variants in Mexico.

REFERENCES

1. King, D.J. and D. Cavanagh. Infectious Bronchitis. In: Diseases of Poultry, 9th ed. B.W. Calnek, H.J. Barnes, C.W. Beard, W.M. Reid and H.W. Yoder, Jr. Iowa State University Press, Ames, Iowa. Pp. 471-484, 1991.

2. Albassam, M.A., R.W. Winterfield and H.L. Thacker. Comparison of the nephropathogenicity of four strains of infectious bronchitis virus. Avian Dis. 30:468-476, 1986.

3. Lin, Z., A. Kato, Y. Kudou and S. Ueda. A new typing method for the avian infectious bronchitis virus using polymerase chain reaction and restrictIon enzyme fragment length polymorphism. Arch. Virol. 116:19-31, 1991.

4. Keeler, C.L. Jr., K.L. Reed, W.A. Nix and J. Gelb Jr. Serotype identification of avian infectious bronchitis virus by RT-PCR of the peplomer (S1) gene. Avian Dis. 42:275-284, 1998.

5. 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 Dis. 37:194.202, 1993.

6. Shi, Q., Wang, C. and Keirs, R.W. Genetic Relationships of Infectious Bronchitis Virus Isolates from Mississippi Broilers. Avian Dis. 44:66-73, 2000.

7. Nagi, S.A., K. Karaca and W. Jia. Significance of genetic recombination in the emergence of IBV variants. Abstract, p. 152. 130th Annual Meeting, American Veterinary Medical Association, Minneapolis, M.N. 1993.

8. Kingham, B.F., C.L. Keeler, Jr., W.A. Nix, B.S. Ladman, and J. Gelb, Jr. Identification of avian infectious bronchitis virus by direct automated cycle sequencing of the S-1 gene. Avian Dis. 44:325-335, 2000.

EVALUATION OF COMMERCIALLY PRODUCED INFECTIOUS BRONCHITIS VIRUS VACCINES AGAINST AN IBV FIELD ISOLATE OBTAINED FROM BROILERS IN CALIFORNIA

M. P. Martin, P. S. Wakenell, and P. Woolcock

University of California at Davis, School of Veterinary Medicine, Dept. Population Health and Reproduction
1 Shields Ave. 1114 Tupper Hall, Davis, CA 95616

SUMMARY

A field-origin isolate of infectious bronchitis virus (IBV) with CA99 serotype was isolated in the winter of 1999 from several broiler flocks in Northern California. The virus was shown to cause late onset respiratory disease and increased airsacculitis condemnation in affected flocks, despite their vaccination status with established IBV vaccination programs. Different commercial IBV vaccines were evaluated for prevention of respiratory disease associated with the field isolate challenge.

INTRODUCTION

Infectious bronchitis virus (IBV) is a highly contagious virus in chicken flocks, which can cause significant disease in commercial poultry. IBV can affect the general health of a flock by producing respiratory disease, renal disease, increased mortality, or production loss. Respiratory signs include coughing, sneezing, tracheal rales, sinusitis, and nasal discharge. IBV can also predispose the host to secondary respiratory infections. The virus may also invade the epithelium causing urolithiasis and increased mortality. Production can be affected in both broiler and layer chickens through decreased weight gain, decreased feed efficiency, increased mortality, increased condemnation, transient reduction of egg production in adult birds, oviduct damage in chicks causing permanent loss of egg production, and decreased egg quality.

Continual mutation of the virus, results in formation of new serotypes that can cause disease in spite of standard IBV vaccination programs. New isolates commonly develop from straight mutations from an individual virus or from recombination of multiple viruses (1,3). Vaccination programs for IBV therefore become costly and difficult to implement due to the high risk of vaccination failure in the face of challenge with a novel virus (2). IBV vaccines selected for an individual flock typically represent the virus isolates that are most likely to be found, given the geographical region and the flock characteristics (including flock genetics and flock use).

In Northern California in the winter of 1999, several broiler chicken flocks were infected by a field-isolate IBV called CA99. Infected flocks suffered from a significant increase in late-onset respiratory disease and condemnation due to airsacculitis. Vaccination programs used in the affected flocks were not reliably effective in the prevention of disease. A study was designed to test two specific vaccination protocols to determine if either was effective in the prevention of respiratory disease in birds challenged with the field isolate. The results of the study would be directly applicable to the prevention of further economic losses in broiler flocks in the endemic area.

REFERENCES

1. Cavanagh, D., Davis, P. J., and J. K. A. Cook. Infectious bronchitis virus: evidence for recombination within the Massachusetts serotype. Avian Pathology. 21:401-408. 1991.

2. Hofstad, M. S. Cross-immunity in chickens using seven isolates of avian infectious bronchitis virus. Avian Diseases. 25:650-654. 1981.

3. Ignjatovic, J., Sapats, S. I., and F. Ashton. A long-term study of Australian infectious bronchitis viruses indicates a major antigenic change in recently isolated strains. Avian Pathology. 26:535-552. 1997.

MOLECULAR INVESTIGATION OF ORNITHOBACTERIUM RHINOTRACHEALE OUTBREAKS IN COMMERCIAL POULTRY

Armando L. Hung and Arnaldo Alvarado

Laboratory of Clinical Pathology and Molecular Biology, Faculty of Veterinary Medicine, University of San Marcos, Lima, Peru

Ornithobacterium rhinotracheale (ORT) is a recent recognized bacterium which is the causative agent of an emerging respiratory tract disease of chickens. Outbreaks of respiratory disease associated with ORT have been reported in USA, The Netherlands, France, Spain, Germany, Korea and South Africa. The clinical symptoms include lacrimation, nasal exudates, sinusitis, dyspnea, edema in facial subcutis and growth retardation. Lesions observed are pneumonia, tracheitis, airsacculitis, peritonitis with foamy exudate and arthritis. Diagnosis of ORT infection is based on isolation, serology and PCR.

In our country, Peru, ORT has recently been recognized associated with outbreaks of respiratory disease, which in most cases were diagnosed as infectious coryza or secondary *Escherichia coli* infection. This paper reports on studies carried out in Peru on use of PCR and RAPD in outbreaks of respiratory disease in broiler, layer hens and broiler breeder farms.

Tracheal and air sacs swabs were collected from birds affected with respiratory disease for culture and PCR for ORT. Bacteria were grown on 5% sheep blood agar incubated at 37^{o}C with 5% CO2 and their biochemical profile determined. Typing of isolates was performed with a specific pathogen free (SPF) chicken serum against ORT serotype A in agar gel precipitation and an immunoperoxidase assay. ORT cells were suspended in STE buffer containing

proteinase K and SDS for DNA extraction. For PCR, primers OR16S-F1 –GAG AAT TAA TTT ACG GAT TAA G- and OR16S-R1 – TTC GCT TGG TCT CCG AAG AT were used. A reaction volume of 50 ul contained 5 ul of 10X PCR buffer, 3 ul of 25 mM MgCl2, 0.5 ul of each dinucleoside triphosphate, 0.5 U of Taq, 52 pmol of each primer and 2 ul of template DNA. Samples were subjected to an initial denaturation step at 94^{o}C for 5 min, followed by 45 cycles at 94^{o}C for 30 sec, 52^{o} C for 1 min, and 72^{o}C for 1 min 30 sec, with a final cycle at 72^{o}C for 7 min. PCR was also applied to clinical samples of tracheal swabs from broiler breeders with respiratory disease.

RAPD analyses was performed using a ERIC 1 primer (ATG TAA GCT CCT GGG GAT TCA C) with all the isolates. Volume reaction was as above, 0.5 Taq, 154 pmol of primer, with adenaturation step of 94^{o}C for 4 min, 35 cycles at 94^{o}C for 1 min, 52^{o}C for 1 min 30 sec, 72^{o}C for 1 min 30 sec, and a final step at 72^{o}C for 10 min. PCR products were examined in 1% agarose gels with 1 x TAE buffer, stained with 10 ul of ethidium bromide and exposed to ultraviolet light and photographed using Polaroid film.

All of 25 ORT isolates belonged to serotype A and were positive in the ORT-PCR, with all positive PCR amplicons being the predicted size of 784 bp. The PCR also produced a 784 bp amplicon with the clinical samples and failed to detect DNA of isolates of *H. paragallinarum* and *P. multocida*. Variations were observed among the ORT isolates in the RAPD –PCR fingerprinting, showing that this technique is capable to recognize differences intraspecies.

The ORT-PCR in this study is recommended as a rapid, sensitive and specific assay to diagnosis respiratory disease caused by ORT and differentiate from infectious coryza and avian cholera, and the RAPD-PCR for monitoring ORT outbreaks.

(Accepted for publication in *Avian Diseases*)

THE EPIDEMIOLOGY OF MYCOPLASMA GALLISEPTICUM IN NORTH CAROLINA

Algis Martinez, Jean-Pierre Vaillancourt, David Ley

Department of Farm Animal Health and Resource Management, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough St., Raleigh, North Carolina, 27606

An epidemic of mycoplasmosis caused by *Mycoplasma gallisepticum* (MG) occurred in North Carolina broiler and turkey breeders in the fall of 1999. This was followed by several cases in chicken and turkey grow-outs (vertical transmission in broilers and horizontal transmission in turkeys). Twenty-three broiler farms in Western North Carolina were quarantined because progeny of infected broiler breeder flocks from the East was placed in the field. However, to date, the majority of cases have occurred in the Eastern part of the state. The highest number of cases was recorded in December, followed by the lowest number in January. This was likely due to limited testing during this first month of the year because of a major snowstorm that prevented access to farms for several days. Of 76 cases in the East, 35 were commercial turkey flocks, 17 were broiler breeders, another 17 were broilers, and 7 were turkey breeders. At least four different strains of MG have been identified by DNA fingerprinting. The most prevalent (Strain B) was first discovered in broiler breeders in mid production.

Since March, the vast majority of confirmed MG cases have been commercial turkey flocks. Typically, infected turkey flocks first showed upper respiratory signs before testing could confirm the presence of MG. On average, flocks were 13 weeks of age at onset of disease (median=12 weeks). The youngest turkey flocks showing clinical signs were 6 weeks old and the oldest were 19 weeks of age. Some flocks showed signs consistent with MG a few days before processing (normally at 20 weeks), but these cases could not be confirmed by serology or isolation.

A case-control study is ongoing. Control farms (MG negative) are matched with MG-positive farms based on location, type of production, and age of the flock at the time the MG flocks were found positive. Preliminary results indicate that relationships among farm workers and biosecurity issues are at the core of this epidemic. Risk factors found to be statistically associated with the disease are 1) farm density (number of farms per square mile); 2) not requiring coveralls from visitors; 3) not requiring plastic or rubber boots from visitors; and 4) having a direct relationship with MG-positive farms (i.e., helping growers of MG farm then returning to visit own birds without changing clothing or boots; borrowing equipment from MG-positive farms; sharing dead bird disposal facilities).

Although the investigation is still ongoing, current evidence suggests that inadequate MG monitoring

(interval between testing and delays in obtaining results) in 1999 and people movement coupled with the lack of biosecurity on several farms have greatly contributed to this epidemic.

(The results of this investigation will be submitted for publication in *Avian Diseases*)

THE RELATIONSHIP BETWEEN MYCOPLASMA GALLISEPTICUM (MG) IN GEESE AND MG OUTBREAKS IN BROILER-BREEDER FLOCKS

S. Levisohn[A], I. Gerchman[A], A. Frummer[B] and Y. Weisman[A]

[A]Division of Avian and Aquatic Diseases, Kimron Veterinary Institute, Bet Dagan, Israel 50250
[B]Beer Tuvia Poultry Diagnostic Laboratory, POB 443, Kiryiat Malachi, Israel

Recent advances in DNA-based diagnosis (PCR) and molecular typing by Random Amplification of Polymorphic DNA (RAPD) have greatly advanced our ability to study epidemiology of *Mycoplasma gallisepticum* (MG) infection. We applied these methods to study a possible association between MG-associated respiratory infection in fattening geese and MG infection in broiler-breeder flocks.

Numerous mycoplasma species may be present in geese, some of which are apparently unique to water fowl whereas others are recognized also in chickens and turkeys. MG has been occasionally reported in geese, although not in Israel. In particular, care must be taken to differentiate between MG and the closely related *M. imitans*, frequently found in geese (1).

Recently MG was diagnosed in three separate outbreaks of clinically severe respiratory disease in geese. Diagnosis of MG was by PCR and isolation from tracheal swabs and/or air sac lesions. Differentiation of MG from *M. imitans* was by PCR-RFLP (2), directly from clinical samples or by analysis of isolates.

Molecular typing of MG isolates from geese by RAPD indicated a clear association with outbreaks in poultry breeder flocks. Two different RAPD types were present in separate outbreaks in the goose flocks, each of which was identical with RAPD types of turkey and chicken MG isolates. In one case, the RAPD type of MG isolates from the diseased geese and from a broiler-breeder flock nearby were identical. Circumstantial evidence points to the possibility that geese may in some cases serve as a "missing link" between broiler-breeder MG outbreaks.

REFERENCES

1. Bradbury, J. M., O. M. Saed, C. A. Yavari, J.P. Dupiellet and J.M. Bove. Mycoplasma imitans sp. nov. is related to Mycoplasma gallisepticum and found in birds. Int. J. System. Bacteriol. 43: 721-728. 1993.

2. Kempf, I. DNA amplification methods for diagnosis and epidemiological investigations of avian mycoplasmosis. Avian Pathol. 27: 7-14. 1998.

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

CONCURRENT NEWCASTLE DISEASE VIRUS AND MYCOPLASMA GALLISEPTICUM INFECTION IN AN EASTERN NORTH CAROLINA COMMERCIAL TURKEY FLOCK

Babak Sanei [A], Shannon Jennings[B] and H.John Barnes [A]

[A]College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606
[B]P.O.Box 856, Warsaw, NC 28398.

High mortality and severe respiratory signs in a tom turkey flock in eastern North Carolina were investigated. The farm was divided into two sections with different designs, 800 feet apart. Section A contained 4 houses. Each house in this section was divided into brooder and grower sections by a wooden

wall. Section B of the farm contained 2 brooder barns and 4 grower barns separate from each other. Birds were 5 weeks of age when they developed clinical signs. An older flock on the farm had similar signs that developed just after this flock was placed onto the farm. Turkeys in section B had swollen sinuses with up to 3 % daily mortality in the most affected house. There was no response to antibiotic treatment. Severe extensive polyserositis was found at necropsy. Clinical signs and mortality were much less severe in section A. Newcastle disease virus (NDV) was isolated from tracheal swabs taken from birds in both sections. Attempts to isolate *Mycoplasma gallisepticum* (Mg) from the birds in section B were unsuccessful at 5 weeks of age, however PCR tests for Mg were positive (birds in section A were not tested). Serological tests for Mg, *Bordetella avium*, and NDV were also done. Only antibodies to NDV were demonstrated. Tracheal swabs were also taken from birds in both sections at 10 and 15 weeks of age for PCR tests and isolation of Mg was attempted at 15 weeks. Mg was isolated and PCR results were positive for birds in section B; birds in section A remained negative. At 15 weeks of age, serological tests (rapid serum plate agglutination test and hemagglutination-inhibition test) revealed positive Mg reactions for birds in both A and B sections. However, compared to section A, serums from turkeys in section B had more positives on both tests. In conclusion, we believe the high mortality rate and severe clinical signs that continued through 15 weeks of age in section B of the farm was due to concurrent infection with ND virus and Mg. In contrast, the milder respiratory signs and lower mortality rate in section A most likely resulted from Mg infection that occurred after the acute stage of ND infection at 4-5 weeks of age.

(Paper will be submitted for publication in *Avian Diseases*)

MYCOPLASMAS FROM A EUROPEAN PERSPECTIVE

Janet M. Bradbury

University of Liverpool, Department of Veterinary Pathology, Leahurst, Neston UK CH64 7TE

MYCOPLASMAS IN COMMERCIAL POULTRY

There appears to have been a resurgence of certain mycoplasma infections in European poultry over recent years. Although the primary breeders remain mycoplasma-free, *Mycoplasma gallisepticum* (Mg) infection occurs from time to time in commercial breeders and it is also reported to be increasing in layers on continuous production sites, causing egg production losses. Some European countries use Mg bacterin vaccines to alleviate losses in layers and live vaccines are currently undergoing trials in some. So far no mycoplasma vaccines are licenced for use in the United Kingdom.

Control measures for Mg in chickens and turkeys and for *M. meleagridis* in turkeys were included in the 1990 European Union Directive (90/539/EEC) governing intra-Community trade in poultry and hatching eggs and imports from third countries. However *M. synoviae* (Ms) was not included in this Directive and, possibly as a consequence, its prevalence appears to be increasing throughout Europe. It is especially common in table egg layers where there appears to be little incentive to control it. Some producers claim that it has no effect on health and productivity while others treat potentially infected flocks with tetracyclines. However there is also concern in some countries that Ms strains may have increased in virulence.

In our laboratory a study is underway to establish the national prevalence of Ms in layers using ELISA to detect antibodies in yolk. This large scale survey will be followed by a comparison of cohorts of positive and negative flocks to determine if Ms infection is causing any significant economic loss. A serological survey carried out recently in Brittany in France revealed that many layer flocks were infected with Ms and might provide a reservoir of infection for transmission to other poultry (Kempf, personal communication).

Unexplained Ms "breaks" also occur in commercial broiler-breeder flocks and in turkey breeders despite the fact that they are kept under conditions of strict biosecurity. A case-control study is underway to investigate the possible causes of such breaks in UK breeders. Each case farm is matched with three control farms and their mycoplasma status is confirmed by serology, culture and PCR examination on samples from 60 birds per flock. An extensive questionnaire, which covers all the current hypotheses with regard to spread of mycoplasmas, is completed with the appropriate farm manager. Multivariate analysis should then reveal the main risk factors involved in such breaks.

MYCOPLASMAS IN BRITISH GAME BIRDS

It has been suggested that game birds may be involved in transmission of mycoplasmas to some commercial poultry flocks. The pheasant (*Phasianus colchicus*) and the red-legged partridge (*Alectoris rufa*) populations have recently increased dramatically in the UK and incidents of upper respiratory disease, which are often attributed to mycoplasmas, are not uncommon in these birds. The clinical signs consist mainly of conjunctivitis and sinusitis and are seen in both species. Occasionally there is also involvement of the lower respiratory tract. The morbidity is high and affected birds lose condition although mortality is variable and may be influenced by other infections. Diagnosis of mycoplasmosis is usually carried out by the rapid serum agglutination test, and a survey amongst UK practitioners revealed that many affected birds were testing positive for Ms. We therefore investigated the mycoplasma status of sixty two incidents of respiratory disease in pheasants and 12 in red-legged partridges. Conventional cultural methods were used and all isolates were typed by immunofluorescence. In addition, commercial PCR kits were used to detect Mg and Ms (IDEXX Laboratories, Maine, USA).

Mg was detected in 31% of incidents of respiratory disease in pheasants and in 67% of incidents in partridges. It was more readily detected by PCR than by culture. Mycoplasmas that were frequently isolated were *M. glycophilum*, *M. pullorum*, *M. gallinaceum* and *M. iners*. Ms was not cultured from any sample during this survey and only one of 120 diseased game birds examined was PCR positive for Ms.

Thus the investigation suggested that Mg, but not Ms, might be involved in much of the respiratory disease seen in UK game birds. Furthermore the role of Mg as a primary pathogen in respiratory disease has been demonstrated experimentally in the chukar partridge (*Alectoris graeca*) in the US (6) and in the red-legged partridge in the UK (3).

Although this survey of respiratory disease failed to incriminate game birds as carriers of Ms it is still possible that they could act as transient hosts for this mycoplasma and it is worth noting that we have isolated Ms from the tracheas of normal pheasants which were captured close to an infected broiler-breeder farm (2).

MYCOPLASMAS IN BRITISH WILD BIRDS

In a separate study we have examined UK wild birds for evidence of mycoplasma infection (1), bearing in mind the large scale epidemic of Mg that has occurred in the American house finches in the Eastern US and in Canada (5). Three groups of wild birds were examined for mycoplasmas as described above for the game birds. The first group were random casualties collected in Scotland and consisted of 43 birds representing 14 species. The group included 22 finches. Mg was detected by PCR in two crows (*Corvus corvus corone*) and one rook (*Corvus frugilegus*) but in none of the other birds.

The second group consisted of 53 corvids and nine feral pigeons, which were shot on a game estate that had a persistent Mg problem. Some of the corvids had been shot whilst feeding on maize put down for the pheasants. In this batch 50% of rooks and 38% of crows were Mg positive by PCR. Another corvid, the jackdaw (*Corvus monedula*) also proved to be PCR positive for Mg in three of 23 birds examined, but the pigeons were all negative. A different Mg PCR (4) was used to confirm the results in several positive and negative samples and all were reproducible. Mg was not isolated but this may have been due to the presence of other faster-growing mycoplasmas. *M. columborale* was the most commonly isolated mycoplasma species.

The third group of wild birds examined was a miscellaneous collection of 60 corvids and songbirds collected from three geographically distinct areas in England. In this group Mg was again detected by PCR but only in three of 16 corvids from the eastern part of the country where game birds are common.

Thus, although Mg was not isolated from any of the wild birds, there is good PCR evidence that British corvids are susceptible to infection with this organism. As with the game birds, there was no evidence of Ms in any of these wild birds. Further studies, including molecular epidemiology, are now needed to establish the true role of Mg and Ms in game and wild birds.

REFERENCES

1. Bradbury, J. M., C. M. Dare, C. A. Yavari, and A. Forrester. Evidence of Mycoplasma gallisepticum in British wild birds. Proc. 13th Int. Congress, Int. Org. for Mycoplasmol, Fukuoka, Japan. p. 253. 2000.

2. Bradbury, J. M., C. A. Yavari, and C. M. Dare. Detection of Mycoplasma synoviae in clinically normal pheasants. Vet. Record. In press. 2001.

3. Ganapathy, K., and J. M. Bradbury. Pathogenicity of Mycoplasma gallisepticum and Mycoplasma imitans in red-legged partridges (Alectoris rufa). Avian Pathol. 27:455-463. 1998.

4. Lauerman, L. H. Mycoplasma PCR assays. In: Nucleic acid amplification assays for diagnosis of animal diseases. L. H. Lauerman, ed. American Association of Veterinary Laboratory Diagnosticians, Turlock, CA. pp.41-42. 1998.

5. Ley, D. H., J. E, Berkhoff, and J. M. McLaren. Mycoplasma gallisepticum isolated from house finches (Carpodacus mexicanus) with conjunctivitis. Avian Dis. 40:480-483. 1996.

6. McMartin, D. A., A. J. DaMassa, W. D. McKeen, D. Read, B. Daft, B., and K. L. Lam. Experimental reproduction of Mycoplasma gallisepticum disease in chukar partridges (Alectoris graeca). Avian Dis. 40:408-416. 1996.

NEWCASTLE DISEASE OUTBREAK IN SPRING 2000 IN MEXICO

Ernesto Soto[A], J. Garcua[A], A. Ortiz[B], B. Lozano[A] and D. Sarfati[A]

[A]Laboratorio Avimex Sa De Cv. Maiz 18, Mexico, D.F. Mexico
[B]Fes – Cuautitlan, Universidad Nacional Autonoma De Mexico. Mexico

HISTORY

Newcastle disease (ND) was first seen in Mexico in 1945. Four epidemics or major outbreaks occurred during the 20th century affecting the country: the first one occurred from 1945 to 1948; the second from 1951 to 1953; the third from 1971 to 1972; and the last one from 1984 to 1985. Velogenic viscerotropic strains of Newcastle disease virus (vvNDV) have been isolated in all epidemics. The most relevant strains isolated in the past were named Chimalhuacan, Iztapalapa and Queretaro.

THE OUTBREAK

Mexico produces around 2.7 million broilers per day with and average live weight of 2.5 kg (5.5 pounds) and 85 million eggs per day. The area called La Laguna produces 12% of the national broiler production (324,000 broilers per day) and 7% of the national eggs production (6 million eggs per day). La Laguna is located in the north central part of Mexico, between the states of Coahuila and Durango. The area has a total of 271 houses: 170 houses for broilers and the rest for layers and breeders. vvNDV in La Laguna was not isolated since 1995. The area was considered free of NDV on March 1996.

The first report of high mortality in commercial farms during 2000 occurred on March 24. A vvNDV was isolated from a 50,000 broiler farm on March 28. Mortality reached 60% on this farm. Birds died showing clinical signs of ND. No other respiratory virus was isolated.

From March to May, vvNDV was isolated in 93 broilers farms; 13.6 million (M) broilers were eliminated and properly buried. Positive farms were not repopulated for at least two months.

Four companies were affected. The first one eliminated a total of 12.6 M birds from 83 farms during April and May. The second eliminated a total of 0.640 M birds from seven farms in April. The third eliminated 0.125 M birds from two farms also in April. The last farm eliminated 0.075M birds from one farm in May.

The disease affected mainly broiler flocks, but fighting cocks and backyard chickens were also severely affected. In the only ostrich farm in the area all young birds died. The virus was not isolated from any breeder or commercial layer flocks. Backyard turkeys or wild birds were not affected.

Starting in April, more than 6300 samples were sent every month to the diagnostic laboratory. Eighty-six isolations of vvNDV were obtained in April and only a few in May. The last isolation of a vvNDV was obtained May 11, 2000.

ECONOMIC IMPACT

The poultry industry in La Laguna was not allowed to sell live birds, meat, or eggs outside the area until November (six months after the last vvNDV isolation in the area). The economic impact was estimated at 50 M USD for the poultry industry and 25 M USD for the Department of Agriculture, for a total of 75 M USD.

EPIDEMIOLOGY

The information recorded during the outbreak shows that some mortality was observed at the beginning of the year in fighting cocks and backyard chickens, but that was not reported and no samples were sent to the lab.

It is highly probable that the virus was introduced into the area by fighting cocks, which is a very common practice in the country. People travel with the cocks through different states to fight them and after one or two weeks go back home, carrying surviving and new birds.

Generally speaking, biosecurity measures in the area were relaxed. The largest company where hatching broiler chicks with very low ND maternal antibodies (less than 2 $\log_2$), and its vaccination

program for ND was adequate to very low. Birds were vaccinated at one day of age with live virus, B1 strain, by spray, with a quarter of the regular dose. Revaccination was done at 14 and 30 days of age with the same strain, with half dose, by the drinking water route. HI titers were usually under 2 $\log_2$.

Other companies vaccinated breeder flocks two or three times with oil emulsion vaccines combined with live virus during the first 20 weeks of age. The chicks hatched with high maternal antibodies (average of 5 $\log_2$). Broilers were usually vaccinated with oil emulsion vaccines at one or ten days of age. Live LaSota strain with full dose are applied by eye drop at ten days, with revaccination by the water route at 28 to 35 days. HI titers are usually above 5 $\log_2$.

High mortality in commercial birds was first seen in a farm belonging to the largest company. Within the same week more farms from the same company were affected. After three weeks, farms from all companies were involved in the outbreak.

The isolation of a vvNDV forced the government and the poultry industry to stop the outbreak by rapid depopulation of affected commercial flocks as well as affected backyard chickens and fighting cocks.

CONCLUSION

The fast reaction of everyone involved in the outbreak helped for a rapid control of the situation in the area. The economic impact of the outbreak was 75 M USD. No other state in the country reported high mortality or the isolation of a vvNDV during the whole year.

THE EFFECT OF AN EARLY NCD CHALLENGE ON BROILER BREEDER'S PERFORMANCE AND ON THEIR OFFSPRING

S. B. Buys

P.O. Box 237, Olifantsfontein, South Africa, 1665

During February 1998 a breeder flock contracted NCD at 41 weeks of age. Production dropped 9% and recovered over a period of 9 weeks. Mortality reached 2.3% for a week instead of the normal 0.2%. The percentage discolored or misshapen eggs increased by 5% during the 9 week period.

Eight weeks later a flock reared on one site was placed on two laying sites 45 km apart. These two flocks contracted NCD respectively at 27 and 28 weeks. Mortality was normal and the flocks dropped 16% and 18% respectively.

From May 1998 nine consecutive flocks contracted NCD at 26 – 27 weeks of age. The 9 flocks only showed a drop in production, (between 11 and 28%) discolored and misshapen eggs but no increase in mortality.

Two of the 9 flocks mentioned were again reared on one unit and placed on two laying units 45 km apart. Both these flocks contracted NCD within 5 days of each other.

On inspection of these flocks rearing NCD antibody titers some houses on all 9 farms showed increased NCD antibody titers as early as 5 weeks of age. In some of these flocks a few birds with twisted necks were reported but mortality was normal.

The conclusion was reached that these flocks already became infected during rearing, and the virus was harbored in the birds. The stress associated with production appeared to induce a viremia despite relative high NCD titers with a drop in production the only visible clinical sign and the NCD antibody titer rose from 2 log 8 to 2 log 11 plus. The hatchability of the normal eggs laid during the drop in production was reduced by 4% on average.

Virus isolation attempted during the acute phase was never successful. With the last 3 flocks SPF birds where placed with the flock at 23 weeks of age. In each of these flocks the SPF birds contracted NCD and died following the drop in production. NCD virus isolation out of the SPF birds by means of embryonated eggs was successful.

In the company's second operation 3 flocks became infected with NCD and experienced drops in production that varied between 11% and 15%. Unexplainable increases in mortality post 28 days of age occurred in one or two broiler houses on different broiler farms. NCD was confirmed in these broilers. On inspection, it was found that these houses were filled with chickens from the NCD affected flocks that hatched between 29 and 39 days after the onset of the NCD drop in production. This on egg infection was contained by fogging eggs with a third generation quaternary ammonium compound (QAC) disinfectant after collection and twice during the setting period.

CONCLUSION

It would appear that birds can harbor NCD virus for a period of time. In such birds an acute viremia can

be induced by the stress of production. This effect can be overcome by using an oil emulsion NCD vaccine at 5 weeks and a high concentration NCD broiler oil emulsion vaccine (0.5ml/bird) at 18 weeks of age.

The on egg transmission of NCD virus can be successfully eliminated by fogging eggs with a QAC disinfectant.

FIELD TRIALS WITH AN OIL EMULSION VACCINE AGAINST NEWCASTLE DISEASE, INFECTIOUS BRONCHITIS AND EGG DROP SYNDROME IN MEXICO

Ernesto Soto, Manuel Gay, Bernardo Lozano, David Sarfati, Juan Garcia and Alejandro Suarez

Laboratorio Avimex Sa De CV. Mexico

INTRODUCTION

Diseases can affect poultry industry in many ways; killing birds and decreasing its productivity level are probably the major economic problems. Countries or farms with low biosecurity measures are usually exposed to a larger number of diseases.

Newcastle disease (ND) has been recognized in many countries of Asia, Africa, Europe and the Americas. Only Oceania seems to remain free of the disease. ND virus (NDV) can cause high mortality in birds with no immunity, as well as a marked drop in egg production in birds with low immunity. Vaccination of laying hens and breeders against ND always requires more than one dose of vaccine to maintain immunity through their lives. The immune response is usually estimated by the HI titers obtained.

Infectious bronchitis (IB) is distributed worldwide. IB caused by Massachusetts (Mass) strains affects laying hens and breeders flocks, and can cause declines in egg production and quality, even in absence of respiratory signs. In addition to production declines, the number of eggs unacceptable for setting is increased, the hatchability is reduced, and soft-shelled, misshapen, and rough-shelled eggs are produced. Inactivated oil emulsion vaccines are used primarily at point of lay in breeders and layers, and usually given after priming with live virus. The immune response is usually estimated by the ELISA titers obtained.

The infection with adenovirus group III or egg drop syndrome (EDS) affects mainly white and brown commercial layers, but heavy breeders are probably more severely affected. The disease causes a drop in egg production (by up to 40%) as well as eggs with shell defects, which include a loss of color in pigmented eggs, thin-shelled, soft-shelled, or shell-less eggs. The disease has been recognized in different countries in Europe, Oceania, Asia and Africa, as well as Mexico and countries from South America. Only inactivated oil emulsion vaccines are used against EDS in commercial layers and breeders before the beginning of lay.

Immunization of birds prior to the onset of production with vaccines containing two ore more killed antigens has been a practice for many years. Inactivated vaccines usually conferred high levels of protective antibodies of long duration that can be achieved.

Postvaccinal serology is usually used to confirm successful application of vaccine an adequate immune response by the birds.

Objective. To demonstrate the safety, potency and immunogenicity features of an inactivated vaccine against ND, IB and EDS in heavy breeders under field conditions.

Methodology and Materials. 25,000 heavy breeders, eighteen weeks old, were vaccinated subcutaneously in the mid back portion of the necks, using 0.5 ml per bird. 500 pullets were kept non-vaccinated controls within the same flock.

During the growing period, all birds received simultaneously four doses of a combination of active NDV LaSota strains, and IB Mass and Connecticut strains, via the drinking water, as per normal handling field practices.

The vaccination crew was asked to comment on any difficult or observation they had had during the injection procedures, such as emulsion high density.

Birds were observed for 30 days after vaccination to possible adverse reactions in site of injection or mortality due to vaccination.

Every 10 weeks, 50 birds of each group were bled, and the serum samples were tested for ND, IB and EDS by serological techniques in the laboratory.

At 34 weeks of age, potency test was performed for each disease.

Results. The vaccine was applied manually using 1.0 ml syringes with number 19, 20 and 21 needles, 0.25 and 0.50 inches long. No problems were reported by the vaccination crew.

After 30 days, none of the birds showed swelling or any other undesirable effect in the site of injection. No birds died from vaccination. The injectability and the safety tests were satisfactory.

HI test were performed for ND and EDS; ELISA was carried out for IB. Results are shown in table 1. A good serological response for the three antigens was detected after the vaccine was applied. No antibody increase was detected in the control group, meaning no field exposure towards these viruses. The immunogenicity tests for ND, IB and EDS were satisfactory.

At 34 weeks of age, 25 birds were challenged with a vvNDV (Queretaro strain) in isolation units, with 200,000 CELD 50% / 0.2 ml by the intramuscular route. Birds were observed for 21 days after challenge. Mortality and egg production were recorded daily. Results are shown in table 2. The potency test against ND was satisfactory.

At 34 weeks of age, a second group of 25 birds were challenged in a separated isolation unit, with a virulent Mass 41 strain of IB using 10^5 CEID 50%/0.2 ml, by the intramuscular route. Birds were observed for 21 days after challenge. Records of mortality and egg production were taken daily. Results are shown in Table 3. The potency test against IB was satisfactory.

At 34 weeks of age, a third group of 25 birds were challenged in a separated isolation unit, with a virulent adenovirus group III, JPA strain, with $10^{5.9}$ CEID 50% / 0.2 ml, by the intramuscular route. Birds were observed for 21 days after challenge. Prime quality egg production and mortality rates were recorded daily. Results are shown in table 4. The potency test against EDS was satisfactory.

CONCLUSION

The inactivated oil emulsion vaccine was safe and induced a good serological response for ND, IB and EDS in commercial birds under field conditions.

The vaccine was efficacious and gave a good protection against mortality and drop in egg production or quality of eggs under exposure to virulent viruses.

Table 1. Serology results. GMT from 50 field serum samples from control and vaccinated groups.

WEEK	ND-HI $\log_2$		EDS- HI $\log_2$		IB-ELISA	
	Control	Vaccinated	Control	Vaccinated	Control	Vaccinated
18	4.3	4.3	0.0	0.0	2500	2500
28	4.5	7.8	0.1	10.1	2440	5690
38	4.1	8.1	0.0	11.1	2130	7100
48	3.9	8.4	0.1	10.3	1610	6960
58	3.1	8.5	0.0	9.6	1080	6600

Table 2. Potency test for ND. 25 birds in each group.

GROUPS *	ACCUMULATED EGG DURING 21 DAYS		ACCUMULATED MORTALITY DURING 21 DAYS	
V-NC	448	100%	1/25	4%
C-V	428	95.5%	1/25	4%
C-NV	237	52.9%	12/25	48%

Table 3. Potency test for IB. 25 birds in each group.

GROUPS *	ACCUMULATED EGG DURING 21 DAYS		ACCUMULATED MORTALITY DURING 21 DAYS	
V-NC	448	100%	0/25	0%
C-V	440	98.2%	0/25	0%
C-NV	316	70.5%	0/25	0%

Table 4. Potency test for EDS. 25 birds in each group.

GROUPS *	ACCUMULATED EGG DURING 21 DAYS (ACCEPTABLE FOR SETTING)		ACCUMULATED MORTALITY DURING 21 DAYS	
V-NC	448	100%	0/25	0%
C-V	446	99.9%	0/25	0%
C-NV	246	54.9%	0/25	0%

*V-NC = Vaccinated, non-challenged, C-V = Challenged, vaccinated, C-NV = Challenged, non-vaccinated.

MORPHOMETRIC EVALUATION OF THE PATHOGENICITY OF DIFFERENT STRAINS OF NEWCASTLE DISEASE VIRUS TO THE RESPIRATORY TRACT

Anilton Cesar Vasconcelos[A], Jairo Eduardo S. Nunes[A], Euripedes Batista Guimaraes[A], Tatiane Alves Paixão[A], Max Augusto Jorge[B], Nelson Rodrigo S. Martins[B]

[A]Laboratory of Apoptosis, Institute of Biological Sciences, Federal University of Minas Gerais, Postal Box 2486, 31270-010, Belo Horizonte, MG, BRAZIL. Http://www.icb.ufmg.br/~pat/Apopt, e-mail: anilton@icb.ufmg.br
[B]School of Veterinary Medicine, Federal University of Minas Gerais, Postal Box 2486, 31270-010, Belo Horizonte, MG, BRAZIL. Phone: (5531) 34992887 or (5531) 91147841, Fax: (5531) 34992879, E-mail: anilton@icb.ufmg.br

Different strains of Newcastle disease virus (NDV) are classified according to their virulence in velogenic, mesogenic and lentogenic (1). The available methods to classify NDV can distinguish strains with significant difference in their pathogenic potential, but sometimes can generate discrepant results when dealing with strains of similar virulence. Vaccinal strains of Newcastle disease virus (NDV) are among the lentogenic, which can show individual variation in virulence to the respiratory tract of the chicken (4).

The inflammatory lesions more frequently found in trachea of birds exposed to NDV include mucosal swelling, hyperemia, edema with dissociation of fibers and infiltrate of lymphocytes and heterophils. They seem to be associated to the virulence of the NDV strains (4, 3).

The aim of this study was to compare the pathogenicity of La Sota (LS), Ulster (UL) and VG-GA (VG) vaccinal strains of NDV, by a morphometric analysis of tracheal thickness and inflammatory reaction, using. 44 day-old SPF chickens free from antibodies anti-NDV. Commercial vaccines containing LS, UL and VG strains were used via intra-tracheal. Vaccines were acquired with the same lot number and titled in embryonated eggs of SPF chickens, for adjustment of the vaccinal dose to 6.5 log10 IEED50/chicken. The treatments were maintained in independent rooms and the control birds received PBS pH 7.0. Chloramphenicol was administered in water (130 mg/l) to the birds for 4 consecutive days, until 48 hours before the beginning of the experiment, to minimize eventual infection bacterial after vaccination.

From 0 to 4 and at 6 and 8 days post vaccination, at least 3 chickens of each treatment were killed by electrocution and fragments of the anterior trachea were collected and processed to histological analysis. Slides of 4μm thick containing 3 sections of the same specimen were stained by Hematoxilin - Eosin (HE) and evaluated in light microscopy for the intensity of the lesions. Morphometric evaluation of the tracheal thickness was conduced by measuring 3 segments, distant from each other by 250 μm, being the intermediate segment the thickest of the section. Data were collected with the aid of an image analyzer (Kontron KS-300 v2.0, Zeiss Kontron Electronics). Data were analyzed statistically by Student's t test.

Tracheal lesions varied depending on the vaccinal strain. Strains LS and UL induced more severe lesions, and VG was less pathogenic to trachea. More frequent tracheal lesions included depletion of goblet cells, epithelial erosion, hyperemia and inflammatory infiltration of mononuclear and heterophils. These findings are in agreement with Santos (8), who observed similar lesions in chickens vaccinated via nasal with the LS and VG strains, seven days after vaccination.

Tracheal inflammatory infiltrates were more intense and disperse in chickens vaccinated with LS than with UL and VG, when infiltrates were more focally distributed. This finding supports the reports of Abdul-Aziz & Arp (2), who evaluated the progression of tracheal lesions in turkeys exposed to LS. Other lesions such as cellular hypertrophy of the mucous glands and intracellular edema of the cylindrical ciliated epithelia, as described by Kelleher et al. (7) and Hamid et al. (5) were absent.

LS strain induced the thickest trachea during the whole experiment (Graph 1). At the third and fourth day after vaccination, when tracheas were the thickest, LS and UL strains did not differ in their virulence. Both LS and UL strains were more virulent than VG strain, inducing swollen tracheas and causing more intense inflammatory lesions. Tracheas from birds inoculated with LS showed slower recovery after the inflammatory peak at the third and fourth day after vaccination, agreeing with a previous study that showed intense edema in trachea as quantified by the hydric content. (6). VG strain induced the smallest thickening of the tracheal mucosa, confirming the low pathogenicity of that strain to the respiratory tract of the chicken (9).

CONCLUSIONS

Histological lesions and tracheal thickness varied depending on the vaccinal strain. La Sota strain induced higher tracheal swelling than the others did, during most of the time of the experiment. Maximum swelling of tracheal mucosa occurred at the third and fourth day after vaccination. At that time, La Sota and Ulster showed similar pathogenicity to trachea, causing greater swelling of tracheal mucosa than VG strain. Tracheas from birds inoculated with LS showed slower recovery after the inflammatory peak at the third and fourth day after vaccination. LS and UL strains were more pathogenic to the respiratory tract; therefore, they should not be used during the season that respiratory infections are more incident.

REFERENCES

1. Allan, W.H.; Lancaster, J.E.; Toth, B. The production and uses of Newcastle diseases vaccines. FAO, Rome. 1973. 114 p.

2. Abdul-Aziz, T. A. & ARP, L. H. Progression of the trachea in turkeys exposed by aerosol to La Sota strain of Newcastle disease virus. Avian Dis., 27(4): 1131-1141, 1983a.

3. Abdul-Aziz, T. A. & ARP, L. H. Pathology of the trachea in turkeys exposed by aerosol to lentogenic strains of Newcastle disease virus. Avian Dis., 27(4): 1002-1011, 1983b.

4. Alexander, D.J. Newcastle disease and other paramyxovirus infections. In: Diseases of Poultry, 9th ed Calnek, B.W.; Barnes, H.J.; Beard, C.W.; Reid, W.M.; Yoder, Jr., H.W. (Eds.). Iowa State University Press. pp. 496-519. 1991.

5. Hamid, H.; Campbell, R.S.F.; Wall, L. Studies of pathology of velogenic Newcastle disease: Virus infection in non-immune and immune birds. Avian Pathol., 20(4): 561-575, 1991.

6. Jorge, M.A, et al. Tracheal humidity as a method of evaluation of the virulence of vaccine strains of Newcastle disease virus. In: Annals of the 16th Research Meeting of the School of Veterinary Medicine UFMG, Belo Horizonte: FEP-MVZ Editora. p. 65. 1998

7. Kelleher, C.J.; Halvorson, D.A.; Newman, J.A. Efficacy of viable and inactivated Newcastle disease virus vaccines in Turkeys. Avian Dis., 32(2): 342-346, 1998.

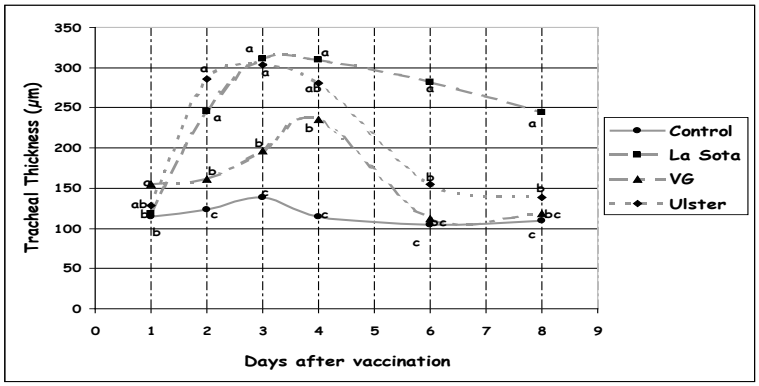
8. Santos, B.M. Avian paramyxovirus: Serological and histopathological studies of the La Sota and VG-GA NDV strains. D.Sc. thesis. Federal University of Minas Gerais, Belo Horizonte, Brazil, 1995.

9. Villegas, P. and Glisson, J. Isolation and characterization of the lentogenic enteric Newcastle disease virus. Proc. 39th West. Poult. Dis. Conf., Sacramento, California. p.26-27. 1990.

Table 1. Average thickness (in μm) of tracheas of control and vaccinated chickens (La Sota, VG-GA and Ulster). Different letters in a line mean statistical differences.

Days after vaccination	Vaccinal Strains			
	Control	La Sota	VG-GA	Ulster
1	114,06^{b}	117,84^{b}	154,98^{a}	128,90^{ab}
2	123,91^{c}	245,80^{a}	160,59^{b}	286,10^{a}
3	138,75^{c}	311,24^{a}	196,13^{b}	303,25^{a}
4	114,47^{c}	309,81^{a}	234,87^{b}	280,73^{ab}
6	103,95^{c}	281,68^{a}	112,01^{c}	154,79^{b}
8	110,15^{c}	244,00^{a}	118,23^{c}	138,87^{b}

Graph 1. Average thickness (in μm) of tracheas of control and vaccinated chickens (La Sota, VG-GA and Ulster). Different letters in a line mean statistical differences.



NEWCASTLE AS A POTENTIAL CAREER ALTERING EXPERIENCE (ANALYSIS/SUMMARY OF 5-STATE NEWCASTLE TABLE TOP EXERCISE)

Conley Byrd DVM, State Veterinarian

Arkansas Livestock & Poultry Commission, #1 Natural Resources Drive, Little Rock, AR 72205
(501) 907-2400 office, (501) 907-2425 fax, cfbyrd@arlpc.org

On Wednesday 13 December 2000 in the middle of a snowstorm in Fayetteville, Arkansas, forty individuals from twelve different states representing many levels of industry, state, and federal agencies participated in a tabletop exercise. It created, as many questions as it did answers to the multitude of problems that would arise if an outbreak of Exotic Newcastle were to occur in the intensive poultry production area of Northwest Arkansas, Southwest Missouri and Northeast Oklahoma.

The following are some of the significant points that became apparent during the planning for and in our participation in this exercise:

Newcastle is too explosive a virus to be handled alone. Any outbreak, anywhere, will have major economic consequences. The longer it lasts the greater the costs. Every action or inaction that delays recognition and subsequent eradication will increase expenses. Industry survival will require that we get it right the first time, but even getting it right will likely be a career altering experience.

The first and most dangerous time for spread will be from the time an outbreak occurs till it is detected. The second most dangerous time will be the period from detection to confirmation. Because of the adverse consequences, no one will be willing to call it Newcastle, until it is confirmed. Detection to confirmation, if samples are sent promptly to NVSL (National Veterinary Services Laboratory) is virtually a fixed time period of five to ten days from arrival to virus isolation and confirmation. Each company should have a clearly defined biosecurity plan to be implemented immediately upon suspicion of disease that minimizes people, poultry, equipment, and litter movements to prevent spread of disease. There is a critical need for grower and service personnel to be educated to signs and symptoms of Exotic Newcastle Disease and Highly Pathogenic Avian Influenza. In areas where vaccination occurs, the explosive nature of the disease could be masked resulting in delay of recognition.

Any outbreak will require cooperation of industry and numerous governmental (state and federal) entities. The more entities involved (for example when quarantine areas cross multiple state and county jurisdictional lines and/or when more than one company are involved) the greater the need for every one to agree on the same game plan and for it to be well coordinated. Currently companies as well as state and federal agencies are inadequately prepared to deal with the consequences of even a small, well-contained outbreak. The questions, "What do we have to do?" and "What should we do?" need to be diligently explored within each entity and all necessary authority and corresponding responsibility clearly defined at all levels.

With each planning interaction and exercise there has been a growing awareness of the activities and responsibilities that will have to be coordinated for effective action. This will decrease the stress level and potential mistakes that would be made by each of the entities during an actual outbreak. It should be realized that each training activity is not an activity that can be put on the shelf and forgotten, but should be part of an ongoing, continuous dialog and preparation for an event we hope will never happen. Interestingly, mutual trust and respect have been the immediate by-products of this partnering activity and continue to result in increase communications and cooperation in other unrelated areas of mutual concern.

Currently there are less total federal personnel employed in USDA/APHIS/Veterinary Services today than there were individuals deployed during the Avian Influenza outbreak in Pennsylvania in 1984. Similar reductions of personnel have been occurring in state agencies and will probably accelerate as Brucellosis is finally eradicated. As a result, there is a growing need for a "FEMA" (Federal Emergency Management Agency) model of interaction in which the poultry industry would be empowered to deal with an outbreak and the state would assist in coordinating activities between companies, supplying additional personnel, and provide an oversight validation of eradication. Further support from the federal level would be provided when local and state resources are depleted as well as final verification of eradication to our international trading partners.

As part of this empowerment and to reduce the time between discovery and confirmation, consideration should be given to authorizing poultry

veterinarians to become Foreign Animal Disease Diagnosticians. This could be accomplished by training sessions at Plum Island or Ames, Iowa; or by some form of special training held in conjunction with other poultry veterinary meetings.

Since FEMA and their state counterparts deal with emergencies on a daily basis it makes sense to try to work within the model and experience of those entities to deal with animal emergencies as well. This is especially true since they have funding and animal agriculture does not. It is our experience that state emergency managers are interested in working with us, but at this time FEMA does not deal with animals other than in a limited way, primarily as it impacts the evacuation of people in a disaster. It has been proposed that FEMA's ESF-11 (Emergency Support Function – 11) be expanded from primarily a food distribution role to include agriculture and animal health. Within USDA, proposed drafts are being formulated and discussed as to its role as the lead agency in dealing with these types of emergencies. This would, if approved, legalize additional funding and resources from the federal level and subsequently strengthen state emergency management involvement as well. In using this model, it has been suggested that training in ICS (incident command system) and interaction with EOC (emergency operations center) for poultry, state, and federal veterinarians and personnel would be beneficial to coordinating their interactions and response to foreign animal disease emergencies.

The extent of economic consequences of an outbreak in commercial poultry will depend upon several unknown variables. Both domestic and international markets will be impacted. The likely immediate reaction will be for other states to embargo and/or impound any live poultry and all processed poultry originating from the state where an outbreak occurs. The growth of international export and small profit margin makes the industry vulnerable to fluctuations of, and thereby, increasingly dependent on those markets. As a result the industry will have to conform to OIE's (Office of International Des Epizooties) international standards and requirements. These international standards will force restrictions on domestic movements to prove that we have the disease under control. "Regionalizaton", because of the time needed to implement it and the requirements that would be necessary to prove a negative, i.e. no infection, may provide little relief. Poultry veterinarians and company officials need more information on the affect that OIE and international trade laws have on their management decisions, especially as it relates to animal health and disease.

Cost analysis projections should be made and presented to Congress demonstrating the economic benefit of providing funding for early eradication of any suspicious diseased flocks, versus having to wait for Secretary of Agriculture or Presidential Emergency Declaration before federal funds can be made available for eradication. It is estimated that each one-cent decline in price will result in an annual decline of broiler receipts of two-hundred-and-fifty million dollars. Depending on the local density of the poultry industry different multipliers are used to project what effect the decreased income would have on the local economy. In addition to direct losses by the company and their employees, growers would also be negatively affected resulting in defaults on bank loans, car payments, and utility payments with subsequent impact on grocery and retail stores, orthodontists and elective medical expenses, college tuition, and tax collections. Direct governmental costs of maintaining quarantine areas are projected to exceed a million dollars per month for each county involved. Some form of federal insurance should be explored for immediate use to reimburse companies and growers in depopulating exposed or highly suspect flocks and to provide financial relief for theirs as well as state and local government expenditures.

In the belief that, "a man with an experience, should never be at the mercy of a man with a theory", we visited with individuals that have lived through the Newcastle experience in California and elsewhere. The handout is the essence of that gleaned experience and was used extensively in our exercise. We offer it as a starting point and challenge you to visit with each other, your companies, your governmental counterparts, and your emergency management officials to develop a plan that can we can all agree upon as a common starting point if we face an outbreak. We only request that you share results of your discussion with us so we can strengthen ours.

A SYSTEM IN USDA, VETERINARY SERVICES FOR EMERGING ANIMAL HEALTH ISSUES . . . IT'S EMERGING!

Miloslav Muller, DVM, MPVM, Victoria E. Bridges, DVM, MS

US Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Centers for Epidemiology and Animal Health, Center for Emerging Issues, Fort Collins, CO

New pathogens are being discovered which threaten the health and productivity of the world's animal population. Some recent examples include Nipah virus and Hendra virus. In addition, known pathogens, such as West Nile virus and Muscovy duck parvovirus, have shown up in the US for the first time. Emerging animal health issues such as these are of concern due to their negative impacts on the health and productivity of livestock and poultry. Also of great concern are emerging diseases that present a risk to public health.

USDA's Veterinary Services' (VS) Strategic Plan (covering fiscal years 2001 - 2003) has incorporated emerging issues under three major goals. These goals cover safeguarding the US from the occurrence of adverse animal health events, monitoring the health and productivity of US animal populations, and enhancing the health status of US animal populations by anticipating and responding to new or emerging threats. Specific objectives in accomplishing these goals include the ability to rapidly detect emerging animal health issues, monitoring and assessing global animal health events, establishment of a central process for gathering, reviewing, and evaluating data, and incorporating monitoring and surveillance for emerging issues with all other types of monitoring and surveillance.

There are many examples of current specific VS activities regarding emerging animal health issues. The Centers for Epidemiology and Animal Health includes the Center for Emerging Issues, whose work is focused on the identification and analysis of emerging animal health issues. In recent years emergency program activities have been broadened in focus to include emerging animal diseases in addition to known foreign animal diseases. A third example of VS' emphasis on emerging issues is the newly developed Emerging Diseases Epidemiologist position for the Florida Area Office that has been advertised.

In November 1999, an Emerging Disease Issues Working Group met, with the purpose to develop a more integrated and coordinated VS approach for dealing with emerging animal disease issues in the US. The group determined that the goal of an emerging disease system should be to quickly identify, assess, and respond to emerging animal disease issues in order to prevent or limit the sudden and negative economic, food security, and public health effects of those issues. Key elements of such a system include identification, assessment, and response, as well as responsibility for implementing responses. Strengths of the current system within VS were identified as well as development of strategy and important next steps to improve the system.

Emerging animal health issues have to be identified and addressed in order to keep the US animal population healthy and marketable around the world. The system used for the identification, assessment, and response for emerging issues must be integrated and coordinated, with the communication and responsibility being what establishes and maintains that integration and coordination. While such a system is still a work in progress within VS, there is much that is currently being done in the realm of emerging disease issues throughout VS.

VELOGENIC VISCEROTROPIC NEWCASTLE DISEASE (VVND): A THREAT FOR THE UNITED STATES?

Benjamín Lucio-Martínez

Unit of Avian Health, Department of Microbiology and Immunology, College of Veterinary Medicine Cornell University, Ithaca, NY 14853

The year 2000 outbreak of velogenic viscerotropic (VVND) in Mexico has raised concerns about the consequences of introducing VVND into the United States (US) commercial poultry population.

If the VVND virus (VVNDV) is highly pathogenic and infects a large population of chickens before it is controlled, the consequences for the US poultry industry will be devastating.

Based on the Mexican experience, typical ND vaccination programs in the US are unlikely to protect against highly pathogenic VVNDV. In addition, the vaccines required to protect against these strains are likely to reduce broiler productivity (3).

Perelman in 1976 (6) found differences in the pathogenicity Mexican VVND isolates for vaccinated chickens, and that a high vaccine titer is required for adequate protection. Among six isolates compared, five caused 100% mortality in unvaccinated chickens. On the other hand, the protection afforded by $10^{4.5}$ embryo infective doses (EID) of live La Sota given by eye drop varied from 55% to 99%, depending on the challenge strain (Table 1). It was necessary to administer 10^{6} EID given by eye drop to obtain 97% protection against the most virulent of the VVND isolates (intracerebral and intravenous pathogenicity index of 1.88 and 2.77, respectively) (Table 2).

The Merial 1999 vaccine survey (1) reports that, 75% of the egg layers in the US receive four live ND vaccines, 18% three and 7% two. Only 21% receive killed ND vaccine, and only 26% are given a live boost during lay. Broiler producers shy away from respiratory reactions and most broilers in the US receive one live B1B1 vaccine at one day of age. A small percentage is re-vaccinated at 14 days of age with B1B1, La Sota or VGGA (Linares, Salem and Takeshita, personal communications)

In Mexico, protection against VVND is typically achieved in broilers with the simultaneous application of oil-emulsified vaccine and a live La Sota in the first two weeks of life, with a live La Sota as a booster at around 4 weeks of age. Some flocks receive a third live La Sota at 6 weeks of age. Layers receive at least one more live La Sota and an oil-emulsified vaccine before onset of production.

VVND may be introduced in to the US by different means: migratory workers, migratory poultry advisers, migratory birds, live poultry or poultry products, and live birds.

1. Migrant workers traveling from Mexico are frequently mentioned as a risk in the introduction of VVND to the US. It would be irresponsible to dismiss the possibility of migratory workers introducing VVND to commercial poultry in the US. But, historic information on VVND, avian influenza, and migrant workers suggests that it is unlikely that VVND may be introduced into the US poultry industry from Mexico:
    a. VVND has been endemic in Mexico at least since 1948.
    b. None of the US VVND episodes has had its origin in Mexico.
    c. Avian influenza H5N2, detected in Mexico in 1994 has not been introduced into the US.
    d. Workers have moved from Mexico to the US in significant numbers since the late 40's with the officially sanctioned "bracero" program.
    e. Sixty percent of the Mexican migrant workers originate from Central Mexico (Michoacan, Jalisco, Guerrero, Zacatecas, San Luis Potosi, Aguascalientes, Guanajuato, Queretaro, Tlaxcala and Puebla) where VVND and avian influenza are endemic (Mexican Embassy) (2).
2. There is no doubt that migratory poultry advisers have the potential to be in close contact with VVND-infected flocks. But, the risk that migrant poultry advisers will introduce VVND to the US is greatly diminished by the precautions that their education dictates.
3. Even though ND has been documented in only 236 species o birds, it is believed that all 8000 avian species are susceptible to NDV infection (4) the transmission to poultry has been documented in very few instances.
4. Due to importation regulations and cost of production, live poultry is not imported from Mexico into the US. A limited number of poultry products (chicken meat) are imported to the US from VVND-free zones in Mexico.
5. Two of the most wide spread instances of VVND in the US have been related to importation of live birds. The first one, the California outbreak in 1971, which severely affected the poultry industry (7) and the shipment of birds out of a Florida aviary to Illinois, Indiana, Michigan, Texas, California and Nevada (5) which did not affect poultry.

REFERENCES

1. Anonymous. 1999 Merial Vaccine Survey. In: Ch. Hofacre (ed.). The Poultry Informed Professional. Dept. of Avian Medicine, U. of Georgia. No. 35. March 2000. pp. 1-10.

2. Anonymous. Embassy of Mexico in the United States of America. U.S.-Mexico Binational Study On Migration.
http://www.embassyofmexico.org/english/4/3/migration3.htm

3. Castillo R., D., B. Lucio M. y A. Antillón R. Efectos producidos por dos cepas vacunales del virus de la enfermedad de Newcastle sobre la enfermedad respiratoria crónica complicada. Veterinaria Méx. 4:123-126. 1973.

4. Kaleta, E.F., and C. Baldauf. 1988. Newcastle disease in free-living and pet birds. p197-256. In D.J. Alexander (ed.). Newcastle disease. Kluwer Academic Publishers, Boston, Mass.

5. Panigrahy, B., D.A Senne, J.E. Pearson,M.A. Mixson, and D.R. Cassidy. Occurrence of velogenic viscerotropic Newcastle disease in pet and exotic birds in 1991. Avian Dis. 37:254-258. 1993.

6. Perelman A., B. Importancia de las cepas de desafío en la evaluación de las vacunas contra la enfermedad de Newcastle. Veterinaria Méx. 7:57-61. 1976.

7. Utterback, W.W., and J.H. Schwartz. Epizootiology of velogenic viscerotropic Newcastle disease in southern California 1971-1973. J. Am. Vet. Med. Assoc. 163:1194-1195. 1973.

Table 1. Mortality after challenge with 6 different VVND strains in unvaccinated chickens or chickens vaccinated by eye drop with $10^{4.5}$ EID_{50} at 15 days of age, and challenged when 30 days old.

(% mortality)

Isolate	Vaccinated	Not vaccinated
Chimalhuacán[3]	45	100
Hermosillo[4]	22	100
Culiacán[5]	22	100
Querétaro[6]	1	82
Jilotepec[7]	20	100
Jalisco[8]	16	100

[3] Close to Mexico City, with dense poultry population at the time.
[4] Northwest Mexico (Sonora State) NDV similar to US velogenics
[5] Northwest (Sinaloa State), [6] Central Mexico (isolated back in the 50s), [7] South of Mexico City (Morelos State),[8] West Central Mexico.

Table 2. Fifteen day old-commercial chickens vaccinated with increased doses of La Sota strain, and challenged at 30 days of age by intramuscular injection of 10^6 EID_{50} of Chimalhuacán NDV.

Vaccine Dose (Log_{10} EID)	Mortality (%)
2	96
3	67
4	48
5	36
6	7

T CELLS LYMPHOKINES CONFER PROTECTION TO NEONATAL BROILER CHICKS AGAINST A VELOGENIC STRAIN OF NEWCASTLE DISEASE VIRUS

Julio C. Alfaro, Víctor M. Petrone, Guillermo Téllez, Rubén Merino, Tamas Fehervari

Departamento de Producción Animal: Aves, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, 04510, México, D.F.

SUMMARY

The purpose of the current study was to investigate the effect of prophylactic treatment on one day old broiler chicks with soluble products contained in the supernatants obtained from splenic lymphoid cells cultures stimulated with concanavalin-A originating from immunized chickens with three dosages of 10^8 CFU/ml of *Salmonella enteriditis*, (SE-ILK, *S. enteritidis*-immune lymphokines) against a challenge made with the velogenic Chimalhuacan strain of Newcastle disease virus (NDV). At one day of age, chicks were weighed and injected intraperitoneally with 0.5 ml of these supernatants. Thirty minutes after lymphokines injection, birds were challenged intramuscularly with a dosage of $10^{7.6}$ EID_{50}/1 ml of NDV. On the 3rd, 7th, 14th and 21st days postchallenge, chickens were weighed to obtain daily weight gain (DWG) and were sacrificed to take samples of organs in order to carry out viral isolation. During the experimental period clinical signs and observed lesions to necropsy suggesting Newcastle disease (ND) were recorded. Obtained results showed that SE-ILK administration was able to diminish infection seriousness due to NDV in susceptible birds because of : a) it avoided significantly detrimental effects on DWG due to NDV infection ($p<0.05$); b) it diminished infection seriousness inhibiting clinical signs ($p<0.001$) and lesions ($p<0.005$) appearance; and c) it decreased NDV isolation rate from challenged chicken organs

((p<0.005). This study offers first evidences about SE-ILK supernatant being able to induce protection to newly born fattening chickens against a viral infection.

Viral infections usually cause economic losses to the poultry industry because infected broiler flocks have a poor daily weight gain (DWG) and mortality (4, 5, 7). Cytokines biology understanding could allow their use to make an optimal bird resistance to this kind of infections. Theoretically, prophylactic administration of cytokines to broiler chicks should promote resistance to viral infections. In the current study a lymphokine preparation was made using hyperimmunized birds with *Salmonella enteridis* (SE-ILK, *S. enteritidis* immune lymphokines). Our purpose was to assess the prophylactic effect of SE-ILK on the negative impact produced by Newcastle disease virus (NDV) in neonatal broiler chicks. We infer the intraperitoneal injection of SE-ILK is able to reduce the incidence of NDV and SE-ILK ability to inhibit the virus detrimental effect on body weight gain of broiler chickens.

MATERIALS AND METHODS

Bacterial inoculum. A primary poultry isolate of phagotype 13 *S. enteritidis* was used. A stock solution of the appropiate bacterium (1 x 10^9 cfu/ml) was prepared.

Lymphokines preparation. SE immune lymphokines were prepared as previously described Kogut and Slajchert (2), and Téllez *et al* (6).

Viral inoculum. The velogenic Chimalhuacan strain of NDV used in this experiment was analyzed by determining the mean embryo infective dose (EID_{50}). The titer was $10^{7.6}$ EID_{50}/ml.

Experimental design. Two hundred one-day-old broiler chicks (Hubbard x Hubbard) were used. They were randomly placed in 5 groups of 40 birds each, in electrically heated batteries located in isolation units. The first group was treated with SE-ILK but not challenged (SE-ILK); the second group was treated with SE-ILK and challenged with NDV ($SE\text{-}ILK_{VENC}$); the third group was treated with NILK and challenged ($NILK_{VENC}$); the fourth group was non treated – challenged (NDV) and the fifth group was non treated – non challenged (negative control). At one day old, birds were weighed and the average weight by group was obtained. On that same day, chicks of SE-ILK treated groups were intraperitoneally injected with 0.5 ml. In the same way NILK was injected in chickens of the corresponding group. Chicks were challenged with a single dosage of NDV ($10^{7.6}$ $DIEP_{50}$/0.1 ml/chick) 30 minutes after supernatants application. At 3 days old, 10 chickens of each group were sacrificed to carry out viral isolation [VI] (1) from organ samples (trachea, lung, brain and cecal tonsils). Remaining birds of each group were weighed at 7, 14, and 21 days old to obtain the average weight per group. Ten chickens of each group was sacrificed on each of these days to perform VI from organ samples. Pathological changes observed in necropsy were reported. Presentation of clinical signs and/or mortality due to infection by NDV was recorded daily. Chi-squared statistical test was used to determine significant differences among groups regarding lesions presence and clinical signs (8). DWG of groups were analyzed by variance analysis using the general lineal models procedure. Statistical differences of means among groups were specified by the Duncan's multiple range test using statistical analysis commercial program SAS® (3). The Chi-squared test was used to determine significant differences among groups, regarding isolation rate of NDV from challenged birds (8).

RESULTS

Clinical signs significant reduction ($p< 0.001$) was observed in the $SE\text{-}ILK_{NDV}$ group compared to the positive control group. Only one chicken (2.5%) of the $SE\text{-}ILK_{VENC}$ group presented torticollis and clonic convulsions, although there was no mortality. No gross lesions were observed in $SE\text{-}ILK_{NDV}$ group. Lesions inhibition in the $SE\text{-}ILK_{NDV}$ group was significant ($p< 0.001$). No significant difference was noted among SE-ILK, $SE\text{-}ILK_{NDV}$ and negative control groups DWG. DWG of $SE\text{-}ILK_{NDV}$ group was significantly higher ($p< 0.005$) compared to that positive control group during the course of the study (Table 3). In the $SE\text{-}ILK_{NDV}$ group a significant decrease was observed ($p<0.005$) in the number of chickens infected with NDV compared to positive control group. In the $SE\text{-}ILK_{NDV}$ group, 3 out of 40 chickens (7.5%) NDV was isolated.

DISCUSSION

Under current intensive poultry production conditions, birds are exposed to a great variety of potentially pathogen microorganisms. Due to this intense antigenic exposure, no vaccination calendar or antibiotic therapy are effective and practical. Under such conditions, use of cytokines as non-specific elements to increase potential of the immune system might result in a more practical solution in protection against infectious diseases. This is one of the first reports that informs about the bird cytokines prophylactic effect *in vivo* with broiler chickens against a specific and common virus of commercially exploited birds, as well as about treatment efficiency with SE-ILK in inhibition of infection produced by NDV. Despite other mechanisms have been considered regarding infectious diseases prophylaxis and

protection such as use of vaccines and/or antibiotics, the use of lymphokines might be able to counteract and eliminate viral infections. Results obtained concerning virus isolation thus show it: reduction in number of virus isolations from chickens treated with SE-ILK has been significant. Birds treated with lymphokines allowed inhibition to infection because the virus could not be isolated from chicken tissues treated with SE-ILK. Infection inhibition was reflected in the absence of the disease clinical stage and in getting an adequate DWG in treated chickens. Finally, prophylactic administration of a lymphokines supernatant of immunized birds with *S. enteritidis* was able to diminish significantly the infection severity produced by a NDV velogenic strain in commercial broiler chicks.

REFERENCES

1. Alexander, D. J. Newcastle Disease. In: A laboratory manual for the isolation and identification of avian pathogens, 3rd ed. H. G. Purchase, H. Lawrence, H. Domermuth, J. Pearson, eds. Kendall/Hunt Publishing Company, Pennsylvania. pp. 114-120. 1989.

2. Kogut, M. H., T. Slajchert. T lymphocytes confer protection in chickens against Eimeria tenella by protection of lymphokines. Immunol Infect Dis. 2:69-79. 1992.

3. Luginbuke, R. C., S. D. Schlotzhaver. SAS/STAT guide for personal computers, 6th ed. SAS Institute Inc, Cary, North Carolina. pp. 555-573. 1987.

4. Sharma, J. M., K. Karaca, T. Pertile, M. Suresh. Avances recientes en la inmunología aviar y la inmunomodulación. Memorias de la XIX Convención Nacional Anual ANECA; 1994 mayo 4-7; Puerto Vallarta (Jalisco) México. México (DF): Asociación Nacional de Especialistas en Ciencias Avícolas de México, AC. pp. 297-300. 1994.

5. Sharma, J. M., K. Karaca, S. Erickson, B. Winslow, D. E. Junker, J. K. McMillen. Efecto del interferón recombinante sobre la inmunosupresión viral en pollos. Memorias de la XXI Convención Nacional Anual ANECA; 1996 mayo 1-5; Cancún (Quintana Roo) México. México (DF): Asociación Nacional de Especialistas en Ciencias Avícolas de México, AC. pp. 147-148. 1996.

6. Téllez, I. G., H. Kogut, B. M. Hargis. Immunoprophylaxis of Salmonella enteritidis infection by lymphokines in Leghorn chicks. Avian Dis. 37:1062-1070. 1993.

7. Tizard I. Uso de inmunomoduladores en medicina veterinaria, con especial énfasis en la inmunomodulación en las aves de corral. Memorias del Curso de Actualización Avances en Inmunología Aviar; 1996 marzo 15; México (DF) México. México (DF): Asociación Nacional de Especialistas en Ciencias Avícolas de México, AC. pp. 57-65. 1996.

8. Zar, J. Bioestadistical analysis. 3rd ed. New Jersey:Prentice-Hall Inc. 1996.

NECROTIC ENTERITIS: EFFECTS OF VARIOUS DIETS ON CLOSTRIDIUM PERFRINGENS AND ITS TOXINS

C. B. Annett, J. R. Viste, E. Simko

Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, Saskatchewan S7N 5B4

Necrotic enteritis caused by *Clostridium perfringens* type A is a persistent problem affecting up to 37% of rapidly growing broiler chickens in the commercial poultry industry. There are several factors known to precipitate outbreaks of necrotic enteritis, including high levels of wheat, barley or fishmeal in the diet. Alternatively, broilers on predominantly corn-based diets have lower incidence of necrotic enteritis.

Currently, antibiotic feed additives or digestion enhancers are widely used by the commercial producers of broiler chickens to control necrotic enteritis. There is growing concern about the medical impact of antibiotic use as feed additives due to transferable antibiotic resistance in human and animal pathogens. If pressure is placed on poultry producers to eliminate antibiotics in feed, the economic effects due to rising necrotic enteritis in broilers and/or reduced consumption of wheat or barley-based diets could be disastrous to Western Canada. Therefore, there is a need to develop an alternative to antibiotic feed additives. Prior to developing a suitable alternative, it is very important to determine the pathogenesis behind the development of necrotic enteritis in broilers fed wheat or barley-based diets.

We examined the proliferation of *Clostridium perfringens* in thioglyocollate media containing one third of supernatant obtained from *in vitro* digested corn-based, wheat-based and barley-based poultry

grower diets and we compared it with Clostridial proliferation in thioglycollate containing only digestive enzymes. A predetermined number of *C. perfringens* (3.23×10^6) was inoculated into each medium and following six hours of anaerobic incubation at 40°C, colony form units (CFUs) were counted, and the number of bacteria per ml of media were determined. Statistical analysis (Kruskal-Wallis one-way nonparametric analysis of variance) revealed that Clostridial proliferation in media containing digested wheat-based ($5.8 \times 10^8 \pm 1.23 \times 10^8$ CFU/ml) and barley-based ($5.95 \times 10^8 \pm 3.44 \times 10^8$ CFU/ml) diets were significantly higher ($p<0.05$) than proliferation in thioglycollate containing digested corn-based diet ($3.77 \times 10^8 \pm 5.1 \times 10^7$ CFU/ml). Toxin production at 6 hours of incubation is currently being investigated, as we hypothesize that *C. perfringens* toxin production will be enhanced by the barley and wheat diet supernatant or inhibited by the corn diet.

We conclude that growth of *Clostridium perfringens* type A is significantly greater when grown in wheat- or barley-based diets compared to a corn-based diet.

(The full paper from this work is being submitted to *Avian Diseases* in 2001)

CORRELATION BETWEEN HEMAGGLUTINATION INHIBITION (HI) TEST AND ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR NEWCASTLE DISEASE

K. Blasco[A], G. Servin [B], and M. Tamayo[A]

[A]Fort Dodge Animal Health México
[B]Indpendent Consultant, Ojo de Agua Mexico

During the last year several cases of Newcastle Disease (ND) were reported in different parts of the world. The disease produces different clinical signs that may vary considerably with the infecting virus. In addition, the type of bird, immune status age and different conditions as geographic distribution in our country may also affect the disease signs while the present of other microorganisms may hide or exacerbate the clinical signs. We can find different signs such as sudden death, severe respiratory disease, nervous signs or even the absence of clinical disease. In Mexico basically there are two types of commercially available vaccines for ND, live and inactivated. Live lentogenic vaccines are derived from field strains such as Hitchner B1 and La Sota. Inactivated vaccines also are derived from lentogenic and mesogenic strains. After the outbreaks many questions came to our mind. Is the vaccination scheme adequate? Are the birds responding to the vaccines used? Is the vaccinating crew making a good use of handling and application? Is there an outbreak? Is there an immune problem in my flock? Do I have comparative studies in my farm/company? Unfortunately, in some cases not every question had an answer. Even under field conditions, the vaccination by itself is insufficient to bring about effective control of Newcastle Disease and must be accompanied by good hygiene and biosecurity. In farms/companies with poor management and with secondary infections a mild Newcastle can produce a high mortality that can mimic a highly pathogenic Newcastle Disease.

In order to control the disease, it is important to acquire serologic information from the flocks. Serology is an important tool in diagnosis, and it is important to know how to obtain the best blood samples and have good handling too. Therefore, although Newcastle Disease has been present since 1926, it is important to go back and check the different factors that are important to have a correct diagnosis. For serology it is important to know the age and size of the flock, the amount of sera that I need to send to the laboratory and how I'm going to preserve and send the samples. Why is the age of the birds important? Because I need to obtain enough blood the easiest way. Why enough blood? Sometimes when we are in the field we don't think if we are having enough sera to run the tests. It is important to have at least 3 to 5 ml of whole blood in order to have at least 0.5 ml of sera. In some cases the test needs to be repeated or we need retention sera. How should we handle the samples? It is important to have them in a horizontal position in order to take the clot later on. Another important point is to identify the samples, age, farm, and vaccination scheme in order to have a correct diagnosis.

A wide range of tests may be used to detect antibodies to NDV in poultry sera. Currently the HI test is more widely used. Different results are presented using ELISA and HI test from different flocks in Mexico. If we are working with HI, all the serology should be worked with this test. We either use

one of them in order to establish our parameters. We should submit samples at different seasons of the year in order to have a complete history of the flock. The value of any serologic method in the diagnosis of disease is dependent on the immune status of the flock, so we must have information from the complete vaccination scheme. We must remember that in order to express a correct result we should link them with clinical signs and production data.

CONJUNCTIVITIS & RHINITIS IN 1-DAY-OLD TURKEYS DUE TO EXCESSIVE EXPOSURE TO HYDROGEN PEROXIDE TOXICITY IN THE HATCHER

R.P. Chin

California Animal Health & Food Safety Laboratory, Fresno Branch Lab, 2789 S. Orange Ave., Fresno, CA 93725

Hydrogen peroxide is currently being used as an effective disinfect as part of a hatchery sanitation program. Significant reduction in bacteria can be accomplished when 3% hydrogen peroxide is used as a fog within incubators. It does not appear to affect hatchability, livability, body weight or feed conversion in chicken flocks. The Material Safety Data Sheet reports potential health effects to include irritation, redness and pain when there is eye contact, and irritation and blistering to the mouth, throat and abdomen when ingested in large quantities. This paper reports the development of conjunctivitis and rhinitis in 1-day-old turkeys when exposed to excessive amounts of hydrogen peroxide in the hatcher.

Six 1-day-old turkeys were submitted to the California Animal Health & Food Safety Laboratory, Fresno Branch Lab, with clinical signs of eyelids being shut closed and eyes appear to be swollen. The birds had come directly from the hatcher. Grossly, there was mild to moderate, unilateral or bilateral, swelling of the eye with a slight loss of feathers on the conjunctiva. In addition, the eyelids were adhered together and there was subcutaneous hemorrhaging around the eye. Caseous material was found within the nasal turbinates. Histologically, there was severe necrosis of the conjunctiva and nasal mucosa with severe heterophil infiltration. Caseonecrotic debris was found within the nasal turbinates. *Enterococcus durans* was isolated from the conjunctiva.

It was subsequently discovered that the hydrogen peroxide fogging system was inadvertently left on the manual setting during the hatching period. The normal amount applied should be 15ml/hr of 3-5% hydrogen peroxide in the Chick Master hatchers. Inadvertently, the system was on continuous delivery for 17 hours, during which 15ml/min (900ml/hr) of hydrogen peroxide was applied. The system was turned off 12 hours prior to pull time. The hatchability from the affected hatchers was 79.4% (average for that day was 81.7%.) From the 45,360 eggs set, there were 645 (1.42%) dead-on-the-tray and 325 (0.71%) poults were culled due to the eye lesion. Poults showing mild to moderate conjunctivitis were delivered to the field and there were no subsequent eye problems reported in the flock.

IMMUNOPROPHYLAXIS AGAINST SALMONELLA ENTERITIDIS INFECTION BY LYMPHOKINES IN BROILER CHICKS

Julio C. Alfaro[A], Víctor M. Petrone, Guillermo Téllez, Rubén Merino, Tamas Fehervari

[A]Departamento de Producción Animal: Aves, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, 04510, México, D.F.

SUMMARY

Broiler chicks were treated prophylactically with the soluble products from concanavalin A-stimulated T-lymphocytes from *Eimeria tenella*-infected chickens in order to investigate the effect of such prophylactic treatment on organ invasion by *Salmonella enteritidis*. Chicks were randomly assigned in three groups: group a) experimental group (*S. enteritidis*-infected, treated): at one day of age chicks were injected intraperitoneally

with *E. tenella*-immune lymphokines, and thirty minutes after the lymphokines injection all chicks were challenged *per os* with 10^5 colony-forming units of *S. enteritidis*; group b) positive control group (*S. enteritidis*-infected, not treated) and group c) the negative control group (*S. enteritidis*-not infected, not treated). At 24 hours post-challenge all chicks were humanely euthanized, and their liver, spleen and cecal tonsils collected. Isolation of *S. enteritidis* was attempted from these tissues. The treatment of chicks with lymphokines resulted in reduction of *S. enteritidis* isolation from liver-spleen ($P<0.05$) but not from cecal tonsils ($P>0.05$). These results demonstrate that the prophylactic treatment with *E. tenella*-immune lymphokines reduces the *S. enteritidis* organ invasion but not the intestinal colonization in neonatal broiler chicks.

Previously it has been reported that the intraperitoneal prophylactic administration of soluble products from Con-A stimulated T-lymphocytes of *Salmonella enterititidis*-infected chickens (SE-ILK, *S. enteritidis*-immune lymphokines) is able to confer protection against *S. enteritidis* infection (5). However, SE-ILK can confer prophylactic protection against other nonspecific microorganisms antigenically different. In some studies, when SE-ILK was intraperitoneally administered to broilers (6) and Leghorn chicks (4) before challenge, they conferred protection against *S. gallinarum* and *S. typhimurium*, respectively. These data suggest that host-resistance mechanisms may be less dependent of specific response to antigen. To shown that lymphokines confer nonspecific protection, soluble products from Con-A stimulated T-lymphocytes of *Eimeria tenella*-infected chickens (ET-ILK, *E. tenella*-immune lymphokines) were administered in *S. enteritidis* susceptible chicks. The purpose of this paper was to evaluate nonspecific protection induced by ET-ILK against *S. enteritidis* organ invasion in neonatal broiler chicks.

MATERIAL Y METHODS

Inffective material. A strain of Mexican indigenous *E. tenella* (MOR-80-QRO) to obtain ET-ILK was used. It was provided by Dr Moreno R. (DPA: Aves, FMVZ-UNAM). A primary poultry isolate of PT-13 *S. enteritidis* strain from the National Veterinary Services Laboratory, Ames, Iowa, was used for challenge. A stock solution of the appropriate bacterium (1×10^5 cfu/ml) was prepared.

Lymphokines preparation. ET immune lymphokines were prepared as previously described by Kogut and Slajchert (1).

Experimental design. One hundred and twenty chicks (Arbor Acres x Arbor Acres) at one day of age were used and randomly assigned in three groups of 40 birds each, in electrically heated batteries located in isolation units. The experimental groups were: 1) negative group, 2) positive group and 3) experimental group. Chicks from experimental group were injected intraperitoneally with ET-ILK (0.5 ml). Thirty minutes after lymphokine injection, all birds were challenged with *S. enteritidis* (10^5 cfu). Chicks were killed and organs (liver, spleen and cecal tonsils) cultured for *S. enteritidis* at 24 hours post-challenge. Briefly, specimens of liver and spleen were collected aseptically and cultured separately from other organs. Organs were incubated for 24 hours at 37ºC in tetrathionate broth. After incubation, the broth was streaked on BGA plates, incubated for an additional 24 hours at 37ºC, and examined for the presence of *S. enteritidis* colonies. Chi-square analysis (7) was used to determine significant differences between groups in *S. enteritidis* colonization rate.

RESULTS

A significant reduction ($p<0.001$) in the number of *S. enteritidis*-organ-culture positive chicks injected with ET-ILK was observed as compared with controls, although there were no significant differences ($p>0.001$) in the colonization rate of *S. enteritidis* in cecal tonsils between groups.

DISCUSSION

The purpose of the present study was to determine if the administration of avian lympokines (ET-ILK) can regulate the host response against a *S. enteritidis* challenge. The results here demonstrate that the prophylactic treatment of chickens with the soluble products from Con-A stimulated *E- tenella*-immune T-cells can induce significant protection against infection with *S. enteritidis* as evidenced by a significant reduction in the number of *S. enteritidis*-organ culture positive chicks at one day post-challenge. Although the mechanism by which SE-ILK confers protection is presently unknown, it is possible that the major effector cell in protection of chicks is the polymorphonuclear cell (PMN). In studies by McGruder (2, 3) a marked granulocytophilia in the peripheral blood was observed within 4 hours following injection of SE-ILK. The factor (s) in SE-ILK and ET-ILK responsible for the increased resistance by PMN to *S. enteritidis* organ invasion have not been identified. It is postulated that at least one component may be a colony-stimulating factor (CSF), such as granulocyte-CFS, granulocyte-macrophage-CFS, or multy-CFS. All three cytokines are known to induce an increase in the number of PMN in peripheral blood and infection places, as well as augment the PMN effector functions (2, 3). Further studies to purify and identify the effect lymphokines

are in progress. It might be worthwhile to study species specificity of the lymphokine-induced protection together with a systematic investigation into the mechanisms by which the lymphokines cause protection.

REFERENCES

1. Kogut, M. H., T. Slajchert. T-Lymphocites confer protection in chickens against Eimeria tenella by production of lymphokines. Immunol. Infect. Dis. 2:69-79. 1992.

2. McGruder, D.E., P. M. Ray, I. G. Tellez, M. H. Kogut, D. E. Corrier, J. R. DeLoach, B. M. Hargis. Salmonella enteritidis (SE) leucocyte-stimulated soluble factors: effects on increased resistance to Salmonella organ invasion in day old Leghorn chicks. P. Sci. 72:2264-2271. 1989.

3. McGruder, D. E., M. H. Kogut, D. E. Corrier, J. R. DeLoach, B. M. Hargis. Comparision of prophylactic efficacy of Salmonella enteritidis-immune lymphokines against Salmonella enteritidis organ invasion in neonatal Leghorn chicks. Avian Dis. 39:21-27. 1995.

4. Ray, P. M., E. McGruder, M. H. Kogut, B. M. Hargis. The specificity of Salmonella enteritidis immune lymphokines and its protection against other Salmonella serovars in day old Leghorn chicks. J. Immunol. 150:315-320. 1993.

5. Téllez, I. G., M. H. Kogut, B. M. Hargis. Immunoprophylaxis of Salmonella enteritidis infection by lymphokines in Leghorn chicks. Avian Dis. 37:1062-1070. 1993.

6. Wong, G. R. Inmunoprofilaxis mediante linfocinas aplicadas in ovo y en pollos de engorda contra la infección por Salmonella gallinarum (tesis de maestría). México (DF) México: Facultad de Medicina Veterinaria y Zootecnia. UNAM. 1996.

7. Zar, J. Bioestadistical analysis. 3rd ed. New Jersey:Prentice-Hall Inc. 1996.

CARBOHYDRATE CELL RECEPTORS FOR ADHESINS OF HAEMOPHILUS PARAGALLINARUM

R.P. Fernández[A], S.A. Sánchez[A], and V.E. Soriano[A, B]

[A]Centro de Investigación y Estudios Avanzados en Salud Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México. Toluca 50000, México

[B]Departamento de Investigación y Desarrollo Avícola, Biosíntesis Laboratorios S.A., Toluca 50130, México

The adherence of bacteria to mucosal surfaces is the initial event in the pathogenesis of most infectious diseases due to bacteria in animals and humans (1). The ability of bacteria to adhere to mucosal epithelium is dependent on the expression of adhesive molecules or structures, called adhesins, that allow attachment of the organisms to complementary molecules on mucosal surfaces, the receptors (2). Most of the *Pasteurellaceae* family organisms can employ multiple molecular mechanisms of adherence (or multiple adhesins) to initiate infection. Surprisingly, the receptors on host mucosal surfaces have yet been identified in very few cases (2). Carbohydrate cell receptors have been identified in most studies reported (4). The purpose of the present work was to determine the role of several carbohydrates and a glycoconjugate on the *in vitro* adherence ability of *Haemophilus paragallinarum* to chicken tracheal epithelial cells.

Carbohydrates D-glucose, D-mannose, D-fructose, D-arabinose, D-rafinose, and glycoconjugate N-acetyl-D-glucosamine were included in this study. *H. paragallinarum* bacteria were treated with 5 mg of each carbohydrate and glycoconjugate and *in vitro* adherence tests were carried out as previously reported (3). Bacteria adhered to thirty tracheal epithelial cells were counted and the adherence means compared by the Tukey test.

Adhesion of *H. paragallinarum* to chicken tracheal epithelial cells was unaffected by D-maltose, D-rafinose, and N-acetyl-D-glucosamine ($p>0.01$). Adhesion was significantly reduced by D-glucose, D-arabinose, and D-fructose. However, adhesion of bacteria treated with D-mannose was highly significant reduced ($p<0.01$).

The inhibitory action of D-mannose, mainly, suggests that D-mannose residue constitutes at least part of the adhesin receptor for *H. paragallinarum* on chicken tracheal epithelial cells.

REFERENCES

1. Beachey, E.H. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. J. Infec. Dis. 143:325-345. 1981.

2. Jacques, M., and S.E. Paradis. Adhesin-receptor interactions in Pasteurellaceae. FEMS Microbiol. 22:45-59. 1998.

3. Fernández, R.P., G.A. García, G.P. Ochoa, and V.E. Soriano. Adherence of Haemophilus paragallinarum to chicken tracheal epithelial cells. Proc. 48th West. Poult. Dis. Conf., Vancouver, British Columbia, Canada. Pp.111-112. 1999.

4. Glorioso, J.C., G.W. Jones, H.G. Rush, L.J. Pentler, C.A. Darif, and J.E. Coward. Adhesion of type A Pasteurella multocida to rabbit pharyngeal cells and its possible role in rabbit respiratory tract infections. Infec. Immun. 35:1103-1109. 1982.

RESISTANCE INCREASE IN LEGHORN CHICKS AGAINST A VELOGENIC STRAIN OF NEWCASTLE DISEASE VIRUS THROUGH LYMPHOKINES PROPHYLACTIC USE

Julio C. Alfaro, Víctor M. Petrone, Guillermo Téllez, Rubén Merino, Tamas Fehervari, Felipa Galindo

Departamento de Producción Animal: Aves, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, 04510, México, D.F.

SUMMARY

One day old Leghorn chicks were prophylactically treated with lymphokines supernatants obtained from lymphocytes cultures proceeding from infected chickens with *Salmonella enteritidis* (SE-ILK, *S. enteritidis*-immune lymphokines). The objective of the current study was to investigate such treatment effect in the presence of an infection with the velogenic Chimalhuacan strain of Newcastle disease virus (NDV). Supernatants were obtained from splenic lymphocytes cultures of immunized chickens with three dosages of 10^8 ufc/ml of *S. enteritidis* and stimulated *in vitro* with concanavalin-A (7.5 μg/ml). Leghorn chicks were injected intraperitoneally with 0.5 ml of SE-ILK at one-day old; thirty minutes later they were challenged with a dosage of $10^{7.6}$ EID_{50}/ml of NDV. Obtained results showed that SE-ILK administration was able to confer resistance to birds because: a) it significantly diminished infection severity by inhibiting appearance of clinical signs ($p<0.001$), lesions ($p<0.005$) and histopathological changes ($p<0.005$) suggesting Newcastle disease; b) it decreased NDV isolation rate from challenged bird organs ($p<0.005$); and c) it potentiated and even accelerated ($p<0.005$) primary response by antibodies in Leghorn chicks in the presence of a NDV infection.

The role of cytokines in immune response includes intensity regulation in the inflammatory, cellular and humoral branches, and determination of the kind of response prevailing after challenge (2, 3). During the last 10 years, using experimental models both *in vitro* and *in vivo*, it has been demonstrated that a considerable number of factors produced by leukocytes is able to give protection against bird parasitic and bacterial diseases (4, 6, 7) Understanding birds' lymphokines biological functions could allow their use to handle adequately the avian immune system in the presence of viral infections. In the current study it was analyzed the prophylactic treatment effect with lymphokines supernatant obtained from lymphocytes cultures originated from chickens infected with *Salmonella enteritidis* (SE-ILK, *S. enteritidis*-immune lymphokines) about infection severity produced by a velogenic NDV strain in Leghorn chicks having no immune experience with this agent.

MATERIALS AND METHODS

Bacterial inoculum. A primary poultry isolate of phagotype 13 *S. enteritidis* was used. A stock solution of the appropriate bacterium (1×10^9 cfu/ml) was prepared.

Lymphokines preparation. SE immune lymphokines were prepared as previously described by Kogut and Slajchert (4), and Téllez *et al* (7).

Viral inoculum. The velogenic Chimalhuacan strain of NDV used in this experiment was analyzed by determining the mean embryo infective dose (EID_{50}). The titer was $10^{7.6}$ EID_{50}/ml.

Experimental design. Two hundred one-day-old Leghorn chicks (ISA Babcock B300) were obtained from a light breeder flock whose immunization program did not include vaccination against Newcastle disease (ND). They were randomly placed in 5 groups of 40 birds each, in electrically heated batteries located in isolation units. The first group was treated with SE-ILK but not challenged (SE-ILK); the second group was treated with SE-ILK and challenged ($SE\text{-}ILK_{VENC}$); the third group was treated with SNT and challenged (SNT_{VENC}); the fourth group was non-treated – challenged (NDV, positive control) and the fifth group was non-treated – non-challenged (negative control). Blood samples were obtained from five one-day old chicks per group to get serum and determine

antibodies levels through a serologic test of hemagglutination inhibition. On that same day, Leghorn chicks of groups treated with SE-ILK were intraperitoneally injected with 0.5 ml. Similarly, NILK was injected to birds of the corresponding group. Chicks were challenged with a single dosage of NDV ($10^{7.6}$ $DIEP_{50}$/0.1 ml/chick) 30 minutes after supernatant application. At 3-day old, 10 chicks of each group were sacrificed to carry out viral isolation [VI] (1) from organ samples (trachea, lung, brain and cecal tonsils). Samples from brain, spleen, thymus, bursa, cecal tonsils, proventriculus and trachea were taken from 2 birds per group for histological study. On the 7^{th}, 14^{th} and 21^{st} days blood samples were obtained from 5 birds per group to get serum and determine antibody levels, and 10 birds were sacrificed to carry out VI from organ samples. Organ samples were taken from 2 birds per group on each of these days for histological assessment. Observed pathologic changes during necropsy were reported. Clinical signs and/or mortality presentation were recorded daily. Chi-squared statistical test was used to determine significant differences among groups regarding appearance of lesions, histological changes, and clinical signs (8). The Chi-squared statistical test was used to determine significant differences among groups regarding isolation rate of NDV from challenged birds (8). A logarithmic transformation was carried out with the obtained data and the antibodies titers geometric mean was obtained by group. Analysis of variance was applied to results using the general linear models procedure. Statistical differences of means among groups were specified using the Duncan's multiple range test (5).

RESULTS

Three birds (7.5%) of the SE-ILK_{NDV} group presented torticollis and clonic convulsions, although there was no mortality. Clinical signs significant reduction ($p< 0.001$) was observed in the SE-ILK_{NDV} group compared to the positive control group (Table 1). No pathological changes were observed in SE-ILK_{NDV} group during experimental stage. Lesions inhibition in SE-ILK_{NDV} group was significant ($p< 0.001$). No degenerative changes were observed in SE-ILK_{NDV} group ($p<0.001$). In the SE-ILK_{NDV} group a significant decrease was observed ($p<0.005$) in the number of chicks infected with NDV compared to positive control group. In the SE-ILK_{NDV} group, 1 out of 40 chicks (7.5%) NDV was isolated. Serologic response to treatments with SE-ILK varied very much; this was higher in the SE-ILK_{NDV} group compared with the remaining groups only during the first seven days postchallenge. It diminished remarkably after this day. In any case, the SE-ILK_{NDV} group differed noticeably ($p<0.05$) in comparison with the rest of the groups.

DISCUSSION

According to obtained results in the current study, prophylactic therapy with SE-ILK can be able to remarkably decrease the clinical signs, gross lesions and mortality produced by a velogenic strain of Newcastle disease virus (VENC). Although other mechanisms have been considered for protection and prophylaxis against infectious diseases, bird cytokine use should be able to counteract and eliminate viral infections. Obtained results by VI thus show it; existing difference between SE-ILK_{NDV} and positive control groups has been remarkable regarding the number of virus isolations from challenged Leghorn chicks. There are several strategies to regulate the humoral response against Newcastle disease virus (VENC) by means of the use of vaccines with active or inactive virus. However, results suggest that seroconversion could increase its potential with the SE-ILK treatment in infected chickens with NDV. Induced effect by the SE-ILK prophylactic use included regulation in leukocytes proliferation and recruitment, observed by a quick accumulation of lymphoid cells in several organs; intensity regulation of humoral immune response, observed by antibodies titers increase in chicks treated with lymphokines and challenged; and, infection inhibition because virus isolation was not possible from tissues of chicks treated with SE-ILK. Existing balance among these activities was basically reflected in the absence of the disease. Due to this it can be concluded that prophylactic application of SE-ILK is able to decrease remarkably infection severity produced by a velogenic strain of NDV in Leghorn chicks.

REFERENCES

1. Alexander, D. J. Newcastle Disease. In: A laboratory manual for the isolation and identification of avian pathogens, 3rd ed. H. G. Purchase, H. Lawrence, H. Domermuth, J. Pearson, eds. Kendall/Hunt Publishing Company, Pennsylvania. pp. 114-120. 1989.

2. Klasing, K. C. Avian leukocytic cytokines. P. Sci. 73:1035-1043. 1994.

3. Kogut, M. H., R. B. Moyers, J. R. DeLoach. Las citocinas y sus interrelaciones funcionales: potenciación de la respuesta inflamatoria en el control de infecciones sistémicas por Salmonella en las aves. Memorias del Curso de Actualización Avances en Inmunología Aviar; 1996 marzo 15; México (DF) México. México (DF): Asociación Nacional de Especialistas en Ciencias Avícolas de México, AC. pp. 8-12. 1996.

4. Kogut, M. H., T. Slajchert. T-lymphocytes confer protection in chicken against Eimeria tenella by production of lymphokines. Immunol and Infect Dis. 2:69-79. 1992.

5. Luginbuke, R. C., S. D. Schlotzhaver. SAS/STAT guide for personal computers. 6th ed. SAS Institute Inc, Cary, North Carolina. pp. 555-573. 1987.

6. McGruder, E. D., P. M. Ray, G. I. Tellez, M. H. Kogut, D. E. Corrier, J. R. DeLoach, B. M. Hargis. Salmonella enteritidis (SE) immune leukocyte-stimulated soluble factors: ettect on increased resistance to SE organ invasion in day-old Leghorn chicks. P. Sci. 72:2264-2271. 1993.

7. Tellez, I. G., H. Kogut, B. M. Hargis. Immunoprophylaxis of Salmonella enteritidis infection by lymphokines in Leghorn chicks. Avian Dis. 37:1062-1070. 1993.

8. Zar, J. Bioestadistical analysis. 3rd ed. New Jersey:Prentice-Hall Inc. 1996.

AVIAN PASTEURELLA HAEMOLYTICA BIOTYPING, SEROTYPING AND PREVALENCE IN MEXICO

C. González, M.E. Vázquez, R. Campogarrido and V. Sivanandan

Boehringer Ingelheim Vetmedica, S.A de C.V. Research and Development Area. Calle 30 No. 2614, Zona Industrial, Guadalajara Jalisco, México

INTRODUCTION

Since 1974 (1) there is not scientific evidence of prevalence of *Pasteurella haemolytica* in poultry, whereas in field *P. haemolytica* isolations from birds with respiratory and production problems is continuous. In ruminants this microorganism is frequently associated with respiratory disease causing very serious problems. Studies for recognition of different strains of *P. haemolytica* have been based primarily on variances in utilization of certain substrates and by serological identification. For understanding and recognition of the role of *P. haemolytica* disease in poultry it is necessary to obtain the prevalence of this microorganism in the field and to implement special methodologies for identification of avian *P. haemolytica* species.

The objective of this study was to differentiate isolates of *P. haemolytica* from birds with reproductive and/or respiratory problems in several regions in Mexico.

MATERIAL AND METHODS

Sixty-two isolates were obtained from breeders (n=9), broilers (n=2) and layers (n=51). These isolates were obtained from the trachea and palatine cleft; and some of them from ovary, heart, and lungs from birds with respiratory or reproductive problems in several regions of Mexico. Bacterial isolates were propagated from preserved stocks in brain heart infusion broth (BHI) supplemented with 15% glycerol and stored in –70ºC. For biovariant isolates, identification was done according to Jaworski *et al* (2).

RESULTS

We identified 5 different biotypes 1, 2, 4, 7 and U corresponding to 24 biovariants. According to Table 1, 46% of biovariant obtained were biotype 4, 21% biotype 2, 17% biotype U, 8% biotype 1 and 8% biotype 7. Regarding most predominant biovariants, 22% of isolates were biovariant 4^{BCDGSX}, 14% biovariant 4^{CDGSX} and 10% corresponding to biovariant $4^{BCGSX.}$ Biotypes isolated in Veracruz and Jalisco were 4, 2 and U; in Yucatan 4 and 2; Puebla 1, 4 and 7; Nuevo Leon biotype 4; and Queretaro biotypes 1 and 4.

P. haemolytica was isolated in broilers in some occasions with respiratory problems associated with other viral agents (Fowl Pox). In breeders and layers, *P. haemolytica* was isolated during decrease of production period with such clinical signs and lesions as anorexia, peritonitis, hepatitis, severe nephritis, dehydration, and arthritis. No other pathogens were found different than *P. haemolytica*.

We serotyped isolates according to G. Frank methodology (3) using bovine reagents, but results were inconsistent. For this reason we are elaborating with each different avian biotype-specific antiserum in rabbits to obtain better correlation between serotype and biotype.

DISCUSSION

According to our results, prevalence of *P. haemolytica* was in the central and south regions of Mexico, with exception of Nuevo Leon state, located in the north of the country. These results suggest that weather conditions could have an important significance in the disease because this organism was

isolated most frequently in hot and hot-humid regions. On other hand, the lack of isolations in other regions could be due to absence of diagnostic focus for *Pasteurella haemolytica*.

It seems that layers (82%) are the highest susceptible species to this problem, followed by breeders (14%) and the last one broilers with 4%. These results suggest a more predominant reproductive problem.

Most biotypes found were 2 and 4 (T Biotype), which is similar as in ruminants *P. haemolytica* pathogens (1, 4, 5). A careful diagnostic analysis of birds with this kind of problem should play a primordial role in knowledge of disease and establishment of *P. haemolytica* as a primary potential pathogen.

Table 1. *P. haemolytica* biovariants isolated in several regions of Mexico

Biovariant	Avian species			Mexican Region	Total
	Breeders	Broilers	Layers		
1^{α}	2		2	Queretaro, Puebla	4
$1^{\beta\alpha}$			1	Puebla	1
2^{BCDGSX}			3	Puebla, Jalisco	3
2^{CDEGSX}	1			Veracruz	1
2^{CGSX}			4	Puebla, Edo. Mexico, Jalisco, Yucatan	4
2^{CEGSX}	1			Veracruz	1
2^{CGSX}	1			Veracruz	1
4^{BCDESX}			1	Edo. Mexico	1
4^{BCDGSX}			14	Jalisco, Edo. Mexico, Yucatan, Puebla, Queretaro	14
4^{BCDSX}			3	Yucatan, Puebla	3
4^{BCEGSX}			1	Yucatan	1
4^{BCGSX}			6	Yucatan	6
4^{CDEGSX}			1	Yucatan	1
4^{CDGS}			3	Yucatan, Puebla	3
4^{CDGSX}	3	1	5	Yucatan, Veracruz, Queretaro, Nuevo Leon	9
4^{CDGXO}			1	Yucatan	1
4^{CGS}			1	Puebla	1
4^{CX}	1			Veracruz	1
7^{BD}			1	Puebla	1
7^{D}			1	Puebla	1
$U^{BR\alpha}$			1	Jalisco	1
$U^{R\alpha}$		1		Veracruz	1
$U^{R\alpha\beta}$			1	Puebla	1
$U^{RX\alpha\beta}$			1	Puebla	1

REFERENCES

1. Hacking C. William and James R. Pettit. Pasteurella haemolytica in Pullets and Laying Hens. Avian Diseases. 18(3): 483-486. 1974.

2. Jaworski M.D., D. L. Hunter, A. C. S. Ward. Biovariants of isolates of Pasteurella from domestic and wild ruminants. J. Vet. Invest. 10:49-55. 1988.

3. Glyn Frank and G. E. Wessman. Rapid Plate Agglutination Procedure for Serotyping Pasteurella haemolytica. Journal of Clinical Microbiology. 7(2): 142-145. 1978.

4. Ward A.C.S., D.L. Hunter, M.D. Jaworski, P.S. Benolkin, M.D. Dobel, J.B. Jeffress and G.A. Tanner. Pasteurella spp in sympatic bighorn and domestic sheep. Journal of wildlife Diseases. 33(3): 544-557. 1997.

5. Adlam C. and J.M. Rutter. Pasteurella and Pasteurellosis. Academic Press. 1989.

AVIAN PNEUMOVIRUS OBSERVATIONS

David A. Halvorson, H.J. Shin, K.V. Nagaraja, F.J. Jirjis, M.C. Kumar and Daniel P. Shaw

Department of Veterinary Pathobiology, University of Minnesota, St. Paul, Minnesota

Avian pneumovirus (APV) is a cause of mild to severe respiratory disease in turkeys. It has been found in the U.S.A. since 1987 when workers at the National Veterinary Services Laboratories (NVSL) detected it in turkeys from Colorado, where a respiratory condition characterized by coughing, rhinitis and sinusitus had been seen since 1986. In Minnesota a similar condition had gone undiagnosed since February of 1986, and in the spring of 1987 NVSL, using the Colorado isolate as the ELISA antigen, demonstrated ELISA antibodies in Minnesota turkeys.

The virus has continued to affect Minnesota turkeys since that time. Prevalence studies by the Minnesota Board of Animal Health have shown that about 35% of Minnesota turkey flocks each year have been sero-positive for APV. These prevalence studies have shown a correlation of disease incidence and turkey density; the highest density counties have the most APV. In addition we have been able to detect a correlation between APV infection at the farm and the location where the turkeys are processed.

Research has pointed to wild birds as being one reservoir of APV, and field reports substantiate that in some cases there has been clear wild bird exposure prior to an outbreak of APV infection. However, by and large, the source of APV seems to be inexplicable. In the field we have documented that flocks that recover from APV infection can later become APV positive by PCR. Whether or not this indicates the presence of infectious virus is not known. Certainly such a question must be answered in order for us to understand whether recovered flocks are a reservoir of APV.

In Minnesota APV is seen most often in the spring and fall and it has been seen as early as five days of age. Possible reasons for the peak incidence in spring and fall include wild bird activity, general farming activity and the impact of external environmental changes on the turkeys. In Minnesota APV has not been seen in broilers or layers, although broilers have been experimentally infected.

Current control strategies include biosecurity and "controlled exposure". Evidence that biosecurity is successful can be found in the failure of APV to establish itself outside the high-density turkey production area in central Minnesota. Although the virus has been detected in other parts of the state and even in North and South Dakota, these areas have successfully eliminated it. Evidence that biosecurity is not successful can be found in the failure to eradicate APV from that high-density turkey production area.

"Controlled exposure" with a high tissue culture passage virus administered early in life has resulted in reduced APV-associated economic losses to the producer and processor.

EFFECT OF EIMERIA TENELLA INFECTION ON POLIMORPHONUCLEAR LEUKOCYTES' CONDUCT IN PERIPHERAL BLOOD FROM GRANULOCYTOPENIC CHICKENS

Xochitl Hernández V., Victor Petrone G., Guillermo Tellez I.

Departmento de Producción Animal: Aves, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México
Av. Universidad 3000, Ciudad Universitaria C.P. 04510, Mexico, D.F. Tel.52(5)6-22-58-67, Fax.52(5)6-22-58-68.

ABSTRACT

The importance of the immune response mediated by cells in *Eimeria tenella* (*E. tenella*) infections, has been widely demonstrated. However, the behavior of the polymorphonuclear leukocytes in these parasitisms has not been well studied. Forty-eight 1-day-old male and female broiler baby chicks were used in this trial plus 5 more so that way a bacteriological sampling could be performed. The birds were assigned to 4 groups of 12 chickens each: 1) control, 2) treated with 200mg / kg of body weight of 5-fluorouracil (5-FU) as a single dose, 3) infected with 5,000 *E. tenella* sporulated oocysts and 4) treated with 5-FU and infected with *E. tenella*. The 5-FU was administered intravenously at the 17th day of age, 2 days later, the

birds were infected with *E. tenella*. A blood sample was taken from 10 birds of each group at 1,3,5,7,8 and 9 days after 5-FU treatment to obtain the polymorphonuclear leukocytes / total leukocytes ratio (PMN/L_T). Nine birds of each group were slaughtered 7 days post infection with *E. tenella* to value the degree of cecal lesions and the numbers of oocysts in the ceca. The PMN/L_T ratios were similar among groups 2 and 4. A significant reduction was observed ($P<0.05$) on PMN/L_T ratios of days 7 through 9 PI in the birds treated with 5-FU. The group that was infected only with the parasite, showed a biphasic increase on the PMN/L_T ratios ($P<0.05$) at days 1 and 6 post inoculation; in comparison with the control group which could be related with the liberation of the sporozoites from the oocyst for searching a host cell and with a great release of antigenic material that goes with a big tissue damage respectively. The final number of oocysts in the group treated with 5-FU and infected with the parasite, was highly significant (P= 1.611 e-07) and the degree of cecal lesions was higher in comparison with the birds that were only infected. These results show that although the PMN presence is not very important on *E. tenella* infections, they do participate in primary infections and their absence enables the parasite reproduction in the host and that suggests that the PMN/L_T increased ratios could be related with the phagocytosis of the asexual and extracellular stages.

INTRODUCTION

To study the PMN indirectly during coccidia infections, 5-fluorouracil (5-FU) was used in this trial. The 5-FU inducts reversible myeloid suppression on mice mainly on PMN (14). Likewise, Kogut *et al*. reported a temporary granulocytopenia in Leghorn chickens after treatment with 5-FU (7). This trial intends to contribute information regarding the behavior of sanguineous PMN during the primary infection with *E. tenella*, to show if the presence of this parasite induces some response from the circulating PMN in control birds, and to determine if the infection is more severe in birds with granulocytopenia.

MATERIALS AND METHODS

Experimental animals. Fifty-three 1-day-old chicks were mixed-breed, they came from a commercial hatchery and were maintained in battery cages (*Petersime Brood-Unit*) in one experimental unit from the DPA: Aves, FMVZ; UNAM. All birds were fed with balanced non-medicated feed and water *ad libitum*.

Eimeria tenella inocula. A moderately pathogenic *E. tenella* field strain (MOR-80) was used. The oocysts were sporulated and stored as described by Long *et al*. (10). The inoculum was counted with a hemocytometer (16), and 5,000 sporulated oocysts were given orally in a 2 ml volume using a cannula. The control birds were inoculated with sterile phosphate buffered saline solution (PBS).

5-Fluorouracil. The 5-FU (Roche, S.A. de C.V., 03310) was administered as a single dose of 200 mg / kg of body weight in the jugular vein of each bird at 17 days of age, the control birds received the same volume of sterile PBS (pH 7.4).

Hematology. A blood sample was taken from the brachial vein in 10 birds of each group. Sample collections were performed at the same time in each day and in the same order.

Total leukocyte count (TNL). From each bird, 0.1 ml of blood was poured in a vial containing 1.9 ml of Natt and Henrick diluent previously prepared, both were blended softly to obtain a 1:20 dilution (13). TNL was made as described by Terry (19), using a hemocytometer and the formula: **TNL/µl**=(total leukocytes in 9 squares x 1.1) x 20

Differential leukocyte count. At the same time the samples were taken for the total leukocyte count, one drop of blood was collected and a blood smear was made from each bird. It was then stained with Wright's stain. From a total of 100 cells of each smear, the differential count between mononuclear and polymorphonuclear cells was realized (11, 18).

Polymorphonuclear cells / leukocytes ratio. (PMN/L_T) In each count, the PMN/L_T was determined dividing the total number or polymorphonuclear leukocytes between the total number of leukocytes per µl of blood per chicken.

Evaluation of the degree of macroscopic cecal lesions. It was performed following the Johnson and Reid scale (5).

Cecal oocysts count. The ceca of 9 birds were settled in 3 subgroups, each one was weighed and macerated in 20 ml of potassium dichromate (2.5%). From this blend 2 ml, were taken and resuspended in 30 ml of potassium dichromate (2.5%) in a flask. The oocysts were counted using a hemocytometer (6, 18). The average number of oocysts per subgroup was obtained from 6 counts. In each one the oocysts were counted (ON) from the four squares of the corners and the one in the middle of each of the two chamber cells.

Experimental design. At one day of age a bacteriological sampling was performed from the feed, layer from the cages, liver and yolk sac from 5 chickens chose randomly; also, a flotation test was made from feces at day 14 to discard the coccidia presence and at day 15 the birds were randomly distributed and a treatment with 12 chickens each: 1) group control, 2) 5-FU, 3) *E. tenella*, 4) 5-FU / *E. tenella*. The 5-FU was at 17 days of age; 2 days later,

the birds from groups 3 and 4 were challenged orally with *E. tenella*. At 1, 3, 5, 7, 8 and 9 days PT with 5-FU 10 birds from each group were sampled to perform the total and differential leukocyte count. Nine birds from each group were slaughtered 7 days post infection. The degree of severity of cecal lesions was assessed in one cecum of each bird, whereas from the other cecum and its content the oocyst amount was determined.

Statistical analysis. To determine differences among the PMN/L_T ratios, a variance analysis was performed. The significant differences between the mean of each treatment were assessed by the Dunnett multiple test using the statistical software S.A.S. (12). The differences on the amount of oocysts in ceca, as the severity degree of cecal lesions of the groups infected with *E. tenella* after the treatment with 5-FU in comparison with the groups that were only infected with the parasite, were analyzed by using the Kruskall-Wallis test. The Mann-Whitney U test was performed to determine differences between the median of each group (21).

RESULTS

The isolation from the bacteriologic assay that was performed on the day in which the birds arrived was negative. In the two groups treated with 5-FU, the PMN/L_T ratios had the same performance, 3 days PT an increase on the PMN/L_T ratio was observed and turned to normal levels on day 5 and diminished until days 8 and 9 PT when the lowest levels were registered. In a previous study, the same performance was observed, since the 10^{th} day PT the PMN/L_T ratios began to recover and turned to normal levels until the 12^{th} day PT in comparison with the control group. The means from the PMN/L_T ratios that belong to the group which was infected only with *E. tenella* showed a biphasic increase ($P<0.05$) at days 1 and 6 after the infection with the parasite in comparison with the control group (Figure 1). In the two groups that were not infected with the parasite, neither cecal lesions nor oocysts were found in any of the samples, while in the birds treated with 5-FU and infected with *E. tenella*, a significantly higher ($P=1.611^{-7}$, U= 324) amount of oocysts was found in the ceca and in its content in comparison with the group that was infected only with the parasite (Figure 2). No statistical differences were found regarding the degree of severity of cecal lesions of birds infected PT with 5-FU in comparison with the birds that were infected only with *E. tenella*.

DISCUSSION AND CONCLUSIONS

L_T levels, likewise the PMN levels in the control groups coincide with reference values (17). At day 3 PT an increase in PMN/L_T ratios was observed but only in the groups treated with 5-FU. That is why this effect is attributed to the drug. At day 5 PT the PMN/L_T ratios diminished, and continued falling until reaching the lowest levels at days 8 and 9 PT. At day 10 PT the drug's effect stops and the PMN blood levels begin to restore until day 12 PT (4). These results match with those of Donowitz and Quesenberry (1986); and Yaeger *et al*. (1983) who reported that 5-FU reduces the amount of precursor granulocyte cells in a transitory way on mice (3, 20). Kogut *et al*. (1994) reported likewise in Leghorn chickens. Besides they report that the presence of the parasite is not capable of stimulate the PMN production by the progenitor cells that are under the drug's effect, different from what was observed in the groups infected only with *E. tenella*, a biphasic increase on PMN/L_T ratios at days 1 and 6 post infection in comparison with the control group. Rose *et al*., (1979) observed a leukocyte increase in peripheral blood at 3 and 10 days; and 6 and 13 days post infection in chickens and rats infected with *E. maxima* and *E. neschulzi*, respectively (16). The PMN increase observed in this trial can be associated with the *E. tenella* life cycle specifically at the moment when the sporozoites are delivered from the oocyst and invade the host cell (day 1) and at the moment when the second generation of merozoites are delivered from the host cells (day 6). This is the most pathogenic stage of the *E. tenella* life cycle that goes with severe tissue damage and bacterial invasion and with it, the PMN mobilization to the site of the infection (1). However, this increase of PMN could be a resource to promote contact between the effector host cells and the parasite. In the group treated with 5-FU, a higher oocyst production was observed in comparison with the control group; nevertheless, when the parasite is inoculated 2 days after the treatment with 5-FU, coincides with the moment of lowest circulating PMN and possibly in the infection site; which may enable the subsistence and conclusion of the parasite's lifecycle. A significant difference was not observed on the severity of lesions among the two infected groups, possibly because we're talking about a less sensible parameter than the oocyst production. When the amount of PMN diminishes in blood and possibly in the infected tissues, the severity degree of cecal lesions is not reduced so that is why it is suggested that these cells are not directly involved with tissue damage and the participation of other cells like: T cytotoxic lymphocytes (LCD8), who destroy the infected cells (9); T cooperative lymphocytes (LCD4); natural killer cells (NK), likewise soluble factors such as IL-1 and IFN-γ (2); and TNF-α(15) could be more relevant in the appearance of lesions.

REFERENCES

1. Baba, E., H. Wakeshima, K.Fukui, T. Fukata, and A. Arakawa. Adhesion of bacteria to the cecal mucosal surface of conventional and germ-free chickens infected with Eimeria tenella. Am. J. Vet. Res. 53:194-197. 1992.

2. Byrnes, S., R. Eaton, and M. Kogut. In vitro interleukyn-1and tumor necrosis factor alpha production by macrophages from chickens infected with either Eimeria maxima or Eimeria tenella. Int. J. Parasitol 23:639-645. 1993.

3. Donowitz, G.R., and P. Quesenberry. 5-fluorouracil effect on cultured murine stem cell progeny and peripheral leukocytes. Exp. Hemato, 14:207-214. 1986.

4. Hernandez, V. X., G. E. Garcia, H. J. A. Navarro, J. A. L. Quintana, H. M. Kogut, I. G. Téllez. Modelo para la inducción de granulocitopenia en pollos de engorda mediante la administración de 5-fluorouracilo. Revista Veterinaria México 29:233-237. 1998.

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

6. Kao, T., and L. P. Ungar. Comparison of sequential, random and Hemacytometer methods for counting Crytosporidium oocysts. J. Parasitol, 80: 816-819. 1994.

7. Kogut, H. M., I. G. Tellez, M. B. Hargis, E. D. Corrier, and R. J. Deloach, The effect of 5-flourouracil treatment of chicks: A cell depletion model for the study of avian polymorphonuclear leukocytes and natural host defenses. Poult. Sci72: 1873-1880. 1993.

8. Kogut, H. M., M. B. Hargis, E. D. Corrier, and R. J. Deloach. Heterophils are decisive components in the early responses of chickens to Salmonella enteritidis infections. Microb. Pathog 16:141-151. 1994.

9. Lillehoj, H.S., J. M. Trout. CD8+ T cell-coccidia interactions. Parasitol. Today. 10:10-13. 1994.

10. Long, P. L., B. J. Millard, L. P. Joyner, and C. C. Norton. A guide to laboratory techniques used in the study and diagnosis of avian coccidiosis. Folia Vet. Lat. 6:201-217. 1976.

11. Lucas, A.M., C. Jamroz. Atlas of Avian Hematology. Unite States Department of Agriculture, (U.S.D.A.),
Washington, D.C. 1961.

12. Luginbuke, R. C., and S.D. Schlotzhaver. SAS / STAT Guide For Personal Computers. 6th ed. SAS Institute, Cary, N.C. 1987.

13. Natt, P.M., and A. C. Herrick. A new blood diluent for counting the erythrocytes and Leukocytes of the chicken. Poult Sci 31:735-738. 1952.

14. Ophir. A., E. Berenshtein, H.J. Ziltener, and E. Razin. 5-fluorouracil and mast cell precursors in mice. Exp. Hematol. 21:1558-1562. 1993.

15. Ovington, K. S., L. M. Alleva, and E. A. Kerr. Cytokines and immunological control of Eimeria spp. Int. J. Parasitol 25:1331-1351. 1995.

16. Rose, M.E., P. Hesketh, and B.M. Ogilvie. Peripheral blood leukocyte response to coccidial infection: a comparison of the response in rats and chickens and its correlation with resistance to reinfection. Immunol 36:71-79. 1979.

17. Rose, M.E., P. Hesketh, and M. Rennie. Coccidiosis: Rapid depletion of circulating lymphocytes after challenge of immune chickens with parasite antigens. Infect. Immun. 45:166-171. 1984.

18. Talebi, A., and G. Mulchy. Correlation between immune responses and oocyst production in chickens monospecifically infected with Eimeria maxima. Avian Pathol. 24:485-495. 1995.

19. Terry, W. C. Avian hematology and cytology. 1a de. Iowa State University Press. Ames, Iowa. 1988.

20. Yaeger, A.M., J. Levin, and F. C. Levin. The effects of 5-fluorouracil on hematopoiesis: studies of murine megakaryocyte-CFC, granulocite-macrofage CFC, and peripheral blood cell levels. Exp. Hematol. 11: 944-952.1983.

21. Zar, H. J. Bioestatistical Analysis. 3th ed. Prentice Hall, Inc., Upper Saddle River, New Jersey. 1996.

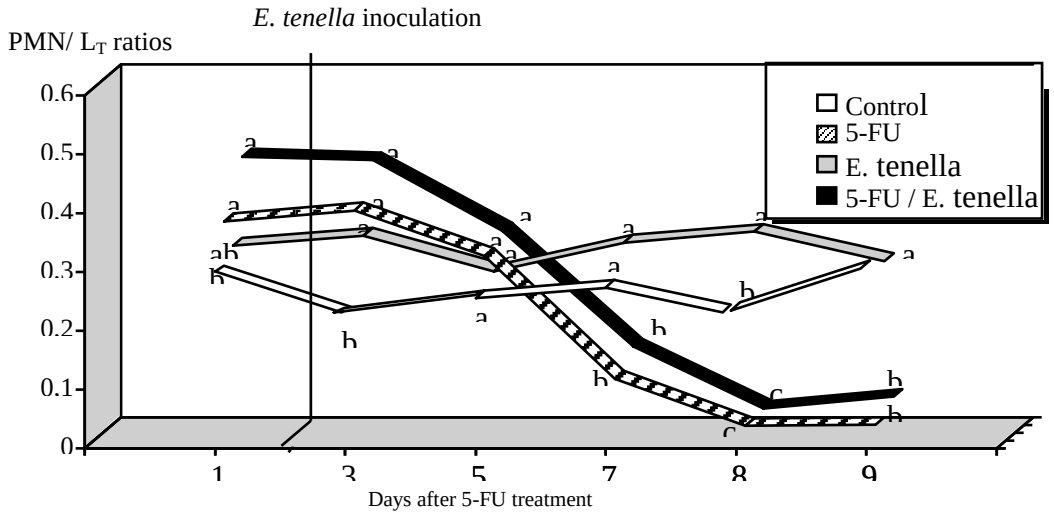


Figure 1. Polymorphonuclear leukocytes / total leukocytes (PMN/ L_T) ratio in peripheral blood of broilers infected with *E. tenella* 2 days after 200 mg / body weight of 5 fluororacil administration. PMN/ L_T shown are the means from 10 chickens by group by day. *= Significance ($P<0.05$) in comparison with the control group.

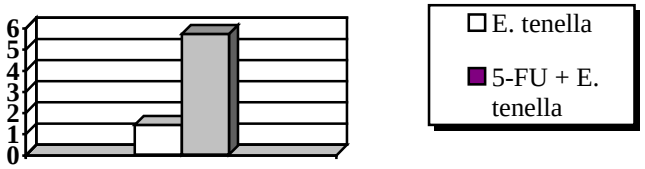


Figure 2. Cecal oocyst production in infected broilers with 5,000 *E. tenella* sporulated oocyst 2 days after intravenously administration of 200 mg / kg body weight of 5 fluorouracil. The data show the means from 9 birds by group. *=Significance ($P = 1.616^{-7}$).

THERMAL EVENTS AND PHYSICAL CHARACTERISTICS OF SIMPLY STACKED POULTRY LITTER: IMPLICATIONS FOR MICROBIAL SAFETY

J. S. Jeffrey[AB], J. C. Lazano[B], E. R. Atwill[A], and E. H. Walton[B]

[A]Departments of Veterinary Extension and Population Health & Reproduction
[B]Poultry Health and Food Safety Laboratory
Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California-Davis, Tulare, CA 93274

Millions of tons of poultry manure are produced in the USA every year. Poultry litter is used widely as a soil amendment, fertilizer or as animal feed. Microbial safety of animal wastes has become a concern due to fears that these products may act as a conduit for the entry of pathogens into the human food chain. We have extensively monitored the temperature (heating) profiles of 5 to 8 ton stacks of poultry litter that were not turned or aerated, over time. In addition we measured water activity, pH and % moisture as elements that may drive the temperature dynamics within a litter stack. The survival of bacterial pathogens (*E. coli*, *Salmonella*, *Campylobacter*) were measured. The findings of this study may have significant impact on how and when litter stacks are monitored for microbial safety and may be used to by producers in developing nutrient management plans.

MATERIALS AND METHODS

The temperature profile i.e., changes in temperature over time and the physical parameters, water activity (Aw), pH, and moisture content (% dry matter, DM) were intensively monitored for six, 5 to 8 ton stacks of poultry litter from broiler chicken facilities. Piles were not turned or aerated after being delivered and instrumented with continuous temperature probes. In addition to continuous probes, manual temperatures were recorded daily at 1 foot to 5 foot depths at a height of 2' from the base of the pile (ground level). The survival of *Campylobacter*, *Escherichia coli* and *Salmonella*, inoculated into 3 piles was measured by repeated sampling and culture over time.

Two predictive models were generated from the raw data to look at, 1) the effect of the pile on the temperature profile (i.e. changes in temperature over time), and 2) the effect of the physical parameters measured (pH, water activity (Aw), % moisture (DM)) on the temperature profile of the pile.

RESULTS AND DISCUSSION

Five of 6 piles reached similar average temperatures of 120 to 155 ^{o} F, but one pile that was dryer and contained less manure was cooler on arrival and throughout the testing period. Individual points within piles reached temperatures greater than 170 ^{o} F. Piles originating from different locations showed a uniform and parallel increase in temperature over time, when measured at a fixed depth and height, 36" and 12", respectively. Temperatures peaked between 2 and 10 days after stacking and declined slowly over the next 10 days. Measuring the pile at incremental depths and heights revealed that areas near the surface heated more rapidly than the deeper portions of the pile. The maximum temperatures generated in the deeper portions of the pile (5' depths) occurred 5 to 7 days after stacking. Also positively correlated with temperature was the height of the measurement from the base of the pile. Temperatures measured near ground level were approximately 5 degrees cooler than temperatures measured at a height of 3 feet from the base of the pile at any point in time (with depth held constant at 3 feet). This means that minimal "safe" temperatures, those necessary to kill microbial pathogens, are depth dependent. And, management guidelines would need to specify the time since stacking and the height and depth at which the measurements should be made.

The pH of the litter piles increased for 7 to 10 days after stacking, then decreased, probably due to early volatilization of nitrogen as ammonia. Litter piles with high pH (basic) generated more heat than acidic piles. A 5^{o} C difference was observed between piles with neutral to acidic pH versus those with basic pH at any point in time. Based on this data, litter treatments that lower pH may also affect the heating process of stacked litter.

The parameter with the greatest single influence on the temperature profile of stacked poultry litter in our model was Aw. Our model predicts that litter piles with higher Aw will be 20^{o} C hotter than those with low water activity. Thus a producer could adjust the moisture of poultry litter before stacking to improve heating. Bacterial survival in stacked litter was poor. The maximum time after inoculation that bacteria were recovered was 2 h for *Campylobacter*, 32 h for *E. coli* and 28 h for *Salmonella*. Given the sensitivity of our testing these data roughly correspond to a 4 to 6 log reduction in *Salmonella* within 28 h; a 5 log reduction in E. coli within 32 h, and a 2 to 3 log reduction in *Campylobacter* in 2 h p.i.

The information from this study should be useful to poultry growers that wish to develop on-farm, good management practices for microbial safety of stacked poultry litter. In addition, this information can be used as a marketing tool for producers supplying litter for crop application and may be used to guide the formulation or revision of regulatory policies for agricultural use of poultry litter.

(This research was funded by the United States Poultry & Egg Association. A full-length article is being prepared for publication in *Poultry Science*)

EVALUATING A CONCENTRATED NEWCASTLE DISEASE OIL VACCINE IN BROILER CHICKENS

Marco A. Juárez, Rubén Merino G, Gerardo M. Nava, Cecilia Rosario C, and Guillermo Téllez I.

Facultad de Medicina Veterinaria y Zootecnia (FMVZ) Universidad Nacional Autónoma de Mexico (UNAM)
Avenida Universidad 3000, Ciudad Universitaria, Mexico, D.F. CP. 04510 * britoco@servidor.unam.mx

SUMMARY

Protection in broilers against Newcastle Disease virus (NDV) is highly related with both maternal antibodies level and field challenge. NDV vaccination must consider chick's age and vaccine dose. One concentrated oil vaccine was applied in chickens of different ages, in order to establish its protection level against Chimalhuacan strain, a native velogenic NDV. Two challenges at time of NDV booster were carried out. Three hundred and sixty broiler chicks were randomized into 8 groups, 45 chicks each. In first vaccination program two groups were immunized at one-day-old with experimental (0.1 ml dose) or commercial (0.3 ml dose) oil vaccines. In second vaccination program two groups were immunized at eight-days-old, with experimental (0.1 ml) or commercial (0.5 ml) oil vaccines. First immunized groups were challenged 21 days post vaccination. Second immunized groups were challenged 28 days post vaccination. Each challenge included positive and negative control groups. Results showed that oil concentrated vaccine was not able to induce protecting antibodies against NDV pathogenic strain challenge. Both commercial vaccines showed well performance. These findings reinforce the fact that in protection against NDV pathogenic strain, care must be taken when applying any oil concentrated vaccine.

Newcastle Disease Virus (NDV) is very contagious and produces large economic losses in the poultry industry (1). In Mexico there is a NDV eradication program. Biosecurity has not been efficient in NDV control. Broiler production time is short and is very important to get a good early protection. While early protection in broilers against NDV is highly related with maternal antibodies level and field challenge, the NDV vaccination should consider chicken age and vaccine dose (2,3). Nowadays, oil emulsion vaccines are applied when chicks are too young, and vaccine dose becomes a problem (2,5). The objective of this study was to evaluate one concentrated oil vaccine applied in chickens of different ages, in order to establish the protection level against pathogenic virus challenge (Chimalhuacan strain), a Mexican enzootic velogenic NDV strain.

MATERIAL AND METHODS

Chickens. Arbor acres x Arbor acres broilers from a single hatchery were used. Four commercial flocks (7,500 chicks each) were placed on separate poultry houses (Table 1). From each flock, 45 birds (three trials, 15 chicks each) were randomly chosen as the treatment group, wing banded, and taken back in the original flock, they were identified as groups A, B, C and D. Positive and negative control birds were hatched from the same hatchery, before challenge. They were provisionally raised in an area where birds were not vaccinated for NDV. All chicks were vaccinated with turkey herpesvirus and SB-1 Marek´s disease virus at hatching, and Massachusetts infectious bronchitis virus at 1-day-old.

Vaccines. One experimental NDV vaccine and two commercial NDV vaccines were used in this experiment.

Serology. Fifteen birds by group were bled every week before and after vaccination. Inhibition hemagglutination (IH) test was carried out in order to evaluate serum samples (4). Results were record as geometrical media titer (GMT) by group, and expressed as logarithm to base two (Log_2).

Experimental design. Vaccination programs were as follows, group A was vaccinated simultaneously at one day old with subcutaneous concentrated oil vaccine (0.1 ml, vaccine 1) and spray NDV (B1 strain), this group was boosted with NDV La Sota strain by drinking water at 21days old. Group B was vaccinated simultaneously at one-day with subcutaneous oil vaccine (0.3 ml, vaccine 2) and spray NDV (B1 strain), this group was boosted by drinking water with NDV La Sota strain at 21-day-old. Group C was vaccinated simultaneously at eight days old with subcutaneous oil vaccine (0.5 ml, vaccine 3) and spray NDV (B1 strain), this group was boosted by drinking water with NDV La Sota strain at 28-day-old. Group D was vaccinated simultaneously with subcutaneous concentrated oil vaccine (0.1 ml, vaccine 1) and spray NDV (B1 strain) at eight-day, this group was boosted by drinking water with NDV La Sota strain at 28-day-old (5).

Challenge. Previously wing banded chickens were transported from experimental farms to a temperature, light, and traffic controlled isolation facility, two weeks post oil vaccination. At 21 days old, A and B groups

were challenged intramuscularly (IM) with NDV Chimalhuacan strain at 0.2 ml of 10^6 embryo infective $dose_{50}$ (EID_{50}) (FMVZ-UNAM). At 28 days old, groups C and D were IM challenged with similar inoculum (10^6 EID_{50}). All groups were challenged again at 56 days old (Table 1). Every challenge included one unvaccinated and challenged group (positive) and one unvaccinated and unchallenged group (negative). All birds were monitored twice daily for clinical signs. Necropsies were performed on died birds from challenge; surviving chickens were euthanatized and necropsied at 15 days post challenge. Gross lesions were recorded.

Statistical analysis. Serologic titers induction were separated by one-way analysis of variance (ANOVA) followed by Duncan´s multiple range test, to separate significantly different GMT, with a significance level of 0.05. Data are reported as GMT and standard error of the mean. Mortality percentages were compared with Chi-squared test ($P<0.01$) (7).

RESULTS

Experimental groups vaccinated with three oil vaccines showed different serologic titers during all serologic evaluation. At 1-day-old, group A immunized with vaccine 1 (0.1 ml) had a 6.21 Log_2 titer, similar to others groups. At seven days old, group A titer was different ($P<0.05$) from B and C groups; but was not different with group D (0.1 ml) titer (Table 2). At 14 days old, antibodies titers of all groups dropped. However, there was no difference between B and C groups, but both groups were different ($P<0.05$) from A and D groups (vaccine 1). At 21 days old, serologic titer from group A was lower when compared with other groups, and during all experiment (Table 2). Titers from group C were higher ($P<0.05$) than B (0.3 ml) and D groups. Titers from A and D groups at 28 days old were different ($P<0.05$) from B (0.3 ml) and C (0.5 ml) groups. At 35 days old, after two weeks of booster with La Sota strain, group A obtained a protective serologic level. Birds from group D acquired this status one week later of booster with La Sota strain (Table 2). At 42 days old group C had higher titers than group D. At 49 days old there were no differences between groups. At last day, all groups showed protective titers. Although, only group A titer was different from group B titer (Table 2). Vaccine 1 (0.1 ml) used in group A did not provide protection against challenge at 21 days, and showed 62.22 % of mortality (Table 3). Group B had only 6.66 % of mortality and was statistically different ($P<0.01$) from positive control group. C and D groups challenged at 28 days old had 4.4 % and 6.6 % of mortality respectively, and were different ($P<0.05$) from positive control group (Table 3). All birds were able against pathogenic strain when challenged at 56 days old (Table 3).

DISCUSSION

In this experiment, vaccines 2 and 3 provided better protection than vaccine 1. Oil concentrated vaccine did not provide protection when applied in either 1 or 8 days old chicks. In this study maternal antibodies against NDV in serum at 1 day old were high. This fact affects vaccination programs at 1 day old (2,4,5,6). Vaccine 2 (0.3 ml) used in B group at 1-day-old, had better performance than vaccine 1 (0.1 ml) in A group. Failure in antibody induction in A group at 21days old was clear. At this time, group A showed the lowest antibody titer. At three weeks old, the field flocks must have at least 90% protection against NDV pathogenic strain. However, at this age group A had high mortality. Group A showed protective titers only at 35 days old, two weeks after booster with La Sota strain. Groups A and D showed high titers in response to booster, because both groups had not well first immunization to make softer the La Sota strain post vaccination reaction. Group C and D had different serologic and protection performance. This difference is explained by one of these factors, oil concentrated vaccine or oil standard vaccine application (2,5). Titers in group C were highest during almost all serologic evaluation; although the titers from group B boosted with La Sota strain one week before than group C were higher at final of serologic evaluation (49 and 56 days old) (2). Both vaccination programs showed well protection during all broiler raising. Vaccination program from group C is frequently used in Mexico (5) because produces high protective antibody titers all time. Group B had 6.66% of mortality at 21 day old-challenge; however this group showed well performance during all serologic evaluation. This immunization program is a very good alternative for 1-day-old chicks that show low sera antibodies level. Vaccination failure in group A and D was observed even when chicks were simultaneously vaccinated at 1 and 8 day old (oil and B1 vaccines). Allan (2) has considered IM challenge for systemic viral replication evaluation. B1 strain replicates in tracheal epithelium where induce well local protection, but restricted systemic protection. Otherwise, the low antigen concentration in vaccine 1 (0.1 ml) was not adequate to induce protective antibodies under field conditions. Vaccination equipment is used to apply large oil vaccine volume (0.5 ml). If oil concentrated vaccine (0.1 ml) is applied with the same needles, it is possible that oil concentrated vaccine wring out around injected site. These findings reinforce the fact that in protection against NDV pathogenic strain; care should be taken when applying any oil concentrated vaccine. All available oil vaccines against NDV should provide

protection during all broiler production period. It is very important in endemic regions, as some states from Mexico and other countries. Uniformity and antigen concentration per dose, and whole flock immunization are important to prevent susceptible population to NDV field pathogenic strain. If high sera antibodies are found at first day is commendable to use C immunization program, but, when titers were lower could be better program B. Oil concentrated vaccine application at any age could not avoid a field outbreak.

REFERENCES

1. Alexander, D.J. Newcastle disease and other paramixovirus infections. In: Diseases of Poultry. 10th ed. B. W. Calnek, H. J. Barnes, C.W. Beard, W.M. Raid, and H.W. Yoder, Iowa State University Press, Ames Iowa. U.S.A. pp 496-513. 1991.

2. Allan, W.H. Vaccination against Newcastle disease with an inactivated oil emulsion vaccine at day-old followed by aerosol application of La Sota vaccine at three weeks. Vet. Rec. 95:54. 1974.

3. Box, P.G. and I.G. S. Furminger. Newcastle disease antibody levels in chickens after vaccination with oil emulsion adjuvant killed vaccine. Vet. Rec. 96:108-111. 1975.

4. Giambrone, J.J. Laboratory evaluation of Newcastle disease vaccination programs for broiler chickens. Avian Dis. 29:479-487. 1985.

5. Gómez, D.M. y B. Lucio. Vacunación contra la enfermedad de Newcastle en pollitos de un día de edad con asperción de virus vivo, con virus muerto emulsionado a ambos. Avirama 2:13-19. 1986.

6. Partadiredja, M., C.S. Eidson, and S.H. Kleven. A comparison of immune responses of broiler chickens to different methods of vaccination against Newcastle disease. Avian Dis. 23:622-633. 1978.

7. Zar, J. Bioestadistical analysis. 2nd ed. ed. Prentice Hall Inc. Englewood Cliffs New Jersey. pp 348-351. 1984.

Table 1. Treatments used in this experiment and oil vaccine assignment per groups.

Group designation	Vaccine number.	Vaccination Day	Booster day La Sota strain	Challenge day Chimalhuacan strain	Birds per house
A	1 (0.1 ml)	1-day-old	21-day-old	21 and 56-day-old	7,500
B	2 (0.3 ml)	1-day-old	21-day-old	21 and 56-day-old	7,500
C	3 (0.5 ml)	8-day-old	28-day-old	28 and 56-day-old	7,500
D	1 (0.1 ml)	8-day-old	28-day-old	28 and 56-day-old	7,500

Table 2. Results of serologic evaluation titers obtained from groups vaccinated with three different volume oil vaccines.

Groups	1 Day	7 Day	14 Day	21 Day	28 Day	35 Day	42 Day	49 Day	56 Day
A	6.21*	2.42	1.53	1.06	1.60	5.66	5.73	5.70	4.80
	± 1.80 a	± 1.25 c	± 1.03 c	± 0.54 c	± 1.18 b	± 1.75 a	± 1.03 ab	± 1.48 a	± 0.94 b
B	6.21	4.72	2.80	2.06	4.60	5.73	5.93	5.93	5.80
	± 1.80 a	± 0.93 a	± 1.25 a	± 0.79 b	± 1.54 a	± 0.59 a	± 0.59 ab	± 0.59 a	± 0.94 a
C	6.21	3.23	2.96	3.13	4.06	6.00	6.13	5.60	5.20
	± 1.80 a	± 1.07 b	± 0.62 a	± 0.83 a	± 0.96 a	± 1.19 a	± 1.40 a	± 0.48 a	± 1.20 ab
D	6.21	2.75	2.11	2.13	1.53	5.26	5.26	5.40	5.33
	± 1.80 a	± 1.06 bc	± 1.32 b	± 0.51 b	± 0.63 b	± 0.88 a	± 0.45 b	± 0.82 a	± 1.29 ab

*Geometrical media titer expressed in logarithm to base two (±SME) with different lowercase superscripts indicate different statistical difference (P<0.05)

Table 3. Results of challenge with one Newcastle disease virus pathogenic strain (Chimalhuacan) after vaccinations.

Group designation	21 day challenged	28 day challenged	56 day challenged
A	62.22%* b	-	0.00% b
B	6.66% c	-	0.00% b
C	-	4.44% b	0.00% b
D	-	6.66% b	0.00% b
Positive control	100.00% a	100.00% a	93.33% a
Negative control	0.00% c	0.00% b	0.00% b

*Mortality percentage with different lowercase superscripts indicates different statistical difference (P<0.01)

MULTIPLEX RT-PCR AND ITS APPLICATION IN EXPERIMENTALLY INFECTED SPF CHICKENS WITH RESPIRATORY PATHOGENS

Yaoshan Pang[A], Mazhar Khan[B], Han Wang[B], Zhxiun Xie[A] and Theodore Girshick[C]

[A]Guangxi Veterinary Research Institute, Nanning, Guangxi, Republic of China
[B]Department of Pathobiology and Veterinary Sciences, University of Connecticut, 61 North Eagleville Road, Storrs, CT 06269-3089
[C]SPAFAS, Inc, 167 Baxter Road, CT 06268

Respiratory diseases multiplex RT-PCR was developed and optimized to identify six major avian respiratory pathogens in our laboratory. In this study, twenty-eight 4 week-old Specific-Pathogen-Free White Leghorn Chickens were divided into 7 groups, each group containing 3 chickens. The following respiratory pathogens were used to inoculate the chickens; Infectious bronchitis virus (IBV) Mass41 (group 1), Infectious laryngotracheitis virus (ILTV) 950802 (group 2), Newcastle disease virus (NDV) B1 Lasota (group 3), AIV T/W/66 (group 4) each containing 10^4 plaque-forming unit (PFU) and *Mycoplasma gallisepticum* (MG) S6 (group 5), *Mycoplasma synoviae* (MS) WVU1853 (group 6) each containing 10^6 color forming unit (CFU). Each chicken was given intra nasally 0.25 ml of 10^4 PFU (for IBV, ILTV, NDV) or 10^6 CFU (for MG, MS) of each pathogen. Group 7 was inoculated intra-nasally with the saline as a control group. Tracheal swabs were taken before inoculation and 2 days interval after post-infection up to two weeks. At the end all birds were euthanized and tissues from the trachea, lungs, ceca and kidneys were collected. Serological [(ELISA, SN (IBV), AGP (ILTV, AIV), HI (MG, MS, NDV)] and isolation of respiratory agents were performed during and at the end of experiment according to procedures defined for each pathogens.

In result the multiplex RT-PCR identified and differentiated chickens infected with the respiratory avian pathogens. Experimental chicken tracheal swabs co-infected by six avian respiratory pathogens were detected by multiplex RT-PCR and detected the co-infected chickens with two or more pathogens.

A CASE REPORT OF ORAL LESIONS IN LAYING HENS

Enrique González[A], Joel Muñoz, Juan Carlos Medina, Alejandro Romero and Javier Lara

Nutek S.A. de C.V.
[A]Investigación Aplicada S.A. de C.V., 7 Norte 416, Tehuacan, Pue. , 75700 Mexico, jlara@grupoidisa.com

SUMMARY

The present study reports a problem of oral lesions in laying hens presented in a farm located in Southeast Mexico. The lesions were observed in a range of gray to black tongue coloring and oral ulcerations. Egg production decreased from 80.6% to 64% for 53 weeks old hens. No apparent disease was observed, and the oral lesions were related with the feed quality. The chemical analysis of the feed showed only the contamination with the mycotoxin Nivalenol. Neither DAS nor T2 toxins were detected. At the beginning of the 54th week a mixture of an organoaluminosilicate with an aluminosilicate was added to the feed. After 3 weeks the lesions disappeared and the production recovered to 80%.

INTRODUCTION

Oral lesions in chickens in the form of ulcers can be associated with productivity losses in the poultry industry. The origin of this kind of lesions is normally attributed to either T-2 toxin or Diacetoxyscirpenol (DAS) (1). Recently Brake *et al* (2) reported evidence that DAS produce oral ulcers, gray-black tongues, decrease in body weight and feed consumption in broiler breeders. DAS and T-2 toxin belong to trichothecene mycotoxins, produced by *Fusarium* species in feeds and grains. By considering that trichothecene mycotoxins comprise a large group of organic compounds, it is very probable that not only both mycotoxins produce this kind of lesion. In fact, it has been reported that some derivatives of the DAS also produce oral lesions (3). However, there is a lack

of literature on the toxicity of other trichothecenes. For example, there is very little information on Nivalenol, which it is considered 10 times more toxic than DON (4).

Nowadays, poultry technicians are well aware of the negative impact of mycotoxins on productivity. Due to this fact, the use of mycotoxins adsorbents has been generalized in the poultry industry and many types of commercial aluminosilicates, when included in the feed, offer good protection of livestock against aflatoxins, but not against other mycotoxins. In the specific case of high levels of T-2 toxin and DAS, aluminosilicates do not give protection against the detrimental effect of these compounds over the animal health (5,6). Therefore, the only solution is the removal of the contaminated feed. However, in most instances this is not possible. As new alternatives, different kinds of adsorbents have been in research and among them are the organoaluminosilcates (7). An organoaluminosilicate is an adsorbent obtained by the chemical reaction of an organic compound on the aluminosilicate surface.

The aim of this study was to follow the evolution of a case of oral lesions in hens under intensive farming conditions and to test the efficacy of a mixture of an organoaluminosilicate with an aluminosilicate to counteract the loss in productivity of the farm.

CASE DESCRIPTION AND METHOD

The problem detected as a loss in egg production was presented in a poultry company from the Southeast area of Mexico. Egg production decreased from 80.6% at the 51st week to 64.9% at the 53rd week. The loss of nearly 20% production prompted a search for the cause of the problem. The examination of hens showed oral lesions in a great percentage of them. The lesions were observed as gray to black tongue and oral ulcers in the lower beak. They were classified in accordance to the degree of severity and were visually scored by the same individual. A lesion score of 1 indicated no visible lesion; the score 2 was seen as black tongue tip, the score 3 was assigned to a lesion that appeared as a clear gray tongue; a lesion score of 4 was a completely black tongue; a lesion 5 was observed as oral ulcers and gray tongue and finally a lesion score 6 was seen as oral ulcers and completely black tongue.

No disease was diagnosed and necropsy of hens did not revealed liver or kidney lesions. Feed contamination with trichothecene mycotoxins was suspected. Therefore, feed samples were collected in the farm and sent to the laboratory. The chemical analysis was done for the trichothecene mycotoxins Deoxynivalenol (DON), T-2 toxin, DAS and Nivalenol by gas chromatography with mass spectrometry detection.

Due to the severity of the case, the inclusion of mycotoxin adsorbents was decided. To have a broad spectrum of mycotoxins adsorption the use of an organoaluminosilicate and an aluminosilicate was suggested. At beginning of the 54th week a mixture of both kind of adsorbents was included in the diet of the flock. The dosage of adsorbents was 2.5 kg of the aluminosilicate and 0.5 kg of the organoaluminosilicate per ton of feed. The hens were fed with this diet for the following three weeks and egg production was recorded daily and accumulated on a weekly basis. In order to evaluate the efficacy of the mycotoxin adsorbents to correct the lesions, 100 hens were selected, identified and examined individually every week for oral lesions during the treatment and the score on the evolution of the lesions was recorded. A total score was obtained as the addition of the percentage of affected hens multiplied by the score assigned. It is important to keep in mind that the new diet was prepared using the same grain that was involved at the beginning of the problem.

RESULTS AND DISCUSION

The analysis of feed showed only contamination with 190 ng/g of Nivalenol. The mycotoxins DON, DAS and T-2 toxin were not detected. It was striking to find only Nivalenol, because there are not reports about oral lesions associated with this mycotoxin. Probably, the presence of Nivalenol at this low level indicates the involvement of undetected *Fusarium* toxins in the lesions.

Figure 1 presents the real and the standard egg production starting at the 51st week and finishing at the 56th week where the treatment was suspended. It can be observed that after the egg production reach the lowest level at the 53rd week, it began to increase reaching almost the standard level at the 56th week. The recovery in egg production began after 1 week of starting the treatment with the adsorbents. This leads to believe that the treatment helped solve the problem.

The weekly examination of the hens allowed evaluating the efficacy of the adsorbents to ameliorate the production drop and to improve the condition of the affected hens. Table 1 shows the evolution of the lesions as a percentage of hens affected in accordance with the score established. Also, it presents the total score of each week. From this score it can be observed that there is a continuous reduction of lesions in the animals until the almost complete disappearance of the black tongue and the oral ulcers. This can be correlated with the recovery in egg production.

CONCLUSION

In this case, the contamination of feed with Nivalenol was the only probable cause of the presence of oral lesions and production drop in laying hens. Perhaps other mycotoxins of the *Fusarium* group were present, but not DON, DAS or toxin T-2. Nivalenol has not been sufficiently studied as a sole cause of these symptoms. From the results of this case, the use of adsorbents was an effective aid in solving the problems of loss of productivity in the poultry industry associated with contaminated feed.

REFERENCES

1. Chi, M.S. and C.J. Mirocha. Necrotic oral lesions in chicken fed Diacetoxyscirpenol, T-2 toxin and Crotocin. Poultry Sci. 57: 807-808. 1978.

2. Brake, J., P.B. Hamilton and R.S. Kittrell. Effects on the trichothecene mycotoxin Diacetoxyscirpenol on feed consumption, body weight and oral lesions of broiler breeders. Poultry Sci. 79: 856-863. 2000.

3. Ademoyero A.A. and P.B. Hamilton. Mouth lesions in broiler chickens caused by scirpenol mycotoxins. Poultry Sci. 70: 2082-2089. 1991.

4. USDA. GIPSA, Technical Services Division. "Grain fungal diseases and mycotoxin reference" p 21.

5. Kubena, L.F., R.B. Harvey, W.E. Huff and D.E. Corrier. Efficacy of a Hydrated Sodium Calcium aluminosilicate to reduce the toxicity of Aflatoxin and T-2 toxin. Poultry Sci. 69: 1078-1086. 1990.

6. Kubena, L.F., R.B. Harvey, W.E. Huff, M.H. Elissalde, A.G. Yersin, T.D. Phillips and G.E. Rottinghaus. Efficacy of a Hydrated Sodium Calcium aluminosilicate to reduce the toxicity of Aflatoxin and Diacetoxyscirpenol. Poultry Sci. 72: 51-59. 1993.

7. Lara, J., J. Muñoz, R. Perez, L. Rivera, J. Chapa and E. Rodríguez. Adsorption of Zearalenone and reduction of its toxicity by an organoaluminosilicate. Proc. X International IUPAC Symposium on Mycotoxin and Phycotoxins. Guaruja, Sao Paulo, Brazil. p. 88. 2000.

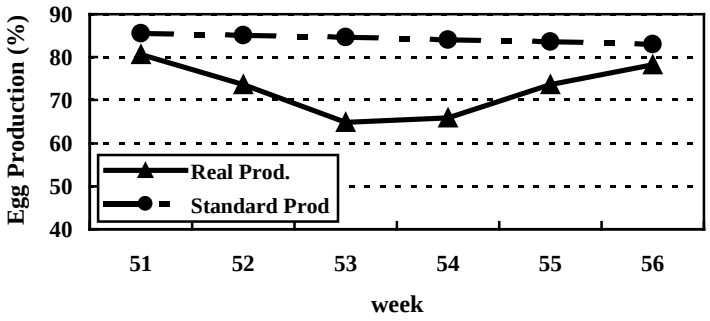


Figure 1. Egg production recorded weekly. The use of the adsorbents started at the beginning of the 54th week.

Table 1. Evolution of the oral lesions at beginning, during and at the end of the treatment with the adsorbents.

	Percentage of affected hens			
	Weeks			
Score	53	54	55	56 (End)
1	4	7	9	10
2	34	29	22	48
3	22	37	43	32
4	13	6	6	3
5	23	21	20	7
6	4	0	0	0
Total Score	329	305	306	249

IDENTIFICATION OF CHICKEN INFECTIOUS ANEMIA VIRUS IN MEXICO, REPRODUCTION OF DISEASE AND SEROLOGY SURVEY IN COMMERCIAL FLOCKS

Nestor Ledesma[A], Tamas Fehervari[A], Ma. Teresa Casaubon[A], Eduardo Lucio[B] and Ferenc Ratz[C]

[A]Department of Animal Production: Poultry, Faculty of Veterinary Medicine and Zootechnique, National Autonomous University of Mexico, Av Universidad 3000 Mexico City, Mexico 04510
[B]Applied Investigation Co. 7 Nte, 416 Tehuacan, Puebla, Mexico 75700
[C]Department of Histopathology and Electonmicroscopy, Central Veterinary Institute, 1149 Budapest XIV Tábornok u. 2 Hungary

SUMMARY

Identification of chicken infectious anemia virus in Mexico, reproduction of disease and serology survey in commercial flocks. Three strains of chicken anemia virus were isolated from chickens originating from broiler and parent stock. Through experimental infection, only one out of the three strains induced low hematocrit values and characteristic lesions for chicken infectious anemia in the thymus and bone marrow of specific pathogen free chicks. The isolates replicated in MDCC-MSB1 cell line. The virus selected as the most pathogenic in experimental infection trials showed resistance to chloroform, heat (75º C for 5 minutes) and passed through a 45 nm filter membrane, but did not pass through the 22 nm filter. These characteristics were similar to the Del Rose reference strain of chicken anemia virus. Thought electron microscopy, the diameter of particles obtained from the pellet of infected cell cultures was between 22 and 27 nm. The serology survey, using a commercial ELISA kit and carried out with samples obtained from different farms all over the country, resulted in widespread seroconversion, indicating that chicken infectious anemia should be considered endemic to Mexico.

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

GENETIC ANALYSIS OF THE HEMAGGLUTININ GENE OF THE AI A VIRUS ISOLATED FROM A SINGLE FARM IN HONG KONG DURING THE 1970'S & 1980'S

Andy O.C. Leung[A], K.F. Shortridge[B], and F. C. Leung[A*]

[A]Department of Zoology, The University of Hong Kong, Hong Kong SAR, China
[B]Department of Microbiology, The University of Hong Kong, Hong Kong SAR, China

ABSTRACT

Southern China has been proposed to be the epicenter for the influenza virus outbreaks because of the close proximity among people and farm animals. Twenty-one samples were collected from a single duck farm during the 1970's and 1980's. The objective is to examine the genetic diversity of the hemagglutinin gene (HA) from these isolates. A set of primers flanking the HA was designed and yield a single PCR product of 1630 bp. PCR products were then sub-cloned and subjected to DNA sequencing for phylogenetic analysis. The results revealed the presence of several lineage and sublineages. Divergence of all the major branches corresponds loosely with the date of the isolation of the virus indicating random mutation of the avian influenza virus and the presence of multiple cocirculating sublineages in the duck farm

*Corresponding author, E-mail: fcleung@hkucc.hku.hk
(A complete paper will be submitted for review and consideration for publication in *Avian Diseases*)

GENETIC CHARACTERIZATION OF THE S1 GENE OF THE CHICKEN IBV IN CHINA

Wu Hongzhuan[A, B], Liu Fuan[B], Xing Caoan[B], Frederick C.Leung[A*]

[A]Department of Zoology, The University of Hong Kong, Hong Kong SAR, China
[B]Department of Veterinary Medicine, South China Agricultural University, 510642, Guangzhou, China

ABSTRACT

Infectious Bronchitis is a highly contagious disease in chickens, and the etiological agent is the Infectious Bronchitis Virus (IBV). One of the possible common failures of vaccination that brings great economic losses to the intensive poultry farming in China may be due to the high mutation rate of the viral genome. In order to reduce the economic losses due to IBV, our aim is to characterize the molecular epidemiology background of IBV in China. In this study, five regional IBV isolates from different provinces in China were isolated and characterized. A pair of primers flanking the whole S1 gene of the IBV was designed according to the published sequence data, and the expected PCR product size is 1760bp. IBVs were propagated in SPF embryonated eggs, and recovered from the allantoic fluid where the embryos died within 48 hrs. RNA was extracted and diagnostic RT-PCR was performed. PCR products with the expected correct size fragment were further confirmed by PCR sequencing. The multiple alignment results demonstrated all IBV strains collected from the 5 regions of China were homologous with strains reported in the Gene bank, including Beaudette strain and Guangdong D41 strain. Differences were also identified among all isolates. Our result indicates that chicken farms at different regions of China should vaccinate their chicken with their respective genotype matched vaccine strains of the particular region.

*Corresponding author, E-mail: fcleung@hkucc.hku.hk
(A complete paper will be submitted for review and consideration for publication in *Avian Diseases*)

QUALITATIVE AND QUANTITATIVE FEATHERING

C.C., López[A], J. Barbi.[B], Arce M.J.[C] and E.G. Avila [A]

[A]Departamento de Producción Animal: Aves, FMVZ, UNAM, 04510 México, DF, Mexico
[B] Novus International de México. Bosque de Ciruelos 194, México 11700 D.F.
[C]INIFAP, Morelia, Michoacán, México.

SUMMARY

A regression analyses was done in fourteen-day old chicks, the dependent variable was the primary remiges and tail coverts feather size and the independent variable was the bird weight there are not a relationship between the length of primary feathers and body weight in 2 weeks-old chicks. The determination coefficient ($p<0.05$) for remiges feather was 10.09%, and for tail coverts 9.12%. However, when length of remiges where the dependent variable, and tail coverts the independent variable, determination coefficient was 41.09%. For that reason, there are more chances that broilers with short feathers will have late feathering even they come from same genetic strain. Feathering was experimentally evaluated using a factorial design with two strains (feather-sexable and non feather-sexable) and the inclusion or not inclusion of two sources of methionine (MHA 88% activity and DL methionine 99% activity). There were significant differences ($p<0.05$) between the two strains, as well as with the treatment with no methionine supplementation, but not between the sources of methionine. A factorial experiment 2 x 3 design (2 source of methionine: MHA and DL-methionine), (3 levels of inclusion: 0.05, 0.10 and 0.20% to the basal diet), with a control treatment and without the addition of methionine. When analyzing the effects of methionine concentration at 21 and 42 days of age, a significant response to the inclusion of methionine in the diets was observed in body weight gain, feed conversion, and feather weight in both periods. In this last day, statistical significance was also present for the feather percentage in relation to bodyweight

RESUMEN

Se realizó un análisis de regresión en pollos de 14 días donde la variable dependiente fue el tamaño de las plumas primarias remeras y las timoneras; y la variable independiente fue el peso de las aves. no hay una relación entre el tamaño de las plumas y el peso, ya que el coeficiente de determinación ($p<0.05$) para las plumas timoneras fue de 10.09% y de las remeras de 9.12%; sin embargo, para el tamaño de las plumas remeras y timoneras como independiente fue de 41.09%. Sin poder encontrar una causante a esto, existen amplias posibilidades de que sean animales con lento emplume a pesar de ser de la misma línea genética y provenir de las mimas reproductoras, Se suplementaron 2 fuentes de metionina a lineas autosexables, no existiendo significancia ($p<0.05$) entre las dos líneas genéticas, así como en el tratamiento que no fue suplementado con la fuente de metionina, pero no entre las fuentes de metionina. En otro estudio factorial 2 x 3 (2 fuentes de metionina: MHA y DL-metionina), (3 niveles de suplementación: 0.05, 0.10 y 0.20% a la dieta basal), con un tratamiento testigo sin la adición de metionina. Al analizar los efectos por concentración de metionina, a los 21 y 42 días de edad. Se observó una respuesta significativa a la inclusión de la concentración de metionina para ganancia de peso corporal, conversión alimenticia y peso de la pluma a los 21 y 42 días de edad (en este último periodo también se presentó significancia estadística para el porcentaje de pluma con respecto al peso corporal.

The most important aspects that have been developed in relation to the conformation, growth, pattern and amount of feathers are: the development of feather-sexable breeds, fast growth rate, higher surface area due to a great muscular development, and efficient feed conversion ratio. Therefore, today's chicken feather weight is proportionally lower than their body weight according with a study done at 49 days of age in a feather-sexable broilers (4.6% in males and 4.1% in females), as compared to birds produced several years ago. Consequently, this results in a "lack of feathers", a condition that is more acute in males. This situation results in problems at the end of the production cycle, and becomes more acute as greater pressure is exerted over carcass quality and as birds are slaughtered at an earlier age.

There are different methodologies for quantitative and qualitative measurements of feathering: In the 70's, a somewhat empirical technique based on feather count taken from a surface area of the litter. Actually, the most common methods involve feather weight alone or related as percentage ofto body weight on wet or dry basis., as well as the third and eighth feather's full length. Others have mentioned more precise aspects, such as tissue nutrient composition based on essential and non-essential amino acids (1). Since feathers are so lightly-weighted, it is likely that a quality problem could not be detected numerically.

Slow feather development, males have a late down molting that comes together with a slow growth of the primary feathers (sickle, axial and covert feathers) and contour feathers. It is common to observe necks of 21-day old males still with down feathers, and naked abdominal lateral posterior regions. At approximately 35 days of age, the situation begins to improve; even some feathers might be found on the litter. At around day 42, chickens are fully feathered. However, first remiges feathers still have an incomplete development at this stage, which may have adverse implications in the plucking process at the slaughterhouse. This is possibly the most commonly observed problem in males, because feather-sexable lines have been widely accepted by poultry producers. It is important to point out to the fact that this is not always recognized as one of the firsts causes triggering different feathering problems in commercial chicken production conditions.

In an study done, feathers were weighted at 21, 35 and 49 days of age showed values for body weight, feather weight and percentage of feathers related to body weight of 572g, 21 g and 3.737% for males; 529 g, 24 g and 4.44% for females at 21 days of age, 1475 g, 69.3 g and 4.70% for males; 1254 g, 68.4g and 5.45% for females at 35 days of age, and 2554 g, 137.1 g and 5.36% for males and 2290 g, 122.9 g and 5.32% for females at 49 days of age.

Remiges growth measurement evaluation may be important in the first week of age to determine bird age, (2) determinated the length of primary wing feather, and the measurement of the third flight feather, a reliable indicator of the age of 4 genetics strains between 1 and 7 days of age. Feather average 9.9 mm at 1 day, 15.6 mm at 4 days, and 27 mm at 7 days of age, with breed variations. The work done in Mexico in feather-sexable broilers showed that there is not a relationship between the length of primary feathers and body weight in 2 weeks-old chicks. The determination coefficient ($p<0.05$) for remiges feather was 10.09%, and for tail coverts 9.12%. However, when length of remiges where the dependent variable, and tail coverts the independent variable, determination coefficient was 41.09%. For that reason, there are more chances that broilers with short feathers will have late feathering even though they come from same genetic strain.

Shape alterations in contour feathering is quite common and usually appears in abdominal and breast areas.where contour feathers loose their banner shape, leaving barbs and barbules separated. Considering bird's body weight, moisture and uric acid will affect the delicate skin without feather protection, which in

turn may become an important cause of economic losses due to downgrading of breast quality.

Dorsal follicular necrosis syndrome. This condition is a slow-feathering problem in broiler chickens characterized by a lack of feathers in the dorsal area has been recurrent, associated to a follicle alteration process caused by gangrenous process at the rachis base. This in turn is followed by a follicle necrosis that leads to an inadequate new feather genesis. No prevention or control measures have been demonstrated. Affected birds kept under observation up to 63 days of age show feather structures that remain incomplete. This condition confers the bird an aspect often called of a "porcupine", not fully covering the skin in this area. This unprotected area turns the skin rough and it is common to see lacerations that can affect the muscle caused by other bird's nails. In consequence, there is an increase in condemnations, and processing is considerably more difficult in the processing plant, as well as the marketing of these birds.

Excess fondling is quite frequent to observe excess fondling behavior (caressing) in breeders and grandparents during growing and development phases. This type of behavior occurs after mid-day or once the feed is finished. However, in broilers it occurs very seldom. Birds dedicate time in excess to caress their feathers. Breast feathers may even appear wet and feathers loose their banner conformation. This condition is also known as "feather licking" or "suckling hens". In extreme cases feather separation may occur. The superior part of the feather´s banner suffers a torsion, giving the impression of a sponged feathering with a rough texture. When this happens, the feather falls more easily.

Effect of total sulfur aminoacids and source of methionine on feathering. Sulfur amino acids play a major role in several metabolic (such as methyl donation) and structural functions (tissue formation such as muscle and feathers). Regular diets include the addition of one source of methionine, although the largest contribution of this amino acid originates in major ingredients (corn, milo, soybean meal, corn gluten meal, meat meal, fish meal, or canola), with near to 80% of the requirement. The remaining 20% is met by the supplemental amino acids. Among the factors that affect methionine and cystine availability are tannin levels (in the case of milo) and inadequate processing (either overprocessing or underprocessing in the case of soybean meal). Availability of methionine hydroxy analog (MHA) (88% activity) and DL methionine (99% activity) has been widely demonstrated. It is difficult to think that this 20% contribution is the cause of the different feathering problems. Feather weight was experimentally evaluated using a factorial design with two strains (feather-sexable and non feather-sexable) and the inclusion or not inclusion of two sources of methionine (MHA 88% activity and DL methionine 99% activity).

Table 1. Percent feathers in two strains with different methionine sources.

	Percent weight of body weight		
Source of methionine	Non feather-sexable	Feather-sexable	Mean
No methionine	4.3	3.9	4.1 a*
DL methionine	4.7	4.1	4.4 b
MHA	4.7	4.2	4.4 b
Mean	4.6 a	4.1 b	

Table 2. Body weight (g), feed conversion (g/g), feather weight (g) and percentage of feather related to body weight at 21 and 42 days of age.

ANALYSIS BY SOURCE								
	21 DAYS			42 DAYS				
	NO MET	DLM	MHA	NO MET	DLM	MHA		
WEIGHT [1/]		616	622		1963 b	1996 a		
CONVERSIÓN [2/]		1.635	1.607		1.976 a	1.948 b		
FEATHER WT. [3/]	30 b	32 a	33 a	107 b	119 a	117 a		
% FEATHER [4/]	4.35 b	4.47 a	4.60 a	4.49 b	4.76 a	4.68 a		
ANALYSIS BY CONCENTRATION OF METHIONINE								
	21 DAYS				42 DAYS			
	METHIONINE ACTIVITY				METHIONINE ACTIVITY			
	0%	0.05%	0.10 %	0.20 %	0%	0.05 %	0.10 %	0.20 %
WEIGHT [5/]	603 b	604 b	618 ab	636 a	1852 c	1946 b	1978 b	2014 a
CONVERSIÓN [6/]	1.65 ab	1.668 a	1.606 bc	1.589 c	2.088 a	1.996 b	1.955 c	1.935 c
FEATHER WT. [7/]	30 b	32 ab	33 a	32 a	107 c	115 b	118 b	122 a
% FEATHER [8/]	4.35	4.49	4.59	4.53	4.50 c	4.62 b	4.69 b	4.84 a

Different subscripts in same row are significant different ($p<0.05$).

These results show significant differences ($p<0.05$) between the two strains, as well as with the treatment with no methionine supplementation, but not between the sources of methionine. An experiment was

conducted with 2,800 broilers from a sexable strain, each one, in a factorial 2 x 3 design (2 source of methionine: MHA and DL-methionine), (3 levels of inclusion: 0.05, 0.10 and 0.20% to the basal diet), with a control treatment and without the addition of methionine.

At 21 days old, the evaluation by methionine source proved significance for the feather weight and feather percentage in relation to body weight, being less in the treatment that was not supplemented with a methionine source. At 42 days of age, the four evaluated variables demonstrated statistic significance ($p<0.05$), obtaining the lowest values by not supplementing the diets with a methionine source.

When analyzing the effects of methionine concentration at 21 and 42 days of age, a significant response to the inclusion of methionine in the diets was divided in 7 treatments with 8 repetitions of 50 chicks observed in body weight gain, feed conversion, and feather weight in both periods. In this last day, statistical significance was also present for the feather percentage in relation to bodyweight (Table 2).

REFERENCES

1. Stilborn H.L., E.T. Moran. Jr., R.M. Gous and M.D. Harrison. Effect of age on feather amino acid content in two broiler strain crosses and sexes. Journal of Applied Poultry Research.. 6:2, 205-209. 1997.

2. Hackl G., and E.F: Kaleta. Methods for the estimation of the age of broilers and effects of water and feed deprivation within the first days of life. Archiv fur Geflugelkunde. 61:6, 280-286. 1997.

NEWCASTLE DISEASE IN MEXICO

R. Cuetos, S. Medina, J.A. Quintana, M. Tamayo

Asociación Nacional de Especialistas en Ciencias Avicolas de México, Ciudad de México

The first outbreaks of Newcastle Disease in the world occurred in Java and Indonesia and in England in the location of Newcastle - Upon Tyne, as reported from Doyle in 1926. Ochi and Hashimoto reported the same disease in 1924 from an outbreak in Korea. During the decades of 1920's and 30's the disease appeared in different countries from Europe, Asia and United States. This was the first panzootic of this viral disease beginning in the Southeast Asia that was spread through commercial trades with England and Continental Europe and then to the world in the following thirty years.

The first outbreak of Newcastle in Mexico was reported in 1946, in a layer facility located in the outskirts of Mexico City. In this outbreak the mortality was 300,000 birds. The identification of this virus was done by Dr. A. Velazquez and Dr. Bankowski. The outbreak coincided with an importation of pullets from California. In those days there was no sanitary control. During the forties, the poultry industry in Mexico was precarious; nevertheless, Newcastle Disease caused severe destruction because there was no biosecurity and vaccines. This helped to disseminate the disease through the whole country. The same thing happened in most of the countries. It was not until the decade of the fifties that it became possible to control the disease with vaccination and good biosecurity. The second panzootic was reported in 1960 and lasted until 1973. In this case the problem was birds (psittacine) that were imported.

This virus as we know, regarding the external envelope, is one of the most resistant, making eradication difficult. It plays an important economic role, difficulting the commerce between countries that have endemic Newcastle Disease. Kaleta & Baldauf demonstrated the presence of this virus in 236 birds from different orders, making this disease difficult to eradicate. Another property of the Newcastle Virus is the stability. It is not possible to mutate. Therefore the lentogenic strains (B1, La Sota) will continue to be nonpathogenic with the same antigenic characteristics.

Newcastle Disease should be considered as one of the most important diseases for the Poultry Industry. In Mexico is compulsory to have adequate vaccination programs along with strict biosecurity measures.

During the past 2 years the world suffered different outbreaks, as the Office International des Epizooties (OIE) mentions. Probably giving place as the fourth panzootic. Some examples are subsequently listed: Australia, April 21, 2000; Austria, March 19, 1999; Belgium, June 4, 1999; Canada, May 21, 1999; Czech Republic, January 22, 1999; USA, January 15, 1999; Netherlands, August 6, 1999; Italy, December 11, 1998; Japan, June 2, 2000; Luxemburg, December 17, 1999; and Mexico, May 2000.

The cause for the outbreak in Mexico was an inadequate vaccine scheme, and also deficient biosecurity measures. According to Mexican needs it's important to have a vaccine with a titer of 10^8 (EID) 50%/ml. When we vaccinate and dilute to a half or a

third it's not possible to have an adequate immune response.

In countries like Mexico, besides having adequate vaccinate schemes and strict biosecurity measures, it is important to have a strict control of game birds, fighting cocks and backyard flocks. The lack of control in these birds can start a new panzootic of Newcastle or any other disease caused by bacteria or virus.

IBD MATERNAL ANTIBODIES AND VACCINATION RESPONSE MEASUREMENT IN BROILER CHICKENS AND PULLETS USING AN ELISA TEST

Jesus Cabriales[A] and Ruben Merino[B]

[A]Boehringer Ingelheim Vetmedica S. A. de C.V.
[B]Poultry Sciences Department, Universidad Nacional Autonoma de Mexico

INTRODUCTION

Infectious Bursal Disease (IBD) is an important immunosuppressive disease of chickens. Most commercial chickens are infected by IBDV early in life and, in unprotected birds, the virus causes serious immunosuppression and mortality. Both broiler chickens and layers are susceptible to the immunosuppressive effect, and thus, predisposes young birds to several bacterial, and viral infections or failure to response to vaccination (3).

Immunization of chickens with vaccines containing either live or inactivated IBDV is the major method used for IBD control. Maternal antibodies level is important in order to know when to start with the vaccination program. Virus neutralization (VN) test is the "Gold Test" in order to detect and quantify neutralizing antibodies IBDV-specific. However, this test is expensive, trained personnel are required, and is time consuming. Currently the ELISA procedure is the most used serological test for IBDV antibodies evaluation in poultry flocks. One "improved" ELISA test has shown a wide cross reaction when detecting antibodies against both classic and variants IBDV strains, besides, this test has also shown a high correlation with VN when measuring antibodies IBDV – specific (4).

This study was carried out in commercial flocks in order to know the maternal antibodies level in young broilers and pullets, as well as the response to their intermediate live vaccination program, using an ELISA test.

MATERIALS AND METHODS

Birds. One Ross broiler (68,000) flock and one Hy-Line W36 pullet (32,000) flock were raised under conventional conditions in commercial farms and were vaccinated with an intermediate IBDV vaccine originated in chicken embryo ([*]Volvac® IBD MLV Inter Lukert CEO).

Vaccination programs. Broilers were vaccinated at 6 and 16 days old by drinking water; pullets at 6, 16 and 26 days old by drinking water.

Serum sampling. Fifteen birds from each farm were bled at different ages before and after vaccination as follows: Broilers were bled at 3, 17, 24, 38 and 52 days old; pullets at 3, 17, 25, 39, 52, and 66 days old. Serum samples from individual birds were collected and stored at 4°C until analyzed by ELISA test.

Determination of anti-IBDV antibodies titers in serum by ELISA. One commercial ELISA kit was used (IBD+ PROFlock PLUS[‡]). This kit has microtiter plates coated with a bursal origin "native" IBDV strain, In brief. ELISA test was carried out as described: after diluting serum samples 1:50, in a non-coated microplate, 50µl from each sample were transferred to the ELISA plate, where 50µl of dilution buffer were previously added to each microwell (final dilution 1:100). After 30minutes of incubation and a washing process, 100µl of conjugate (goat anti chicken IgG conjugated with Horseradish Peroxidase) were add to each microwell. After 30 minutes of incubation and another washing process, 100µ of cromogen/substrate (ABTS) were added to each microwell. After 15 minutes 100µl of stop solution were added to stop color development. With this system, the developed color intensity is directly related with the antibodies level in the serum sample. All ELISA plates were read at 405 nm with a Dynatech MR650 ELISA reader. Optical density (OD) values were converted in ELISA

[*] Boehringer Ingelheim Vetmedica S.A. de C.V.
[‡] Synbiotics Corporation, San Diego CA, USA.

titers, using the software ProFile for Windows® (Synbiotics Corporation).

RESULTS

Figure 1 shows maternal antibodies catabolism and serologic response to IBDV vaccination in broiler chickens measured by ELISA test. Catabolism of Maternal antibodies starts with 7536 Geometric Mean Titer at 3 days old. At 17, 24, 38 and 52 days old, GMT ELISA titers were 161, 56, 7560 and 10352 respectively. Coefficient of variation expressed as percentage, were 39.25, 72.72, 50.45, 18.2 and 5.12 at 3, 17, 24, 38 and 52 days old.

Serologic results in serum samples from pullets are shown in figure 2. ELISA GMT values were 9106, 8050, 4619, 1398, 8790 and 10871 at 3, 17, 25, 39, 52 and 66 day old. Coefficient of variation expressed as percentage, were 12.18, 11.46, 22.38, 65.22, 7.64 and 4.20 at 3, 17, 25, 39, 52 and 66 day old.

DISCUSSION

Monitoring is used to evaluate responses to vaccination, helps in determining the best age for vaccination, predicts levels of maternal immunity in progeny, and detects exposure to virulent IBD viruses (2). In this study, serum samples from broiler and pullets were evaluated with an ELISA test, because of its sensitivity, specificity, and practicality for large scale use. Results from this study showed ELISA maternal antibodies level of 7536 and 9106 (broilers and pullets, respectively) at 3 days old. With conventional ELISA kits, as a rule of thumb, 50 – 60% of the GMT present in a breeder flock can be expected in their progeny. However, there is evidence that using an ELISA kit coated with a native bursal originated IBDV as antigen, maternal immunity transference to the progeny could be as high as 90% (1).

Broiler chickens in this study were vaccinated twice, the first vaccine application was at 6 days old, when antibodies level was 7536 and 39.25 % CV; so it could be expected that this first vaccine did not produce a serologic response, because of the high titer. However, CV indicates that there were chickens with high and low antibodies level. In this case the vaccination program works well, due chickens could be susceptible to an IBDV early infection. Maternal antibodies last for 24 days (56 GMT and 50.45 % CV).

Second vaccination was at 16 days old, while ELISA titer was 161 and 72.72 % CV at 17 days old. Response to live vaccination program was 7536 (18.2 % CV) and 10352 (5.12% CV) at 38 and 52 days old, respectively. These ELISA titers are high and CV is considered excellent, so chickens from this flock are well protected against any IBDV outbreak.

Pullets in this study were vaccinated three times, first vaccination was at 6 days old, when antibodies GMT was 9106 and 12.8 % CV; second vaccination was at 16 days old; - antibodies GMT 8050 and 11.46 % CV at 17 days old-. When antibody level was high, it seems that these two vaccinations did not produce a serological response. Third vaccination was at 26 days old with antibodies GMT 4619 and 22.38 % CV at 25 days old. At 39 days old the lowest ELISA titer was found (1398 and 65.22 % CV), it could be the early response to third vaccination. At 52 and 66 days old ELISA titers were 8790 (7.64 % CV) and 10871 (4.2% CV), respectively. These high titers and low CV's indicate a very well immune response to immunization with an intermediate IBDV vaccine.

It is evident that maternal immunity protects birds against early IBDV infections. Therefore, to know this maternal antibodies level is crucial in order to establish the best age for the first vaccination. If vaccine is applied too early, it could be neutralized by high maternal antibodies. If vaccination is too late, birds could be susceptible to IBDV in the field.

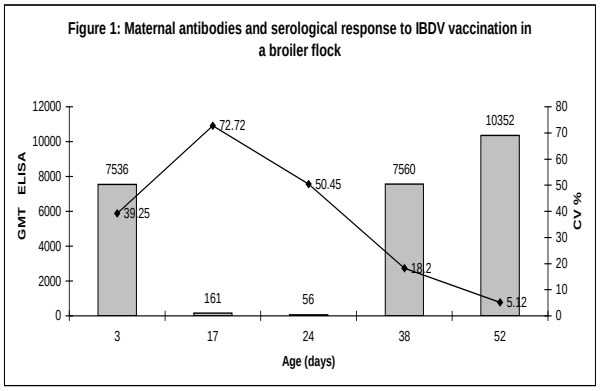
REFERENCES

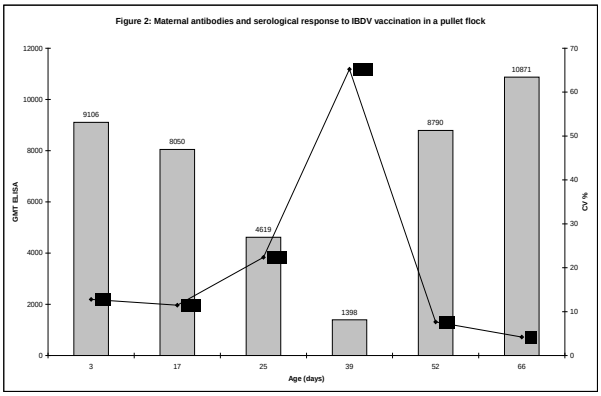
1. Cosenza H. AgroBioTek Laboratorios. Comayagüela, M.D.C., Honduras.

2. Jackwood D.J., Sommer S.E. and Odor E. Correlation of Enzyme - Linked Immunosorbent Assay titers with protection against Infectious Bursal Disease Virus. Avian Dis 43:189-97. 1999.

3. Lukert P.D. and Saif Y.M: Infectious Bursal Disease Virus. In: Calnek B.W., Barnes H.J., Beard C.W., McDougal L.R., Saif Y.M., editors. Diseases of poultry. Ames: Iowa State University Press pp 721-738. 1997.

4. Lamichhane C.M., Kirkeegard L. Jr., Collins W. ELISA provides better detection of antibodies to Infectious Bursal Disease Virus (IBDV). In: Proceedings of the XI International Congress of the World's Avian Veterinary Association. Budapest, Hungary. August 18-22. p 41. 1997.





EXPERIMENTAL USE OF SPECIFIC IMMUNOGLOBULINS AGAINST INFECTIOUS BURSAL DISEASE IN ONE DAY OLD CHICKS

A.Morales G, M. Blancas Lopez Y E. Lucio Decanini

Investigación Aplicada S.A.DE C.V., 7 Norte 416 Tehuacan, Pue. Mexico 75700

INTRODUCTION

Infectious Bursal Disease (IBD) is an acute, highly contagious viral infection of young chickens. Also IBD causes immunosuppression when chickens of three weeks of age or less are infected. IBD was reported firstly in USA in 60's years by Cosgrove (1). From its appearance several efforts have been made to control the disease. One of these was the use of live virus vaccines which were shown to be highly pathogenic. Mildly strains of Infectious Bursal Disease Virus (IBDV) were development as an alternative but they caused mild lesions on Bursa of Fabricius (2). The protection of specific antibodies against lesions in Bursa and immunosuppression by IBDV was an event that modified the concept on vaccination. The use of vaccines killed virus to increase antibodies in breeders and their progeny was quickly extended.

There are several factors that have obstructed a good protection through maternal antibodies because farmers recieved chicks from different breeders and, of course, different quantity and quality of antibodies.

The objective of this paper was evaluate the role of specific immunoglobulins against IBDV from egg yolk from hyperimmunized hens administered with Marek's Disease vaccine in chicks at one day of age and their protection against challenge IBDV strain at 7,14, 21, and 28 days postchallenge.

MATERIALS AND METHODS

Virus. Edgar and 73688 Strains IBDV were propagated in 6 week old SPF birds. A 20 % Bursal tissue homogenate was prepared with each strain and inactivated with 0.1 % Formol during 24 hr at room temperature.

Vaccine. An oil emulsion killed vaccine containing Edgar and 73688 was elaborated. A 60/40 oil-water ratio was used.

Birds. Eight 4 weeks old layer hens (Babcock B300) were vaccinated with a dose of vaccine (0.5 ml) subcutaneously in the neck. A booster vaccination was at 8, 12 and 16 week of age with same amount of vaccine and route of application. All birds were maintained in wire cage for the entire experiment. Food and water were administered *ad libitum*. Every week all birds were bled by wing vein, and antibodies to IBDV by microvirusneutralization test were quantified.

Immunoglobulins. After layer hens began to drop eggs were collected and immunoglobulin extracted by the method described by Jensenius (1). Fifteen ml of diluent to Marek's Disease vaccine were removed and 15 ml of sterile immunoglobulins of IBDV (Igs IBDV) were added, mixed, and this diluent was used to reconstituted Marek's Disease vaccine HVT strain. Also, the pH of diluent was measured with/without Igs IBDV and its effect on Units Forming Plate of Marek's Disease vaccine by methods already described.

Trial. One hundred twenty one day old broiler chicks were divided in three groups of 40 chicks,

identified as A, B and C. Each group was housed in a Horsfall-Bauer isolator unit under negative pressure. Group A received a dose of 0.2 ml of Marek's Disease vaccine with Igs IBD, Group B received a dose of 0.2 ml of Marek's Disease vaccine alone, and Group C was control Group. At 7, 14, 21 and 28 days post vaccination ten birds from each group were challenged with $10^{3.0}$ TCID by ocular route. After 5 days post challenge all birds were sacrificed and necropsied to find typical lesions of IBD, and Bursal Index was determined

Bursal Index. It was determined by following formula bursa weight/ body weight x 1000

RESULTS AND DISCUSSION

Specific immunoglobulins were obtained from egg yolk from hyperimmunized hens with a titer of at least 1:10240 per 0.05 ml. There was not adverse effect on UFP and pH of Marek's Disease vaccine when they were added to diluent.

Table 1 shows geometric mean titers for IBV measured by microneutralization test in chicks with and without Igs IBDV at 1,7,14, 21 and 28 days of age. As it can be seen Ig treated group shown 3 log 2 higher titers than control group after 4 weeks of treatment.

Table 2 shows Bursal Index (BI) in both groups challenged at 1, 2, 3 and 4 weeks post vaccination. Treated group showed a better BI than untreated group until 21 days, but at 28 days there was a better response in untreated group than treated group.

The results obtained here agree with other reported by Lucio, *et al*. in 1996. (2). They found a good protection with immunoglobulins against IBDV administered alone in chicks type SPF for 1-2 weeks. In this paper we found a good protection until 21 days post treatment measured by BI.

We think that immunoglobulins administered with Marek's Disease vaccine could be a good alternative to protect against secondary reactions of IBDV.

REFERENCES

1. Jensenius, Chrjens. Eggs conveniently packaged antibodies. Methods for purification of yolk Ig G. J. Immunol. Methods. 46: 63-68. 1981.

2. Lucio, D.E., A. Morales, A.Toscano, and J.Chapa. Evaluación de la protección conferida por inmunoglobulinas específicas contra el virus de la infección de la bolsa de Fabricio. Memorias de la XXI convención anual ANECA. Cancún.Q.R.México. Pp 178-182. 1996.

3. Lukert, P.D. and Y.M.Saif. Infectious Bursal Disease. In: Disease of Poultry. Fourth Ed. Calnek Eds. Iowa University Press. Pp 721- 738. 1997.

Table 1. Serological response and Bursal Index in chicks vaccinated at one day old with Marek's disease vaccine containing specific immunoglobulins against IBDV after challenge with pathogenic strain of IBDV.

	Treated Group		Untreated Group	
D.P.V. *	G.M. Titer **	Bursal Index	G.M. Titer **	Bursal Index
1	15.9	-----	15.9	----
7	16.3	2.00	14.7	1.76
14	13.0	1.06	10.7	1.06
21	12.4	1.04	8.5	1.00
28	11.4	0.88	8.4	1.02

*. D.P.V. = Days Post Vaccination

**. G.M. Titer = Geometrical Mean 2 log Titer measured by Microneutralization test.

EFFECT OF PROPHYLACTIC ADMINISTRATION OF DEFINED PROBIOTIC ON MORTALITY AND HORIZONTAL TRANSMISSION BY SALMONELLA GALLINARUM IN BROILER CHICKS

Nava M. G.[A], D.J. Nisbet[B], V. K. Lowry[C], and I.G. Tellez[A]

[A]Poultry Science Department, Faculty of Veterinary Medicine and Zootecnia, National Autonomous University from Mexico

[B]United States Department of Agriculture, Agricultural Research Service, Food Animal Protection Research Laboratory

[C]Department of Veterinary Anatomy and Public Health, Texas A& M University

SUMMARY

One day old chicks are easy to be infected because they do not have a mature normal intestinal microflora (NIM) which confers protection against enteric pathogens. "Competitive exclusion"(CE) is one successful method for controlling salmonellae in poultry. Two experiments were carried out to show the prophylactic administration of defined probiotic (defined competitive exclusion product = Premmt^{TM}) effect to reduce mortality and horizontal transmission by *Salmonella gallinarum* (Sg) in broiler chicks. **Experiment I**. 160 one day old chicks were randomly assigned in two groups of 20 chicks each. Control group (A) received orally 0.25 ml of physiologic saline solution (PSS), and group B received orally 0.25 ml of defined probiotic (DP). Both groups were 250,000 UFC of Sg/0.25 ml challenged orally at 3 days old. Mortality percentage was 73.7 and 7.5 in control and DP treated groups, respectively. The prophylactic administration of DP reduces significantly ($P< 0.001$) the mortality in treated (DP) chicks. **Experiment II**. Two hundred forty one day old chicks were randomly assigned in three groups, 20 chicks each. Group C- were challenged with 250,000 UFC of Sg/0.25 ml orally, group D- treated orally with 0.25 ml of PSS, group E- treated orally with 0.25 ml of DP. Both experiments had four replicates. The survivors chicks were killed at 13 days old, in order to identify Sg in organs. The prophylactic administration of DP reduced significantly horizontal transmission of Sg ($P<0.005$) in group E when is compared with groups C and D. The percentage of mortality for groups C, D and E was 80.0, 53.7, and 8.7 respectively. In addition to horizontal transmission of Sg in liver and spleen, in group E was significantly decreased in comparison with group D. The percent of Sg positive isolations from organs in groups C, D and E was 86.2, 83.7, and 35.0 respectively. Prophylactic administration effect of defined NIM (probiotic) in one day old chicks reduces significantly both mortality and horizontal transmission.

In young birds low levels of *salmonellae* could colonize the intestinal tract because they don't have a mature normal intestinal microflora (NIM) that confers protection against enteric pathogens. At the moment, we known that natural resistance is develop with the age, it is related with the establishment of a NIM mature in the intestinal tract of the bird. The complex intestinal microflora is completely developed in the small intestine in the first two weeks of life, but in the cecal tissue it takes more of four weeks. As well, the cecum is the principal site of salmonella colonization (1,2). The slow maturation of NIM has been attributed to sanitary production conditions and substitution of the natural incubation for artificial incubation. In natural conditions, embryos have a sterile gastrointestinal system, but in the moment of hatch it is exposed to intestinal microorganisms from hen by means of the grounds in the shell and in the nest. However, under commercial production conditions with the biosecurity degree in hatcheries the chick has little opportunity to obtain his NIM quickly (3). One of the most successful and promising methods for the control of *Salmonella* infection in the poultry industry is the "Nurmi concept" or "competitive exclusion" (CE). This concept recognized the importance of an early establishment of NIM in young chicks (4). The mechanism of NIM or probiotics in preventing *Salmonella* infection is unknown, but three of the most important mechanisms are the occupation of specific sites that take up *Salmonella* in intestinal mucous, competition for nutrients, and volatile fatty acid production (4). The objective of this work was to show the effects of defined probiotic on mortality and horizontal transmission by *Salmonella* in broiler chicks challenged with *Salmonella gallinarum*.

MATERIALS AND METHODS

Salmonella gallinarum. The bacteria for the challenge was a primary isolation of *Salmonella gallinarum*, resistant to novobiocin (No) and nalidixic acid (AN), obtain from Poultry Science Department FMVZ-UNAM. The inoculum was prepared in Trypticase soy broth during 18 h of incubation. The concentration of CFU of salmonellae was determined by spectrophotometry in order to achieve a concentration of 10^6 CFU/1.0 ml. The concentration of viable cells of *Salmonella gallinarum* was confirmed in brilliant green agar (BGA) plates.

Probiotic. One package of 2000 doses of defined probiotic (defined competitive exclusion product = Premmt^{TM}) was prepared following the maker's instructions. The probiotic was administrated to the chickens 45 minutes later of their preparation.

Experimental design. Two experiments were designed in order to show the prophylactic administration effect of defined probiotic to reduce mortality and horizontal transmission by *Salmonella gallinarum* in broiler chicks.

Experiment I. One hundred sixty chicks one day old were randomly assigned in two groups of 20 chicks each. Control group (A) received orally 0.25 ml of physiologic saline solution (PSS), and group B received orally 0.25 ml of defined probiotic (DP). Both groups were challenged orally at 3 days old, with 250,000 UFC of Sg/0.25 ml.

Experiment II. Two hundred chicks one day old were randomly assigned in three groups, 20 chicks each. Group C- this group were challenged orally with 250,000 UFC of Sg/0.25 ml, group D- only were

treated orally with 0.25 ml of PSS, group E- this group were treated orally with 0.25 ml of DP. Both experiments had four replicates. In order to identify *Salmonella gallinarum* in organs, surviving chicks were killed at 13 days old.

Statistical analysis. To evaluate the differences between percentages groups of positive isolations of *Salmonella gallinarum* and the mortality a Chi-squared test was used.

RESULTS

Experiment I. Mortality percentage was 73.7 and 7.5 in control and DP treated groups, respectively. The prophylactic administration of DP reduces significantly ($P< 0.001$) the mortality in treated chicks, compared with control group.

Experiment II. Mortality was reduced significantly ($P< 0.005$) in the E group (DP) when it was compared with the C group (positive group) and with the D group (negative group). The percent mortality for the groups C, D, and E was: 80.0, 53.7, and 8.7 respectively (Table 1). The horizontal transmission of *Salmonella gallinarum* in liver and spleen of group E was reduced significantly when compared with group D. The percentage of positive isolations to *Salmonella gallinarum* of liver and spleen for the groups C, D and E was 86.2, 83.7, and 35.0 respectively. The number of isolations of *Salmonella gallinarum* in the liver and spleen of the D group was not significantly different when it was compared with the group C (Table 1).

DISCUSSION

The animal and their NIM form a very complex ecosystem, in which many mechanisms affect the composition of intestinal microbiota. The NIM affects the colonization of other bacteria for direct competition of nutrients (nutrients and growth factors); competition for specific receptors in common (points of adhesion); for production of germicides substances (colicins, hydrogen peroxide, bacteriocins, ammonium, enzymes), bacteriophages, antibiotics and development of a restrictive atmosphere (increment in volatile fatty acids production, mainly propionic acid; lactic acid, sulfihidric acid, change in pH) (4,5,6,7,8) In this work we demonstrated that prophylactic administration of defined probiotic (defined NIM), from old chickens to one old day chicks, reduced significantly the mortality in the groups treated with DP (groups B and E). This suggests that DP colonized the intestinal tract of the chicks and inhibited the colonization by *Salmonella gallinarum*. Also, we can see this protective effect when DP administration reduced horizontal transmission of *Salmonella gallinarum* in the groups treated (group E), comparing them with the group D not challenged and not treated with DP. This result coincides with previously work performed by other investigators, in which they showed the effect of several probiotics to reduce the level of colonization and invasion by *Salmonella* spp (4,9,10,11,12,13). On the other hand, Weinack *et al.* showed that administration of CE products in 3 to 9 old days chickens infected with *Salmonella* spp could recover more easily from infection than the chickens not treated with EC products. In the same work, Weinack *et al*. showed that the litter where the chickens are raised is a good vehicle to transmit the NIM to other birds. In field studies Snoeyenbos *et al.* demonstrated that groups treated with EC products at 11 days after challenge with salmonella, the chickens showed a more rapid elimination of the infection than chickens not treated with EC, but the therapeutic mechanism of this phenomenon of reduced infection is unknown to the authors (14). In the present work we did not see this phenomenon, possibly because of the short duration of both experiments.

The level of protection that confers the NIM contained in the DP utilized in this work should be big due to the fact that in the "rechallenge" (horizontal transmission of *Salmonella gallinarum*), the quantity of CFU could be the same or greater than the original challenge dose. Pivnick and Nurmi mentioned that a known challenge dose introduced in chickens, coupled with continuous "rechallenge" of flockmates not infected directly with the challenge dose but exposed to infected chickens, could generate a “rechallenge" dose that could be greater in chickens not infected directly. If the bird received 10^3 cells, it will excrete 10^7 to 10^9 cells per gram of feces at 1 or 2 weeks post challenge. Of course, this will depend on many other factors, like the avian resistance, the challenge age, bacterial characteristics, and another like Duchet-Suchaux *et al.* described (15).

The prophylactic administration of defined probiotic in one day old chicks is capable of reducing the horizontal transmission of *Salmonella gallinarum*. This means that we could apply defined probiotics in the newborn chicks before arrival at the farm, and although they live together with infected birds, infection will be repelled.

REFERENCES

1. Cox NA, Bailey JS, Mauldin JM, Blankenship. Presence and impact of Salmonella contamination in comercial broiler hatcheries. Poult Sci 69:1606-1609. 1990.

2. Goren E, Dejong WA, Doornenbal P, Bolder NM, Mulder AW, Jansen A. Reduction of salmonella infection of broilers by spray application of intestinal

microflora; a longitudinal study. Vet Quarterly 10:249-255. 1988.

3. Leitner G, Heller DE. Colonization of Escherichia coli in young turkeys and chickens. Avian Dis 36:211-220.1992.

4. Stavric S. Microbial colonization control of chicken intestine using defined cultures. Food Tech 41:93-98. 1987.

5. Stanton TB, Savage DC. Motility as a factor in bowel colonization by Roseburia cecicola, an obligately anaerobic bacterium from the mouse caecum. J Gen Microbiol 130:173. 1984.

6. Nurmi E, Rantala M. New aspects of Salmonella infection in broiler production. Nature 241:210-211. 1973.

7. Barnes EM, Impery CS, Cooper MD. Manipulation of the crop and intestinal flora of the newly hatched chick. Clin Nutr 33:2426-2433. 1980.

8. Snoeyenbos GH, Soerjadi SA, Weinak OM. Gastrointestinal colonization by Salmonella and phatogenic Escherichia coli in monoxenic and haloxenic chicks and poults. Avian Dis 26:266-275. 1982.

9. Corrier E, Nisbet JD, Scalan MC, Téllez GI, Hargis BM, Deloanch R. Resistence against Salmonella enteritidis cecal colonization in leghorn chicks by vent lip application of cecal culture. Poult Sci 73:648-652. 1994.

10. Nisbet JD, Corrier DE, Scalan MC, Hollister GA, Beier RC, DeLoach RJ. Effect of dietary lactose and cell concentration on the ability of a continuos-flow derived bacterial culture to control Salmonella cecal colonization in broiler chickens. Poult Sci 73:56-62. 1994.

11. Nisbet JD, Corrier DE, Scalan MC, Hollister GA, Beier RC, DeLoach RJ. Effect of defined continuos-flow derived bacterial culture and dietary on Salmonella typhimurium colonization in broiler chickens. Avian Dis 37:1017-1025. 1993.

12. Corrier DE, Hollister AG, Nisbet DJ, Scanlan CM, Beier RC, DeLoanch JR. Competitive exclusion of Salmonella enteritidis in leghorn chicks: comparison of treatment by crop gavage, drinking water, spray, or lyophilized alginate beads. Avian Dis 38:297-303. 1994.

13. Primm ND, Kristen V, Wykle L, Hofacre CL. Application of normal avian gut flora by prolonged aerosolization onto turkey hatching egss naturally exposed to salmonella. Avian Dis 41:455-460. 1997.

14. Weinack OM, Snoeyenbos GH, Soerjadi-Liem AS, Smyser CF. Therapeutic trials with native intestinal microflora for Salmonella typhymurium infectionsin chickens. Avian Dis 29:1230-1234. 1985.

15. Duchet-Suchaux M, Mompart F, Berthelot F, Beaumont C, Léchopier P, Pardon P. Differences in frequency, level, and duration of cecal carriage between four outbred chicken lines infected orally with Salmonella enteritidis. Avian Dis 41:559-567. 1997.

Table 1. Effect of defined probiotic on mortality and invasion to liver and spleen of chicks in the horizontal transmission by *Salmonella gallinarum*.

Group	**chicks dead /total (%)**	
A B	14.7/20 (73.7)^{A} 1.5/20 (7.5)^{B}	
		Liver and spleen positives to Salmonella gallinarum/total (%)
C	16.0/20 (80.0)a	17.2/20 (86.2)a
D	10.7/20 (53.7)a	16.7/20 (83.7)a
E	1.7/20 (8.7)b	7.0/20 (35.0)b

* Column with different letters (A and B) are significantly different ($p<0.001$) and column with different letters (a and b) are significantly different ($p<0.005$).
** Means of the four replicates.

A- group challenge with *Salmonella gallinarum*, not treated defined probiotic, B- group challenge with *Salmonella gallinarum*, treated with defined probiotic, C- challenge with *Salmonella gallinarum*, D- group not challenge with *Salmonella gallinarum*, not treated with defined probiotic, E- group not challenge *Salmonella gallinarum*, treated with defined probiotic.

HISTOLOGIC EVALUATION OF LYMPHOID AND HEMATOPOIETIC ORGANS IN CHICKEN EMBRYOS INOCULATED WITH HERPESVIRUS AND AN EXTRACT OF AVIAN LYMPHOKINES

Mireya Ortiz[A], V M Petrone[A], T Fehérvári[A], G Téllez[A]

[A]Department of Animal Production: Poultry. Facultad de Medicina, Veterinaria y Zootecnia. Universidad Nacional Autónoma de México, petrone@servidor.unam.mx

ABSTRACT

The development of the immune system in the chicken embryo has been studied from several points of view; however, the effect of a viral antigen applied during embryonic development is undocumented. The aim of the present work was to evaluate the presence and migration of cells, which contribute to the immune response in different stages of embryonic development and day-old chicks. The development of lymphocytes present in the bursa of Fabricius and thymus was evaluated using the histological analysis of the yolk sac, bursa of Fabricius, thymus, liver and bone marrow of 100 chicken embryos free of specific pathogens divided into 5 groups: Group I) Bivalent vaccine of Marek's disease with viral antigen, Group II) Bivalent vaccine of Marek's disease plus lymphokines, Group III) Lymphokines, Group IV) Vaccine diluent and Group V) Negative control. Samples were taken on days 14, 17 and 20 of embryonic development and day-old chicks. An increase in lymphoid follicle development was observed in the bursa of Fabricius of embryos inoculated with lymphokines (Group III) compared to embryos inoculated with bivalent vaccine plus lymphokines (Group II) and to embryos inoculated only with the bivalent vaccine (group I) ($p<0.05$). In addition, a greater amount of granulocytes was found in yolk sac, liver, and bone marrow of embryos inoculated with lymphokines (Group III) than in embryos inoculated with the bivalent vaccine plus lymphokines (Group II) and in those inoculated only with the bivalent vaccine (Group I) ($p<0.05$). Results of the present study suggest that the application of antigens or protein molecules at an early stage, such as on day 10 of embryonic development, increases granulopoiesis in lymphoid organs such as the liver and yolk sac, and also increases the number of lymphoid follicles in bursa of Fabricius.

INTRODUCTION

The aim of the present work was to evaluate cell presence and migration which contribute to the immune response at various stages of embryonic development and in day-old. Histological analysis of embryonic tissues treated with SE-ILK and viral antigens were used to determinate cell types and the number of cells present in the tissues we examined.

MATERIAL AND METHOD

Experimental embryos. One hundred specific pathogen-free embryos of 10 days were used (Alpes®, Tehuacán, Puebla, Mexico).

Bivalent vaccine. The bivalent vaccine (BV) consisted of strains of HVT and SBI, the commercial vaccine used to evade Marek's disease (MD), with titers of 10^3 plate forming units (PFU)/dose respectively, of 200 µl each (INTERVET® Toluca, Edo. de México, MEXICO).

Vaccine diluent. The vaccine diluent was the same as used to dilute the bivalent vaccine by INTERVET®.

Lymphokines. These were raised in birds immunized against *Salmonella enteritis* phagotype 13 (SE-ILK), and prepared as described in Téllez *et al*. (1).

Treatment design. Embryos were randomly divided into 5 groups of 20 embryos each and inoculated via yolk sac on day 10 of incubation, as follows: **Group I**. Embryos inoculated with a complete dose of 200µl of BV-MD. **Group II**. Embryos inoculated with a complete dose of 200µl of BV-MD plus 100µl of SE-ILK. **Group III**. Embryos inoculated with 100µl of SE-ILK. **Group IV**. Embryos inoculated with 200µl of vaccine diluent. **Group V**. Embryos not inoculated.

Sampling schedule. Four samples were taken on days 14, 17 and 20 of embryonic development and 1 day after hatch of yolk sac, liver, BF and thymus.

Samples. Embryos were euthanized as described by Andrews *et al.* (2). Subsequently they were fixed with pins to a paraffin plate, and the necropsy was performed with a magnifying glass to remove the needed organs.

Histology. The right thymus chain was taken, a transversal section of 2mm width of the middle part of

the left liver lobule, and an area of 1 cm^2 of the distal pole of the yolk sac, 5 mm of the femoral bone marrow and the complete BF. The samples were fixed in 10% formalin, buffered to pH 7.4. Once fixed, the organs were processed with the routine technique for paraffin sections and stained with hematoxilin and eosin (3).

Histological evaluation. Yolk sac. Ten perivascular areas were selected and transversally sectioned. Cells were counted to obtain the proportion of granulocytes in relation to the rest of the hematopoietic cells. **Liver**. A transversal section of 2mm width of the middle part of the left liver lobule was used to count perivascular areas to obtain the proportion of granulocytes. **Bursa of Fabricius**. A transversal section of the middle part of each BF was taken to count lymphoid follicles per section of each BF. **Thymus**. A longitudinal section of the right thymus chain was made to obtain the proportion of the cortex and medullar width. The proportion was expressed as the percentage of the thymus medulla considering the sum in mm of the cortex and medullar width as 100%. **Bone marrow**. Ten microscopic fields were selected to count and obtain the proportion of granulocytes with respect to the rest of hematopoietic cells. This sample was only taken in day-old chicks instead of the yolk sac, which was in the process of involution at this point in development.

Titration of the bivalent vaccine. A sample of the BV-MD equivalent to a complete dose of 200µl was taken aseptically and processed for titration of avian herpesvirus HVT and SB2, according to the method by Palya (4).

Bacteriologic control. Liver, thymus, yolk sac, bone marrow and BF samples were taken and processed for bacterial isolation with routine bacteriological methods (5) to prove the absence of aerobic bacteria in embryos during the experiment.

Statistical Analysis Data were transformed by the arcsinus of the square root (6) for statistical analysis of percentage of granulocytes in bone marrow, in the proportion of thymus cortex and medulla, and in the perivascular areas of the yolk sac. Analysis of variance was applied to transformed data and the difference between means was evaluated by means of Tukey's test (7, 8). The Kruskall-Wallis test was applied for comparison of medians of the spaces of the portal region or areas peripheral to the central lobular veins of the liver with granulocytes, and the amount of lymphoid follicles in the BF. Comparison of all groups and differences between groups were analyzed with the Mann-Whitney U test (7, 8). The level of significance was $p<0.05$.

RESULTS

Bivalent vaccine titration. The presence of Marek viruses (HVT+SB1) was confirmed by titration in chicken embryo fibroblast culture, at a concentration of 103 PFU/200µl in a sample taken to inoculate embryos. The samples of lymphokines and vaccine diluent were free of Marek virus as confirmed by isolation.

Granulocyte quantification. The four samples taken for morphological evaluation (on days 14, 17 and 20 of embryonic development and from day-old chicks) showed a significant increase ($p<0.05$) in the mean percentage of granulocytes in liver and yolk sac of embryos inoculated with SE-ILK (Group III), followed by the group inoculated with the bivalent vaccine plus SE-ILK (group II) (Table 1, Figure 1 and 2). In the Group of embryos which received viral antigen (Group I) a significant increase was observed in the number of granulocytes present in yolk sac on days 14 and 17 of embryonic development ($p<0.05$). No cellular differences were found in liver in any evaluation ($p>0.05$). Embryos of groups inoculated with vaccine diluent (Group IV) and the negative control (Group V) always exhibited a lower number of granulocytes in the yolk sac and liver ($p>0.05$).

Quantification of lymphoid follicles. Of the four samples taken for morphologic evaluation (on days 14, 17 and 20 and from one-day chicks) the median of lymphoid follicles was greater in embryos inoculated with SE-ILK (Group III) ($p<0.05$). The treatment of viral agent (Group I) and of viral agent plus SE-ILK (Group II) increased ($p<0.5$) the amount of follicles. This increase was observed from day 10 of inoculation until day 14 of embryonic development. After day 17 of embryonic development no significant difference was found between the groups mentioned above and embryos inoculated with vaccine diluent (Group IV) and negative controls (Group V). The amount of follicles increased from day 11 of embryonic development in all groups.

Quantification of thymus medulla percentage. The thymus showed the least reaction to the different treatments. In the thymus samples of days 14, 17 and 20 of embryonic development and one-day chicks no significant difference between the proportion of medulla and cortex of all inoculated groups ($p>0.05$) (Group I to Group V). However, in the group inoculated with SE-ILK (Group III), the proportion of medulla was numerically the greatest of all groups.

Bacteriological control. The result of organs subjected to bacteriological analysis was negative, showing that no bacterial contamination occurred during the study.

DISCUSSION

Results show that all treatments had an effect on one or more of the parameters measured in the experiment. The effects were dramatic in the SE-ILK group (Group III) and the less evident in the vaccine diluent group (Group IV). The present investigation showed that the treatment increased the number of granulocytes significantly compared with untreated embryos along the investigation. This effect correlated with age, i.e., granulocyte number increased with embryonic development although treatments were applied at early stages. The increase in granulocytes was detected both in yolk sac and in liver. This result confirms that granulopoiesis may be stimulated at early stages by the presence of lymphokines and by viral antigen (BV-MD) or both. However, the greatest stimulating effect of SE-ILK occurred in liver and yolk sac, while in bone marrow, the group that received viral antigen plus SE-ILK (Group II) granulocytes were numerically more than in the group that received SE-ILK only (Group III). This agrees with the report by Kogut *et al*. (9), who found that administration of SE-ILK confers significant protection against infection with *Salmonella* sp if applied to 18 day old embryos or to one-day chicks or turkeys (9).

Studies by McGruder *et al*. (10), and by Kogut *et al*. (11) show that lymphokines administered during the last days of incubation increase granulocyte proliferation, mainly of the heterophils (10, 11). The present work reveals that granulopoiesis can be stimulated if SE-ILK is administrated as early as day 10 of embryonic development. The positive effect of SE-ILK application was detected by day 14 of embryonic development in yolk sac and liver, and the tendency of granulocyte increase was maintained in liver along all of the study. The same positive effect was kept with the presence of viral antigen, since the number of granulocytes observed in embryos inoculated with viral antigen plus SE-ILK (Group II) was significantly higher ($p<0.05$) than the negative control (Group V) and the group inoculated with viral antigen (Group I). In addition to the stimulating effect on granulopoiesis, administration of SE-ILK also increased the amount of lymphoid follicles significantly in BF, which represents a greater amount of lymphocytes. The increase was not detected in the other groups, not even in the group that received antiviral antigen plus SE-ILK (Group II). No significant changes were observed in thymus, since the SE-ILK only produced detectable activation by the histological technique applied to bursa of Fabricius B-lymphocytes. Results of the present study suggest that the administration of antigens or protein molecules at an early stage, i.e., 10 days of embryonic development are capable of producing an increased granulopoiesis in lymphoid organs such as the liver and the yolk sac, as well as a rise in the number of lymphoid follicles in the bursa of Fabricius.

REFERENCES

1. Téllez I. G, Kogut M. H. and Hargis B. M. Immunoprophylaxis of Salmonella enteritidis infection by lymphokines in Leghorn chicks. Avian Dis. 37, 1062-1070. 1993.

2. Andrews E. J, Bennett B. T, Clark J. D, Houpt K. A, Pascoe P. J, Robinson G. W. and Boyce J. R. Report of the AVNA Panel on Euthanasia. JAVNA. 202, 229- 249. 1993.

3. Allen T.C. Hematoxilina y Eosina. In: Prophet EB, Mills B, Arrington JB, Sobin LH, editores. Métodos histotecnológicos. Washington (DC): Instituto de Patología de las fuerzas armadas de los Estados Unidos de América, 55-60. 1995.

4. Palya V. Preparation and testigo of Marek's disease vaccine. In: Food and Agriculture Organization of the United Nations. Manual for the production of Marek's disease, Gumboro disease and inactivated Newcastle disease vaccines. Food and Agriculture Organization Animal Production and Health paper. 1st ed. Rome. 1991.

5. Mallison E. T. and Snoeyenbos Salmonellosis. Jensen MM and Skeeles. Staphylococcosis. Sharma J. M. Marek's Disease. In: Hitchner BS, editor. A Laboratory Manual for the isolation and identification of avian pathogens. The American Assosiation of Avian Pathologists. 3rd ed. 1993.

6. Wayne W. D. Bioestadística, base para el análisis de las ciencias de la salud. México (DF). 3ª ed. Limusa. 1987.

7. Marriott F. H. C. The Interpretation of Multiples Observations. Londres (UK): Academic Press. 1974.

8. Méndez R. I. Modélos Estadísticos Lineales. 2ª ed. México (DF): CONACYT. 1981.

9. Kogut H. M, Wells L. L. and Lowry K. V. Ontogenia de las actividades bactericida y fagocítica de los heterófilos de pollo y pavo, y su estimulación por linfocinas. Memorias de las VII Jornadas Médico Avícolas. 1998 marzo 11-12, México (DF), México (DF): Asociación Nacional de Especialistas en Ciencias Avícolas, 44-47. 1998.

10. McGruder E. D, Ray P. M, Téllez I. G, Kogut M. H, Corrier D. E, Deloach J. R. and Hargis B. M. Salmonella enteritidis immune leukocyte-stimulated soluble factors: effects on increased resistance to Salmonella organ invasion in day-old Leghorn chicks. Poult. Sci. 72, 2264-2271. 1993.

11. Kogut M. H, Téllez I. G, McGruder, Wong R. A, Isibasi A, Ortiz V. N, Hargis B. M and DeLoach J. R. Evaluation of Salmonella enteritidis immune

lymphokines on host resistance to Salmonella enterica ser gallinarum infection in broiler chicks. Avian Pathol. 25, 237-249. 1996.

A PUPIL DEFECT IN ROSECOMB BANTAM CHICKENS

K. Plumer[A], C. Cardona[A, B], H. Shivaprasad[A, C]

[A]University of California, Davis
[B]Veterinary Medicine Extension, Surge III, Rm. 1383, University of California, Davis, Davis, CA 95616
[C]California Animal Health and Food Safety Laboratory, Fresno Branch, 2789 S. Orange Ave., Fresno, CA 93725

A defect in the pupil shape of rosecomb bantam chickens was noticed by a breeder. The pupil in affected birds appeared to be elongated at the lower margin of the eye in most birds and along both the upper and lower margins in a few birds. In all cases, the lesion in the left eye was more pronounced than the lesion in the right eye.

Clinically, the chickens appeared to be normal. Pupillary light responses were normal in affected birds although the affected portion of the iris did not constrict in response to bright light. One chicken was also blind but blindness has appeared in other chickens in the flock with normal pupil shape and no others with the defect were blind. We hypothesize that this defect in pupil shape does not cause any functional abnormalities for the chickens.

Histologically, the lesions were unremarkable. 15-week old birds were examined and the lower aspect of the iris had an excessive amount of mature collagen admixed with the iridal muscle fibers. One-week old chicks, the youngest birds in which the lesion is detectable, were histologically examined and a slight disarray of the iridal muscle fibers was observed.

None of the chickens had any signs of tumors and DNA collected from chicks with the defect were consistently negative for serotype I Marek's disease virus by PCR analysis. No other infectious agents were detected in these chickens.

Based on these findings, no causes for this defect were immediately evident. In order to narrow the possibilities, 28 specific pathogen free (SPF) chicks (SPAFAS, Storrs, CT) were incubated, hatched, and raised to 1-week of age in the rosecomb bantam environment. Twelve rosecomb bantams were incubated, hatched, and raised to 1-week of age at the same time, in the same incubator, hatcher and brooder. The chicks were examined at one week of age. None of the 28 SPF chicks had the pupil defect (0%) and three of rosecomb bantam chicks had the lesion (25%). Based on this experiment, environmental and most infectious agents have been eliminated as possible causes. Most likely, this represents a genetic anomaly.

An analysis of the pedigrees of affected birds was done. The pupil defect was significantly higher in incidence among chickens of the black, black-breasted red and red pyle lines. The brassy and blue color lines had a lower incidence of the lesion. Females were affected in significantly greater numbers (26% of all females were affected) than were males (9% of all males were affected).

EVALUATION OF DIFFERENT MANAGEMENT IN HATCHABILITY OF BROILER BREEDER FERTILE EGGS

Ortiz M. Francisco, Rosario C. Cecilia, Rojas O. Luis A., Quintana L. José A.

Department of Poultry Production, FMVZ University of Mexico, Ciudad Universitaria 04510 Mexico D. F.
quintana98@yahoo.com

ABSTRACT

Two thousand and sixteen fertile eggs fertile were collected from a 37-week-old breeder flock. Four different managements were done to eggs (four trials for each treatment): Treatment 1, eggs were stored 72 hrs at 23 ºC at the farm, and transported in a special vehicle (careful transportation) to the hatchery. Treatment 2, eggs were stored 72 hrs at 23 ºC at the farm and transported in ordinary vehicle to the hatchery. Treatment 3, eggs were transported in the ordinary vehicle to the hatchery, stored 72 hrs at 18 ºC and 80 % of humidity. Treatment 4, eggs were transported in the ordinary vehicle to the hatchery,

stored 72 hrs at 18 ºC and 80 % of humidity, preheated to 30ºC, during 8 hours before hatching. After incubation period (18th day) unfertile eggs were discarded and embryo diagnosis was performed, then fertile eggs were transferred to the hatcher machines. At day 21 hatched eggs were quantified as first and second quality, and unhatched eggs as pipped eggs and eggs in which the embryo did not hatch. The results were analyzed using variance analysis, and means were separated using Tukey test. Significance were determined at P<0.05. The lower percentage of early death was found in treatments 1 and 4. A highest percentage of first quality chick was found in treatment 1 (P<0.05). The treatments 1 and 3 showed the lowest percentage of second quality chicks. These results suggest that fertile eggs storage at 23 ºC for three days from 37-old-week breeders is enough to keep the quality and hatchability features. A careful transportation of fertile eggs allows an increase in hatchability. It is not necessary to preheat fertile eggs when they have been stored at 23ºC for three days.

INTRODUCTION

Embryo development stops when temperature of fertile eggs is around 25 to 27 ºC, this is called "physiological zero" (8). Fluctuation in this range can lead to consequences in egg quality and viability of the embryo. Extremely low temperatures can cause the lost of internal egg characteristics, whereas higher temperatures start cellular development (1,2,7).

Transportation of fertile eggs from breeder farm to hatchery must be done immediately after collection (7). It is important that the company use vehicles in excellent condition, and use responsible and trained personnel who drive on the best roads to avoid bad conditions (5,7). A clean vehicle is required that keeps fertile eggs protected from environmental conditions, contamination, and temperature variations. If the road is too long it is important to keep in mind that the vehicle should have a cooler system in order to maintain a uniform temperature to assure viability of the embryo (5).

MATERIALS AND METHODS

An Arbor Acres 37-week-old breeder flock raised at 2400 m above sea level, and a hatchery at 1292 m above sea level were used in the present study. A total of two thousand and sixteen fertile eggs from the previously described flock were collected. They were randomly assigned into 4 different treatments, each one with 126 fertile eggs (each treatment had four trials).

Treatment 1. 504 fertile eggs were collected, disinfected and stored for 72 h at 23 ºC. They were then transported on a special vehicle to the hatchery.

Treatment 2. 504 fertile eggs were collected, disinfected and stored for 72 h at 23 ºC at the farm. The normal vehicle that belongs to the enterprise then transported them.

Treatment 3. 504 fertile eggs were collected, disinfected at the farm, and then transported on a normal vehicle to the hatchery where eggs were stored for 72 h in the storage room at 18 ºC and 80 % of humidity.

Treatment 4. 504 eggs were collected and disinfected at the farm and transported in the normal vehicle of the enterprise to the hatchery where they were stored for 72 h in the storage room at 18 ºC and 80 % of humidity. After this period, they were preheated for 8 h in order to reach 30ºC before hatch.

Hand disinfection was done using a solution of alcohol with glycerin. The selection and disinfection of the egg was performed immediately. Eggs were collected using a solution of ammonium quaternaries at 40 ºC.

The treatments were transported at 5:00 pm, and lasted 39 minutes (about 30 km).

At day zero, all trials were hatched in the same machine (Chick Master) to 37.7 ºC, humidity of 55%, with vertical ventilation and turning. At day 18 all treatments were candled individually, and infertile eggs were removed. The same day fertile eggs were moved to the hatchery (Chick Master) to 37.2ºC. Eggs were classified into four treatments: infertile, broken, contaminated, and early death. At day 21 the number of hatched and unhatched chicken was quantified. Hatched chickens were classified into four treatments: first or second quality, pipped and unpipped eggs.

The percentages obtained were analyzed using a variance analysis; significant differences among treatments were obtained by Tukey test through Statgraphics program.

RESULTS

A significant difference in hatchability percentages was found. Treatments 1 (88.3%) and 2 (85.6%) were better than 3 (81.1%) and 4 (79.0%) ($p<0.05$).

Treatments 1 and 3 have the less percentage of chickens of second quality, both with 2.82 % against treatments 2 (6.1%) and 4 (5.4%) ($p<0.05$). The highest percentage of dead embryos (not pipped eggs) was observed in treatment 4, whereas less percentage was found in treatment 1.

In many cases a significant difference between treatments was not demonstrated, probably due to a large variance inside treatments. We suggest repeating this experiment using a large amount of trials, and to control variables.

Preheating fertile eggs from 37-week-old breeders hens at 30 ºC after being stored for 3 days at 18ºC in

the storage room could be less strict or even omitted, because the increase in temperature that eggs experience during the transportation to the hatchery could be enough.

CONCLUSIONS

Storage at 23ºC of fertile eggs from 37-week-old breeder hens is enough to maintain the quality and hatchability characteristics. A carefully management of fertile egg could increase the hatchability. Preheating is not necessary when fertile eggs are stored for 3 days in the storage room from 18 to 20ºC.

REFERENCES

1. Brake, J. Optimización del almacenaje de huevos fértiles. Avicultura Profesional,14;6:26-31;1996.

2. Martin S, Powell K. Tecnología de incubación mejora la calidad y viabilidad de los pollitos. Avicultura profesional;16;5:41-43; 1998.

3. Mehereford R. Efectos del transporte en huevos fértiles y pollitos. Desde el huevo hasta el pollito (5). Avicultura profesional;16;3:18-21; 1998.

4. Quintana L JA, Silva M, Ruiz R, Cazares R, Merino R. Memoria XXV Concvención Anual ANECA. Cancún Q. R., México ¿El periodo de conservación del huevo fertil afecta la productividad del pollo de engorda de igual modo que a la incubabilidad 274-277. 2000.

5. Reis, L.H., Gama, L.T. and Chaveiro, M. Effects of short storage conditions and broiler breed age on hatchability, hatchability time, and chik weights. Poultry Science; 76:1459-1466, 1997.

6. Tullet, S.G. Avian Incubation. Poultry Sience Symposium. Chapter 12 egg handling and storage at the farm and plant. Number Twenty-Two. Editor Freeman BM. Editorial Butterworth-Heinemann. 195-203. 1991.

DETERMINATION OF THE PATHOGENICITY OF DIFFERENT ISOLATIONS OF ESCHERICHIA COLI FROM FERTILE EGG AND CHICKENS WITH YOLK SAC INFECTION THROUGH SEROLOGY AND HYBRIDIZATION OF DNA

Rosario CC[A], López CC[A], Castañeda SMP[A], Téllez IG[A], Peñalba GG[B], Eslava CC[C]

[A]Department of Poultry Production, FMVZ, UNAM
[B] Counsellor Independent, [C]Department of Public Health, FM, UNAM. Ciurcuito Exterior cp 04510 Ciudad Universitaria, Mexico, D.F., MEXICO
rosario@servidor.unam.mx

INTRODUCTION

Yolk Sac Infection (ISV) is one of the main causes of death in chickens during the first week of life. The most common bacteria isolated in this infection is *Escherichia coli*. Different from other bacteria that only can produce a type of disease, E. *coli* is able to produce a great variety of syndromes in man and animals. *E. coli* has been used as model to illustrate that bacteria are pathogenic due to the genes that they hold and not by belonging to a specific strain.

The serologic classification of *E. coli* is very similar to the scheme of Kauffman-White for Salmonella spp.:

Somatic Antigens or u "O": There are 175 different somatic antigens.

Capsular Antigens or "K": Exist 70 antigens K internationally recognize.

Flagelar Antigens or "H": There are 56 groups.

Serogrups most commonly identified as pathogens for chickens are: O1, O2 and O78.

There have been described 5 pathogenic groups of *E. coli* producers of diarrhea in human beings based on distinct properties of virulence, different interactions with the intestinal epithelium, and distinct combinations of O:H; all this given by virulence genes. These groups are:

Enteropatogenic *E. coli* (EPEC): They own *eae* gene (attaching-and-effacing) and *bfp* that is responsible for the production of pili which act during the phase of adherence among bacteria.

Enterotoxigenic *E. coli* (ETEC) Strains of *E. coli* that produce at least one of the two groups defined as enterotoxin, ST (heat-stable toxin) and LT (heat-labile toxin).

Enterohemorrhagic *E. coli* (EHEC) They own the gene *eae* that is also at the EPEC strains. Also they produce one toxin than is virtually identical to the toxin of *Shigella* spp. (Shiga-like-toxin).

Enteroaggregative *E. coli* (EAEC) They have one fimbrial structure call Aggregative Adherence Fimbriae I (AAF/I), that participates at the pathogenesis of this group.

Enteroinvasive *E. coli* (EIEC) They elaborate one or more enterotoxins, which play an important role in the pathogenesis of the diarrhea. The gene *ipah* has been chosen to identify this group.

Cytolethal Distending Toxin (CDT) It is a factor responsible for toxic effect in cells of Chinese Hamster Ovary (CHO)

The objective of this paper was to know the occurrence of diverse serotypes of *E. coli* at the YSI and to determine if the isolations were carriers of virulence genes, and to explain their participation in the pathologic process.

MATERIALS AND METHODS

Two hundred and sixty seven strains identified as *E. coli* isolated from different places were used. The number of strains by place is showed in Table 1.

Serological Identification. Serotyping of *E. coli* was performed following the procedure described by Orskov and Orskov (1984), utilizing specific antisera (SERUNAM).

Determination of Virulence Genes. It was performed through the technique of DNA colony hybridization, previously described by Hill and Payne (1984).

RESULTS

At this work, the serogroups found most frequently were O19 (12%), rough strains (OR) (10.86%) of the total (Table 2).

The identification of flagellar antigens of *E. coli* also showed a great variety; however, an important percentage of the strains were not motile (30.7%).

Forty one percent of the strains (110/267) had at least one virulence gene. The commoner was *ipah* gene (46%). Also *eae* and *cdt* genes were found among studied strains.

The highest percentages of death were found between day 3 (21.30%) and 5 (25%), this mortality agrees with the highest number of isolations which were carriers of virulence genes. Also, it could be observed that 6% of strains which were carriers of any gene, were carriers of *ipah-cdt* genes. These strains were only isolated the third day. S*th, agg1, agg2, slt, bfp* and *lth* genes were not found in our strains.

DISCUSSION

Mosqueda and Lucio (1985), point out that the curve of death rate of the YSI lasts until the 7th to 10th day and reaches its maximum level among the 4th and 5th day, decreasing 3 to 5 days later. In this work, it was found that the pattern of death rate agrees with that reported by both authors.

El-Sukhon (1989), serotyped 89.6% of strains of *E. coli* of the total. With the present work, it was possible to identify the serotype of 85.3% of our strains.

Whiteman et al. (1983) and White et al. (1993) reported that serotypes isolated more frequently from chicken diseases are O1, O2 and O78; however, in our work, we were not able to identify serogroup O1. This is possibly due to the strains serotyped by other authors coming from respiratory infections.

Some authors mention that strains which cause YSI are normal inhabitants of the chicken's gut or environment (3). In this work the main serotypes which were carriers of virulence genes do not belong to serogroups O2 and O78, contrary to the previously reported by White et al. (1983).

Carter and Cole (1990), indicate that the invasiveness is not a common mechanism of pathogenicity among avian strains of *E. coli*; however, the predominant gene in our strains was *ipah* (80/267strains).

CONCLUSIONS

The main cause of YSI were *E. coli* due to the curve of death rate by YSI agree with isolation of *E. coli*, both have their peak between day 3 and 5 of chick life.

Serogroups isolated most frequent were O19, OR, O84 and O8. These strains have not been reported as pathogens to chickens.

The most common gene of virulence found in chickens with YSI was *ipah* (invasiveness gene).

This is the first report about serotyping of *E. coli* avian strains in Mexico.

REFERENCES

1. Orskov, F. and Orskov, I. Serotyping of Escherichia coli. En: Methods of microbiology. Vol. 14. London: T. Bergan. Academic Press, 1984: 43-112.

2. Hill, W.E. and Payne, W.L. Genetic methods for the detection of microbial pathogens. Identification of enterotoxigenic Echerichia coli by DNA colony hybridization: collaborative study. Journal of the Association of Official Analytical Chemists 67: 801-807; 1984.

3. Mosqueda, T.A. y Lucio, M.B. Enfermedades comunes de las aves domésticas. México: Universidad Nacional Autónoma de México, 1985.

4. Allan, B.J., Van den Hurk, J.V. and Potter, A.A. Characterization of Escherichia coli isolated from cases of avian colibacillosis. Can J Vet Res 57: 146 –151. 1993.

5. El-Sukhon, S.N. Incidence of Escherichia coli in chicken affections. Indian J An Sci; 60: 263-266.1989.

6. Cloud, S.S., Rosenberger, J.K., Fries, P.A., Wilson, R.A. and Odor, E.M. In vitro and in vivo characterization of avian Escherichia coli. I. Serotypes, metabolic activity, and antibiotic sensitivity. Avian Dis; 29:1084-1093, 1985.

7. Whiteman, C.E. and Bickford, A.A. Avian disease manual. 2nd. ed. USA: American Association of Avian Pathologists, 1983.

8. White, D.G., Dho-Moulin, M., Wilson, R.A. and Whittam, T.S. Clonal relationships and variation in virulence among Escherichia coli strains of avian origin. Microb Pathogen; 14:399-409; 1993.

9. Edwards, P.R. and Ewing, W.H. Studies on coliform type isolated from the organs of fowls. Cornell Vet; 44:50-56; 1954.

10. Carter, G.R. and Cole, J.R. Diagnostic procedures in veterinary bacteriology and mycology. 5th. ed. USA: Academic Press, Inc. Hancourt Brace Jovanovich, Publishers, 1990.

Table 1. Strains of *E. coli* isolated from different samples from a poultry integration.

SITE OF SAMPLING	SAMPLES	STRAINS OF *E. coli*
Breeder Flock	Nest Material	0
	Fertile Eggs	4
Hatchery	Fertile Eggs at 19th day	28
	Fertile Eggs at 21st day	47
Broilers	Chicks with Yolk Sac Infection	188
TOTAL		267

Table 2. Serogroups O isolated at breeders farm, hatchery and broilers with yolk sac infection.

SEROGROUPS	STRAINS	
	NUMBER	PERCENTAGE (%)
O19	33	12.36
OR	29	10.86
O84	24	8.99
O8	17	6.37
O78	13	4.87
O120	11	4.12
O?, O103, O155	10 *	3.75*
O118	7	2.62
O112, O15, O168, O2, 09	6*	2.25*
O146, O22, O44	5*	1.87*
O152	4	1.50
O91	4	1.50
Others	3 or less	2.24

Each one

(This is a summary of an article that will be published in Veterinary Microbiology)

PATHOGENICITY OF DIFFERENT SERO-GROUPS OF AVIAN SALMONELLAS IN SPF CHICKENS

Parimal Roy; A. S. Dhillon; H. L. Shivaprasad; D. M. Schaberg; Daina Bandli and Sylvia Johnson

Avian Health Laboratory, Washington State University –Puyallup 7613 Pioneer Way East, Puyallup, WA 98371-4998, (253) 445-4537(Telephone), (253) 445-4544 (Fax), E-mail: asdhillon@wsu.edu

Three hundred eighty four specific pathogen free (SPF) single comb white leghorn chicks were divided into 16 equal groups. Fourteen groups were inoculated individually with 0.5 ml of broth culture containing

1×10^7 colony forming unit (CFU) of either *S. typhimurium* (1), *S. heidelberg*(4), *S. montevideo* (2), *S. harder* (1), *S. kentucky* (3), *S. enteritidis*-PT 13a (1), *S. enteritidis* PT 34 (1), *S. enteritidis* PT 4 (1) by crop gavage. One group of 24 chicks was inoculated in the same way with 1×10^7 CFU of Salmonella pullorum. Another group of 24 chicks was kept as an uninoculated control group. All the chicks were observed daily for clinical signs and mortality. *Salmonella* re-isolation was done from different organs at 7 and 28 days post inoculation (DPI). All the chicks were weighed individually at 7, 14, 21 and 28 DPI. Two chicks at random from each group were euthanized and necropsied at 7, 14 and 28 DPI for gross pathology. Selected tissues were examined for histopathological changes at 7 and 14 DPI. Dead chicks were examined for the gross and histopathological lesions. Chicks inoculated with S. pullorum had the highest mortality followed by *S. typhimurium, S. heidelberg* (00-1105-2), and *S. enteritidis* PT4 (Chicken-CA). The lowest mortality was observed in groups inoculated with *S. heidelberg* (00-1131-1) and *S. kentucky* (99-583). Other inoculated groups and the uninoculated control group did not show any clinical sign or mortality. Ceca were found to be 100% positive for the re-isolation of *Salmonellas* both at acute or chronic infection compared to other organs. Mean body weights were reduced to 0.67% to 33.23% in the inoculated groups at different weeks compared to uninoculated controls. In some inoculated groups body weights did not differ from uninoculated controls ($p\angle$ 0.0001). The consistent gross and hispathological lesions were peritonitis, perihepatitis, yolk sac infection, and enteritis.

MATERNAL IMMUNITY FOR INFECTIOUS BURSAL DISEASE VIRUS (IBDV)

G.A.Servin[A], K.Blasco[B], M. Tamayo

[A]Independent Consultant, Mexico
[B]Fort Dodge Animal Health

Infectious Bursal Disease (IBD) was recognized in 1957. For nearly 45 years there has been enormous progress and achievement in disease control and poultry production. During this period there have been development of effective vaccines, polymerase chain reaction (PCR), development of ELISA system, and actually new systems that can give us better information as they are based in viral protein 2 to measure antibody response. In the past five years there have been different outbreaks in Latin America with virulent strains, although in Mexico we still haven't faced this problem. The classical disease with high morbidity, severe depression, and white diarrhea is not frequently encountered in the field. The subclinical form of IBD is common. This form of the disease for some time was affecting birds around the second week of age. From serologic information that we get from the field, age of outbreak has moved to two weeks more. This form of disease is more costly because it affects the end of production, and allows some viruses, such as bronchitis (that is usually affecting the birds around this age), to establish themselves in the birds with a reduced immune response. We also observe early challenge that leaves the bird with an impaired immune system susceptible to gangrenous dermatitis, colibacillosis and a high feed conversion.

From the information we get from the field we can say that in Mexico IBDV challenges occur in all broiler growing areas. The level of challenge is variable. Unfortunately many times no isolation or identification of the type of virus is done. We only know by clinical signs and serology of a standard virus. There is information of serology of variants, but when in the field are using vaccine variants by serology you cannot accept the presence of them. There has to be an isolation and identification, which is easily done now by commercial PCR.

In 1968 the first attenuated vaccine was approved, and around 1979 the inactivated vaccines were introduced. The idea was to hyperimmunize the hens in order to provide higher levels of antibodies that could be passed to the progeny. If we review papers from five, ten, or twenty years ago the questions are the same:

- Are the current vaccines adequate to prevent IBD?
- Which program is good for breeders?
- What is an adequate breeder titer for IBD?
- What is an adequate broiler protective titer for IBD?
- What kind of vaccines should we use?
- Should we make "shuttle" programs for IBD?

From field results broiler breeder vaccination programs have not been totally effective. There are many different environments for hens and broilers so it's difficult to establish a standard program. Also, with

the different variables that appear in the field, reproduction of the outbreak or verifying the presence of the disease is very difficult to accomplish.

As industry is concentrating into more birds in fewer companies, need of technical support in the field is needed. We are presenting IBD serologic results of broiler breeders and broilers with different vaccination schemes from different areas. Serology is a "must" that should be done in every complex. It gives us an aerial view of what is going on there. Regarding Gumboro, the next step is to correlate the serology with field results with the "bursometer", production results, and at the end with PCR. Later on we can go to specific points and work on them in order to have a "zoom" of the situation in every complex. We have spent a lot of time working on problem solving and trying new products. Facing all the new changes it is important to monitor our flocks and always remember what is happening in the field. Monitoring the flock not only tells me my virus status, it also helps me to monitor the application for my vaccination program. When we monitor our birds, we should roughly have the same results every time. To obtain the "whole" story of the flock it can take a complete year, but we can save time and money trying to guess what is going on.

After so many years it is important to define a program through application and monitoring. It is the only way we can establish our requirements. When we have a good monitoring program, good vaccination, and good application results, we can expect healthy happy chickens.

IN VITRO SUSCEPTIBILITY OF ORNITHOBACTERIUM RHINOTRACHEALE TO SEVERAL ANTIMICROBIAL DRUGS

V. E. Soriano[A, C], N.A. Vera[A], C.R. Salado[B], and R. P. Fernández[C]

[A]Departamento de Investigación y Desarrollo Avícola, Departamento Técnico[B], Biosíntesis Laboratorios S.A., Toluca 50130, México

[C]Centro de Investigación y Estudios Avanzados en Salud Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México. Toluca 50000, Mexico

As part of the basic characterization of *Ornithobacterium rhinotracheale*, the *in vitro* susceptibility of reference strains and Mexican isolates of this bacterium to several antimicrobial drugs was investigated. Antimicrobial drugs included in this study were sulfaquinoxaline, sulfachloropyridazine, sulfamerazine, sulfamethazine, trimethoprim, fosfomycin, gentamicin, oxytetracycline, amoxicillin, and enrofloxacin. Minimal inhibitory concentrations (MIC) were determined by the broth microdilution method as described (1). We have used the criteria of Blackall *et al*. (2) to define sensibility, intermediate sensibility, and resistance to these antimicrobials drugs as corresponding to MIC levels of <4 μg/ml, 4 to 8 μg/ml, and > 16 μg/ml, respectively. These same criteria were applied to this study except that sensitivity was defined as <8 μg/ml, intermediate sensitivity as 8 to 16 μg/ml, and resistance as ≥32 μg/ml. The MIC values for sulfaquinoxaline, sulfachloropyridazine, sulfamerazine, sulfamethazine, trimethoprim, fosfomycin, gentamicin were similar (≥128 μg/ml) for all reference strains and Mexican isolates of *O. rhinotracheale*. In contrast, there was considerable variation in the MIC values for amoxicillin, enrofloxacin, and oxytetracycline among reference strains. All Mexican isolates, except one for enrofloxacin, showed resistance to these three antimicrobial drugs. Obtained results indicated lower MIC values for amoxicillin, enrofloxacin, and oxytetracycline among reference strains. It can be concluded from these results that acquired antimicrobial resistance is exceptionally frequent in *O. rhinotracheale* similarly as reported by others (3, 4).

REFERENCES

1. Blackall, P.J. Antimicrobial drug resistance and the occurrence of plasmids in Haemophilus paragallinarum. Avian Dis. 32:742-747. 1988.

2. Blackall, P.J., L.E. Eaves, and D.G. Rogers. Biotyping of Haemophilus paragallinarum isolates using hemagglutinin serotyping, carbohydrate fermentation patterns, and antimicrobial drug resistance patterns. Avian Dis. 33:491-496. 1989.

3. Devriese, L.A., J. Hommez, P. Vandamme, K. Kersters, and F. Haesebrouck. In vitro antibiotic sensitivity of Ornithobacterium rhinotracheale strains from poultry and wild birds. Vet. Rec. 137:435-436. 1995.

4. Fitzgerald, S.L., J.M. Greyling, and R.R. Bragg. Correlation between ability of Ornithobacterium rhinotracheale to agglutinate red blood cells and susceptibility to fosfomycin. Onderst. J. Vet Res. 65:317-320. 1998.

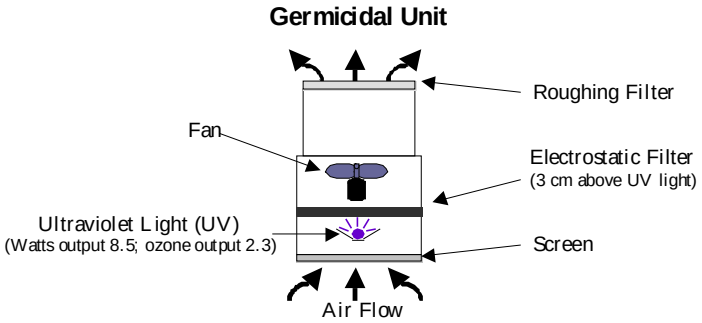
EFFICACY OF A GERMICIDAL AIR FILTRATION UNIT IN TRAPPING AND KILLING MICROORGANISMS IN THE AIR IN PENS OF CHICKENS

J.L. Spencer, B. Rennie and J.A. Devenish

Canadian Food Inspection Agency, Animal Diseases Research Institute / Centre for Plant Quarantine Pests, 3851 Fallowfield Road, P.O. Box 11300, Station "H", Ottawa, Ontario K2H 8P9

A germicidal air filtration unit (GU)* was evaluated for its capacity to trap and kill microorganisms in the air in a pen of 40 caged chickens. As illustrated in the figure, air was drawn upward into the GU through a screen that prevented passage of feathers. Air then passed through an electrostatic (E) filter that trapped the dust. An ultraviolet light, mounted on a track, passed back and forth across the undersurface of the E filter. Finally, the air passed through a roughing (R) filter that was installed for evaluating the efficacy of the E filter. At low or high speed the fan drew approximately 450 or 750 cu. ft. of air per minute, respectively. In these studies the unit was operated for 24 hour periods with the fan operating at high or low speed and with the UV light on or off. A portion of each filter was put in a stomacher bag and 9 ml of 0.1% buffered peptone water was added per g of filter. After mixing, serial dilutions of the dust suspension were assessed using standardized tests for total aerobic, total coliform and fecal coliform counts as well as total mold counts.

In two trials at low fan speed with the UV light off, there were about 10^4 colony forming units (cfu) of bacteria per g of E filter and this was about 10 fold higher than what was detected on the R filter. With the UV light on, the levels of total aerobic bacteria on both E and R filters were below the detectable limit of 100 cfu per g. Likewise, coliforms and molds were reduced below the detectable limit in filters exposed to UV light. In two trials at high fan speed there were 10^2 to 10^5 cfu of bacteria on all filters with the UV light on or off. However, the number of bacteria on E filters exposed to UV light was 100 or 1000 fold lower than on those that were not exposed. These and other preliminary studies to be reported suggest that the GU could reduce the level of pathogens in the air in a poultry facility.



*Product of Engineering Dynamics Limited, 137 Young Drive, R.R. 1, Carleton Place, Ontario, Canada, K7C 3P1

EFFICACY OF AEROSOL VACCINATION OF AVIAN PARAMYXOVIRUS TYPE 1 IN COMMERCIAL PIGEONS

K. H. Tonooka[B], J. S. Jeffrey[A], M. C. Bland[C], G. L. Cooper[C], P. R. Woolcock[D], and A. Hunter[B]

[A]Department of Veterinary Extension and Population Health & Reproduction
[B]Poultry Health and Food Safety Laboratory
University of California-Veterinary Medicine Teaching and Research Center, Tulare, CA 93274
[C]California Animal Health and Food Safety Laboratory System, Turlock, CA 95380
[D]California Animal Health and Food Safety Laboratory System, Fresno, CA 93725

Paramyxovirus Type 1 (PMV-1) has a worldwide distribution and a large host range (1,2,3). Frequent outbreaks among racing pigeons stimulated the testing of killed vaccines (Hitchner B1 strain) for protection against PMV-1 infection with highly reliable results (5). For commercial squab, the aerosal route may offer a less labor intensive means of vaccine administration. Therefore in this study, the use of aerosol vaccination in commercial pigeons was evaluated when challenged with a field strain isolated in the central valley of California. Intravenous challenge was performed four weeks after the third dose of aerosol vaccine. Vaccination was by coarse droplet of Hitchner B1 followed by B1/Lasota at three week intervals. Four groups of pigeons were included in the study: vaccinated and challenged (VC, n=10); vaccinated and unchallenged (VU, n=10); unvaccinated and challenged (UC, n=10); and unvaccinated and unchallenged (UU, n=9). The birds were observed for 6 days. Necropsy was performed on day six post inoculation (PI) in the challenged groups and on day 7 in the unchallenged groups.

Clinical signs of illness were evident by day 4 PI in the UC and VC groups. Symptoms included loose fecal droppings, shivering, tucked heads and lethargy in both groups. By day 5, increased depression, reluctance to move, and torticollis were observed. Seven of 10 birds in the UC group died or were humanely euthanized before day 6, while two of 10 died in the VC group. No mortality occurred in the two unchallenged birds. Weight loss was noted in all groups; highest in the UC group and lowest in the UU group. At necropsy, lesions in the virus challenged groups were predominately in the pancreas, kidney, and brain. The histologic findings of pancreatic necrosis, tubular nephrosis and interstitial nephritis, lymphocytic cuffing and gliosis in the brain were consistent with reported cases of PMV-1 infection of pigeons (4,6,7,8). Among the unchallenged birds, no brain or pancreas lesions and few mild kidney lesions were observed. Vaccination titers in the VU birds were negative for 7/10 birds and ranged from 1:4 to 1:128 in the remaining birds. Titers remained negative in the UU group and less than 1:8 in the UC group. Titers ranged from 1:4 to 1:4096 in the VC group.

In summary, vaccinated challenged birds demonstrated clinical signs and pathology consistent with PMV-1 infection. There was 20% mortality, diarrhea, depression, torticollis and histologic lesions of the brain, pancreas and kidney. We concluded that aerosol vaccination was not effective in protecting pigeons against PMV-1 virus challenge in this trial. Based on this study we would not recommend aerosol vaccination as an alternative to the use of killed vaccine.

REFERENCES

1. Alexander, D.J. Newcastle disease and other paramyxovirus infections. In: Diseases of Poultry, 10th ed. B.W. Calnek, H. J. Barnes, C.W. Beard, L.R. McDougald, and Y.M. Saif, eds. Iowa State University Press, Ames, Iowa. Pp. 541-569. 1997.

2. Alexander, D.J., and G. Parsons. Avian paramyxovirus type 1 infections of racing pigeons: Pathogenicity experiments in pigeons and chickens. Vet Rec. 114:466-469. 1984.

3. Alexander, D.J., G.W.C. Wilson, J.A. Thain, and S.A. Lister. Avian paramyxovirus type 1 infection of racing pigeons: 3 Epizootiological considerations. Vet Rec. 115:213-216. 1984.

4. Barton, J.T., A.A. Bickford, G.L. Cooper, B.R. Charlton, and C.J. Cardona. Avian Paramyxovirus Type 1 Infections in Racing Pigeons in California. I. Clinical Signs, Pathology, and Serology. Avian Dis. 36:463-468. 1992.

5. Marx, D. A Veterinary Approach to Pigeon Health. The Racing Pigeon Digest Publishing Co. Inc. 1997.

6. Shirai, J., H. Hihara, and M. Maeda. Virus Distribution and Histopathologic Changes in Organs of Chickens Inoculated with Newcastle Disease Virus (Avian Paramyxovirus-1) Isolated from Racing Pigeons. Avian Dis. 32:544-547. 1988.

7. Tangredi, B.P. Avian Paramyxovirus Type I Infection in Pigeons: Clinical Observations. Avian Dis. 29:1252-1255. 1985.

8. Tangredi, B.P. Avian Paramyxovirus Type I Infection in Pigeons: Recent Changes in Clinical Observations. Avian Dis. 32:839-841. 1988.

A POSSIBLE IMMUNOMODULATORY EFFECT OF SAPONINS FROM QUILLAJA SAPONARIA IN CHICKENS

H. Toro[A], C. Borie[A], M.T. Cesario[B], and R. San Martín[B]

[A]Faculty of Veterinary Sciences, University of Chile, casilla 2, correo 15, Santiago, Chile
[B]Faculty Chemical Engineering and Bioprocessing, Catholic University, Av. Vicuña Mackenna 4860, Santiago, Chile
Financed by Fondef I2010

SUMMARY

In a first trial we evaluated the antibody response to Newcastle Disease virus (NDV) adding a highly purified *Quillaja saponaria* saponins (Q-Sap) into the vaccine's excipient. We also measured the antibody response to Infectious Bursal Disease virus (IBDV) vaccine in birds receiving a crude form of Q-Sap in the diet. Our results showed that neither the ocular administration of Q-Sap nor the addition of this phytochemical in the diet of chickens was able to enhance the specific serum antibody response. In a second trial, we evaluated the susceptibility of chickens (with maternal immunity) receiving dietary QP to an experimental challenge with *Salmonella typhimurium*. The addition of QP in the diet resulted in a significantly lower percentage of *S. typhimurium* positive birds 10 days after experimental challenge. The same experiment conducted in specific pathogen free chickens determined similar reisolation values regardless the addition of Q-Sap in the diet. However, significantly lower colony forming units (CFU) of the challenge strain could be detected in the feces of the treated birds. These results suggest an enhancement of the bird's immune response against this pathogen.

INTRODUCTION

Saponins are natural detergents found in many plants including the Chilean soapbark tree (*Quillaja saponaria*). Beside their multiple applications both for human and animal nutrition, odor control, cosmetics, etc., an important area of interest in quillaja saponins (Q-Sap) is their possible effect on the immune system. Since many years saponins are known for their adjuvant activity for parenteral applied viral vaccines (7). There are also reports on increase effectiveness of oral vaccines, enhanced enteric uptake of molecules and direct stimulatory effect on the immune system (8, 2, 1, 14). More recently, Hoshi et al., (4) compared the antibody response of orally immunized chickens using different adjuvants. Their results showed Q-Sap to be a useful oral adjuvant for this specie.

This paper dealt with further investigating the adjuvant effects of Q-Sap following either local or oral administration in chickens as well as their possible effect on enhancement of the non-specific immune response. In a 1st trial we evaluated the antibody response to Newcastle Disease virus (NDV) adding Q-Sap into the vaccine's excipient. Additionally, we measured the antibody response to Infectious Bursal Disease virus (IBDV) vaccine in birds receiving Q-Sap in the diet. In a 2nd trial, we evaluated the susceptibility of chickens receiving dietary Q-Sap to an experimental challenge with *Salmonella typhimurium*.

MATERIAL AND METHODS

***Quillaja* saponins.** Two forms of Q-Sap from a commercial source (Natural Response, Quilpué, Chile) were used. A highly purified registered form of Q-Sap (5) named Supersap[R] was used for local (ocular instillation) delivery. Supersap's content of saponins is 90%. A crude extract of the *Quillaja* tree named "Quillaja Powder" (QP) containing 1.5% (w/w) of Q-Sap and 7% humidity was used for oral administration along with the diet.

Trial 1. Trial 1 considered the evaluation of the antibody response after vaccination against NDV and IBDV at day 10 of age. Both viral vaccines were obtained from a commercial source (Intervet, Boxmeer).

NDV vaccination. One hundred specific pathogen free (SPF) chickens (VALO-Lohmann, Cuxhaven) were hatched and divided into two groups. Both groups were subjected to NDV vaccination by the ocular route at day 10 of age. One group of 50 birds received the vaccine containing the Q-Sap product Supersap[R] incorporated into the vaccine's vehicle at a final concentration of 0,5% (w/v). Each bird received one drop of the vaccine (10③l) in each eye. The two drops

contained one vaccine dose. The control group received the vaccine using the regular vehicle.

IBD vaccination. One hundred SPF chickens from the same source were divided into two groups. Both groups were subjected to IBDV vaccination by the oral route via the drinking water at day 10 of age. One group received QP in the diet from day 1 of age and throughout the experimental period of 35 days. In this group QP was added to the feed at a final concentration of 1000 parts per million (ppm). The control group received regular feed throughout the experimental period.

Serum samples. Blood samples were obtained from all birds by wing vein puncture at days 1, 7, 21, 28, and 35 of age. Sera obtained were tested for specific antibodies using a commercial ELISA-Kit (Idexx, Westbrook, Maine). ELISA's were conducted according to the manufacturer's recommendation.

Trial 2. The susceptibility of both SPF and chickens with specific maternal immunity receiving dietary QP to an experimental challenge with *Salmonella typhimurium* was evaluated by reisolation attempts of the challenge strain from the ceca and from the liver & spleen of the birds.

Challenge strain. A spontaneous nalidixic acid and ryfampicin resistant *S. typhimurium* strain, obtained originally from chickens, was used in all experiments. This *S. typhimurium* nal^r rif^r was inoculated into 10 SPF birds prior to use it in the experiments to test its ability to colonize intestine and invade organs. This strain was successfully isolated from all bird's 5 days after inoculation.

Experiment A. Ninety chickens were obtained from a commercial supplier and divided into 3 groups of 30 chickens. These chickens were the progeny of breeders that had been subjected to 2 doses (10 and 16 weeks of age) of an inactivated *Salmonella enteritidis* vaccine (Biomune, Kansas). The bird groups received QP in respective dosages of 100 and 300 ppm (groups being designated QP 100 and QP 300) mixed with the regular feed throughout the experimental period of 20 days. A control group received regular feed during this period. All groups were orally inoculated with $1.5x10^8$ colony forming units (CFU)/ml of the challenge strain at day 6 of age. Ten days after challenge, all birds were euthanized and sampled for salmonella isolation attempts. Both ceca and spleen & liver were obtained separately from each bird. Isolation was conducted according to standard procedures using XLD agar (Difco, Detroit) adding 20mg/ml of ryfampicin and nalidixic acid (Laboratorio Chile, Santiago). Negative samples were further evaluated in delayed secondary enrichment in tetrathionate broth. Final identification was based on routine biochemical reactions and by agglutination with a Salmonella O antiserum Poly A-I and Vi (Difco, Detroit).

Experiment B. Ninety SPF chickens (Universidad Austral de Chile, Valdivia) were hatched and divided into 3 groups of 30 chickens. These birds were grouped and challenged as described in experiment A. Accordingly, groups received QP in respective dosages of 100 and 300 ppm throughout the experimental period of 20 days. The control group received regular feed. All groups were orally inoculated with $6.5x10^8$CFU/ml of the challenge strain at day 10 of age. Ten days after challenge, all birds were euthanized and sampled for salmonella isolation attempts. Both ceca and spleen & liver were obtained separately from each bird. Isolation was conducted as described above. Additionally, a quantitative recovery of *S. typhimurium* was conducted by serial dilutions in plate count agar added with the mentioned antibiotics.

RESULTS - TRIAL 1

NDV vaccination. Birds vaccinated by eye drop receiving Supersap^R showed a moderate ocular irritation expressed by closed eyes. This was observed for approximately 30 min in the treated group. Both the control and the treated groups showed a significant increase of specific antibodies in serum after vaccination ($F= 10.92$). When comparing the average values obtained at each time after vaccination by analysis of variance no significant differences were evident ($F=1.2$).

IBDV vaccination. Both chicken groups, those receiving QP orally and the non-treated controls, increased their antibody levels after vaccination significantly ($F= 55.6$). No significant differences could be detected between the antibody levels detected in the control and the treated group ($F=0.103$).

RESULTS – TRIAL 2

Experiment A. The QP treated groups showed significantly lower reisolation percentages both from ceca and organs from the chickens with maternal immunity as compared with the controls ($p<0.05$). The percentage of reisolation from ceca of the control bird group was 90% while QP 100 and QP 300 showed values of 63.6% and 54.3% respectively. Isolation from organs of the control group was 63.3%. QP 100 and QP 300 achieved 27.3% and 45.7% respectively.

Experiment B. The QP treated groups and the control showed similar reisolation percentages both from ceca and internal organs ($p<0.05$). The reisolation percentages from ceca varied between 91.4 and 97.1%. The reisolation percentages from organs showed no significant differences varying between 40 and 47%.

Four groups were established according to the quantitative recovery of *S. typhimurium* from feces; CFU/g feces: $>10^{10}$, 10^8, 10^6, and $<10^5$. While birds of

the control group showed higher recovery counts of salmonella, birds of the QP treated groups showed significantly less CFU/g feces.

DISCUSSION

Experiments conducted mainly in mice have shown that immunization with protein antigen in the presence of Q-Sap have resulted in increased antibody production (8, 9, 7). Chickens receiving Q-Sap orally immunized with non-replicating antigens such as bovine serum albumin have also shown higher specific antibody titers as compared with other adjuvants (4). *Quillaja* saponins have proven to potentiate the ocular delivery of non-replicating antigens such as insulin (11). However, the present results showed that ocular administration of Q-Sap included in the NDV vaccine was not able to elicit higher specific serum antibodies in the treated birds.

Oral administration of Q-Sap has also shown to stimulate both the humoral and cellular immune responses to concomitantly orally administered inactivated rabies antigen (2). The present results showed no differences of the specific antibody response after IBDV vaccination between chickens receiving dietary Q-Sap and non-treated birds. According to this result no effect on the serum antibody response could be attributed to the administration of dietary QP.

A possible explanation for these results, as compared with the results obtained by other authors, may be that Q-Sap does not interfere with the adherence to specific cell receptors and replication of live viruses. It is also important to consider that different fractions and purification levels of saponins may vary substantially in their physiological effects (12). However, we consider the present results preliminary since other responses, particularly the local specific IgA, were not measured. This response would have been interesting to search due to the fact that a transient local eye irritation was observed. This local effect might have had influenced the local response to the vaccine.

Chavali and Campbell (2) reported that mice fed with saponins showed an increased clonal expansion and lymphocyte function suggesting an immunomodulatory effect of this phytochemical. Additionally, recent studies on a saponin isolated from *Colubrina retusa* demonstrated an *in vitro* direct antimycobacterial activity of this compound (3). Other studies have also shown that saponins influence microbial fermentation in the gastrointestinal tract of animals (10, 13). Katsunuma et al., (6) showed absence of growth of 13 different bacteria strains in media containing 10 *Yucca shidigera* saponins. The minimum inhibitory concentration of this saponin ranged between 14 to 0.7 mg/ml. In the 2nd trial the addition of Q-sap in the diet resulted in a significantly lower percentage of *S. typhimurium* positive chickens (with maternal immunity) 10 days after experimental challenge with this pathogen. A different behavior was observed in SPF chickens, which showed similar reisolation values regardless the addition of Q-Sap in the diet. However, significantly lower CFU of the challenge strain could be detected in the feces of the treated birds. These results also suggest an enhancement of the birds immune response by saponins. It seems important to underline that the challenge doses of *S. typhimurium* used (10^8 CFU/bird) were relatively high. This fact may have influenced the results allowing the speculation that lower challenge doses could have had resulted in a more obvious protective effect.

REFERENCES

1. Campbell, J.B., and J.C. Bede. Antimicrobial and immunomodulatory effects of orally administered saponins. European Adjuvant Group Bulletin 5: 12-31. 1989.

2. Chavali, S.R. and J.B. Campbell. Adjuvants effects of orally administered saponins on humoral and cellular immune responses in mice. Immunobiology, 174: 347-359. 1987.

3. ElSohly, H.N., S. Danner, X.C. Li, A.C. Nimrod, and A.M. Clark. New antimycobaterial saponin from Colubrina retusa, J. Nat. Prod., 62:1341-1342. 1999.

4. Hoshi, S., A. Uchino, N. Saito, K. Kusanagi, T. Ihara, and S. Ueda. Comparison of adjuvants with respect to serum IgG antibody response in orally immunized chickens. Comp. Immunology, Microbiology & Infectious Diseases, 22:63-69. 1998.

5. Kamstrup, S., R. San Martin, A. Doberti, H. Grande, and K. Dalsgaard. Preparation and characterisation of quillaja saponin with less heterogeneity than Quil-A Vaccine, 18:2244-2249. 2000.

6. Katsunuma, Y., Y. Nakamura, A. Toyoda, and H. Minato. Effect of Yucca shidigera extract and saponins on growth of bacteria isolated from animal intestinal tract. Animal Science Journal, 71:164-170. 2000.

7. Kensil, C.R., U. Patel, M. Lennick, and D. Marciani. Separation and characterization of saponins with adjuvant activity from Quillaja saponaria Molina cortex. Journal of Immunology, 146:431-437. 1991.

8. Maharaj, I. K.J. Froh, and J.B. Campbell. Immune responses of mice to inactivated rabies vaccine administered orally: potentiation by quillaja saponin. Canadian Journal of Microbiology, 32:414-421. 1986.

9. Movat, A.M., A.M. Donachie, G. Reid, and O. Jarrett. Immunostimulating complexes containing Quil A and protein antigen prime class I MHC-restricted T lymphocytes in vivo and are immunogenic by the oral route. Immunology, 72: 317-322. 1991.

10. Nevel v., C.J., and D.I. Demeyer. Effect of antibiotics, a deaminase inhibitor and sarsaponin on nitrogen metabolism of rumen content *in vitro*. Animal Feed Science and Technology, 31:323-348. 1990.

11. Pillion, D.J., J.A. Amsden, C.R. Kensil, and J. Recchia. Structure-Function relationship among *Quillaja* saponins serving as excipients for nasal and ocular delivery of insulin. Journal of Pharmaceutical Sciences, 85: 518-524. 1996.

12. Price, K. R., I.T. Johnson, and G.R. Fenwick. The chemistry and biological significance of saponins in foods and feedingstuffs. CRC Critical Reviews in Food Science and Nutrition, 26:27-135. 1987.

13. Wallace, R.J., L. Arthaud, and C.J. Newbold. Influence of *Yucca shidigera* extract on ruminal ammonia concentrations and ruminal microorganisms. Applied and Environmental Microbiology, 60:1762-1767. 1994.

14. Wu, J.-Y., B.H. Gardner, C.I. Murphy, J.R. Seals, C.R. Kensil, J. Recchia, G.A. Beltz, G.W. Newman, and M.J. Newman. Saponin adjuvant enhancement of antigen-specific immune responses to an experimental HIV-1 vaccine. The Journal of Immunology, 148:1519-1525. 1992.

APOPTOSIS IN LYMPHOID DEPLETION INDUCED BY T-2 TOXIN IN THE BURSA OF FABRICIUS OF CHICKENS

Anilton Cesar Vasconcelos[A], Milene Alvarenga Rachid[A], Jairo Eduardo S. Nunes[A], Francisco Glauco A. Santos[A], Nelson Rodrigo S. Martins[B], Adriana Rodrigues Tristão[B], Vera Alvarenga Nunes[B]

[A]Laboratory of Apoptosis, Institute of Biological Sciences, Federal University of Minas Gerais, Postal Box 2486, 31270-010, Belo Horizonte, MG, BRAZIL. Phone: (5531) 34992887 or (5531) 91147841, Fax: (5531) 34992879, Http://www.icb.ufmg.br/~pat/Apopt E-mail: anilton@icb.ufmg.br

[B] School of Veterinary Medicine - Federal University of Minas Gerais, Belo Horizonte, MG, BRAZIL

Apoptosis is a programmed and active type of cell death that requires energy and protein synthesis for its execution (12). It is implicated in physiological regulation of tissue size, playing a homeostatic role opposite to mitosis. It is a sort of "cellular self-destruction" that takes place in normal tissues, such as the turnover of epithelia and leukocytes, cyclic or seasonal involution of gonads and hormone or growth factor removal induced involution (11, 20). This type of cellular suicide can also be triggered by pathological stimuli, as some toxins (1, 5, 2, 6). The T-2 mycotoxins belong to the trichothecenes class, produced by fungus of the genera *Fusarium* (3). Trichothecenes' common substrates are corn, barley, rye, wheat, milo and other cereal crops. Mold growth and toxin production are favored by alternating cool and warm temperatures. Once produced, T-2 toxins are stable in the environment and resistant to the heat and pressure of cooking, feed milling and processing (14). Trichothecenes are potent immunosuppressors, increasing susceptibility to certain infectious diseases (4). Antibody formation is suppressed and mitogen-induced lymphocyte blastogenesis is impaired, depending on the dose and of the exposition period (8, 18). The mechanism of toxicologic damage is not well known and different mechanisms may be operative for each effect. T-2 toxin has a direct effect on the lymphocytes, causing necrosis in the lymphoid follicles and on circulating lymphocytes (8, 9). Although the T-2 toxin has been recently incriminated as apoptosis inductor in mice's lymphoid organs (19, 10), no reference is made to apoptosis as a mechanism of immunosuppression in T-2 toxicosis in birds.

Histomorphometrical studies were conducted in the bursa of Fabricius (BF) to test the involvement of apoptosis as a mechanism of lymphoid depletion and immunossupression in broiler chicks, after the intake of feed containing T-2 toxin of *Fusarium sporotrichioides*. Forty-two one-day-old Hubbard male chicks were randomly distributed in three groups. The treated group (n = 15) received contaminated feed with 2,64 mg/kg of the T-2 toxin. The residual group (n = 12) received the contaminated feed up to 7 or 14 days and received uncontaminated feed during the last week. The control group (n = 15) received only uncontaminated feed. Five animals of control and treated groups and six animals of the residual group were killed and necropsied at ages of 7, 14 and 21 days, when BF was collected for histomorphometric analyses and DNA extraction. Half of each BF was fixed in 10% buffered neutral formalin and processed by routine techniques. Slides were stained by hematoxilin - eosin (HE) and by methyl green - pyronin (MGP) techniques. MGP stains

condensed chromatin in dark green and the cytoplasm in red and facilitates apoptosis detection (13). Some slides were also used for *in situ* identification of the fragmentation of the DNA by inserting labeled nucleotides in the 5'OH terminal portion of fragmented DNA of the cells in apoptosis (TUNEL or Terminal deoxynucleotidil transpherase Uracil Nick End Labeling). The other half of the BFs were stored in microtubes of 1,5 ml at –80° C, for posterior DNA extraction and electrophoresis in agarose.

The identification and quantification of apoptosis was accomplished by light microscopy. Cellular shrinkage, condensed chromatin in perinuclear clumps and the presence of apoptotic bodies were considered as morphological evidences of apoptosis (12). Morphometry (parenchyma / stroma ratio and apoptotic index) was achieved with a light microscope equipped with a camera connected to a Computer (Image analyzer). Data were collected with the aid of an image analyser (Kontron KS-300 v2.0, Zeiss Kontron Electronics GMBH). Data were analyzed statistically by the non-parametric Kruskal-Wallis test (parenchyma / stroma ratio and apoptotic index). The parenchyma / stroma ratio is a great parameter to evaluate atrophic states. It was determined with low magnification (4X objective). Three fields for slide were used. In each field, follicular area and empty space were measured. Stroma area was obtained by subtracting these areas (follicular area and empty space) from the total area of the field. Slides stained with MGP were used to determine the apoptotic index (number of apoptotic cells / total number of cells).

Bursal lymphoid follicles of Control Group were all well cellularized, independently of the experimental period. Both cortical and medullar areas were more defined and denser than in treated and in residual groups.

Histological lesions in BFs of the treated and residual groups were discrete at 7 days, moderate at 14 days and more evident at 21 days. Increased goblet cells were observed in the coating epithelium. Also a diffuse and moderate depletion of lymphocytes in the medullar and cortical area were observed, originating countless empty spaces. Apoptotic bodies were frequently found within the empty spaces as well as lymphocytes with condensed chromatin. Arai et al. (2) reported induction of apoptosis by radiation in bursal lymphocytes, with morphology of apoptotic cells very similar to the ones we saw in this experiment.

The parenchyma / stroma ratio (P/S) of BF of birds of the treated and residual groups were smaller than the ones of the control group at 14 days ($P< 0,05$) (Table 1). At 7 and interestingly at 21 days, P/S from the treated group were similar to the control group. Although the P/S ratio of the residual group did not show differences with the treated group, only the P/S ratio of the residual group was different from control group at 21 days.

The apoptotic indices of the 3 groups evaluated at 7 and 21 days did not differ from each other (Table 2). However, BF of treated and residual groups, sacrificed at 14 and 21 days, showed moderate to intense depletion of the lymphoid follicles, confirmed by lower parenchyma / stroma ratio and by higher apoptotic index, when compared to the control group ($P< 0,05$).

Apoptosis was confirmed by "*in situ*" labeling of the fragmented genome (TUNEL) in apoptotic lymphocytes scattered in bursal follicles of the treated and residual groups. Positive labeling was seen as brownish to dark intranuclear dots, as revealed by the diaminobenzidine (DAB) by peroxidase reaction.

Also electrophoresis of bursal DNA in agarose gel showed the internucleosomal DNA fragmentation characteristic of apoptosis. The gels showed the typical "ladder pattern", by the presence of bands with 200 bp or their multiples. Awhile the treated and residual groups presented more numerous and more intense distal bands, the control group showed denser bands near the migration origin, characterizing lower internucleosomal degradation.

Summarizing, there are evidences that the toxin T-2 causes fragmentation of the DNA and increases the apoptotic index in BF at 14 days. These results allowed us to conclude that apoptosis may be involved in the mechanism of lymphoid depletion caused by T-2 toxin in broiler chicks.

REFERENCES

1. Anilkumar, T.V.; Sarraf, C.E.; Alison, M.R. The biology and pathology of programmed cell death. Vet. Hum. Toxicol., 34(3): 251-254, 1992.

2. Arai, S.; Kowada, T.; Takehana, K. et al. Apoptosis in the chicken bursa of Fabricius induced by X irradiation. J. Vet. Med. Sci., 58(10): 1001-1006, 1996.

3. Baldissera, M.A.; Santurio, J.M.; Silva, J.B. et al. Aflatoxins, ochratoxin A and zearalenone in grains and feed for animal consumption in the South of Brazil. Ciênc. Tecnol. Alim., 12(1): 72-82, 1992.

4. Dohms, J.E., Saif, Y.M. Criteria goes evaluating immunosupression. Avian Dis. 28: 305-310, 1984.

5. Gruber, J.; Sgonc, R.; Hu, Y.H. et al. Thymocyte apoptosis induced by elevated endogenous corticosterone levels. Eur. J. Immunol., 24: 1115-1121, 1994.

6. Hickman, J.A. & Boyle, C.C. Apoptosis and cytotoxins. Br. Med. Bull. 52(3): 632-643, 1997.

7. Hoerr, F. J.; Carlton, W.W.; Yagen, B. Mycotoxicosis caused by the single doses of T-2 toxin or diacetoxyscirpenol in broiler chickens. Vet. Pathol.18: 652-664, 1981.

8. Hoerr, F. J.; Carlton, W.W.; Yagen, B. et al. Mycotoxicosis produced in broiler chickens by multiple doses of either T-2 toxin or diacetoxyscirpenol. Avian Pathol. 11: 369-383, 1982.

9. Holt, S. & Deloarch, R. In vitro effect of T-2 mycotoxin on the immune response of mice. Am. J. Vet. Cattles. 49(9): 1480-1484, 1988.

10. Islam, Z.; Nagase, M.; Ota, A. et al. Structure-function relationship of T-2 toxin and its metabolites in inducing thymic apoptosis in lives in mice. Biosci. Biotechnol. Biochem., 62(8): 1492-1497, 1998.

11. Kerr, J.F.R. Definition of apoptosis and overview of its incidence. Immunol. Rev., 142:1-15, 1993.

12. Kerr, J.F.R.; Wyllie, A.H.; Currie, A.R. Apoptosis: the basic biological phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer, 26: 239-257, 1972.

13. Moffitt, P. THE methyl green-pyronin technique goes demonstrating cell death in the murine tumour S180. Cell Biol. Inter., 18 (6): 677-679, 1994.

14. Osweiler, G.D. Toxicology. Media, Williams & Wilkins, 1996.

15. Prophet, E.B., Mells, B., Arrington, J.B. et al. Laboratory Method in Histotechnology. Washington: American Registry of Pathology, 1992. 275p.

16. Rosenstein, Y.; Kretschmer, R.R.; Lafarge-Frayssinet, C. et al. Immunosuppressive activity of Fusarium toxins; effect on antibody synthesis and skin grafts of crude extracts, T-2 toxin and dyacetoxyscirpenil. Immunology, 36: 111-117, 1979.

17. Sharma, J.M. Overview of the avian immune system. Vet. Immunol. Immunopathol. 30(1): 31-44, 1991.

18. Sharma, R. P. Immunotoxicity of mycotoxins. J. Dairy Sci., 76: 892-897, 1993.

19. Shinozuka, J.; Guanmin, L.; Kiatipattanasakul, W. et al. T-2 toxin-induced apoptosis in lymphoid organs of mice. Exp. Toxic. Pathol. 49: 387-392, 1997.

20. Vasconcelos, A.C., Moro, L., Da Silva, P.M. Apoptose or programmed cellular death and its importance in veterinary pathology. Rev. Port. Ciênc. Vet. 91(520): 182-193, 1996.

Table 1. Average values of the parenchyma / stroma ratio, according to the treatment and to the experimental period.

	Period (days)		
Groups	**1-7**	**1-14**	**1-21**
Control	$1,95^{Ab}$	$10,44^{Aa}$	$6,85^{Aa}$
Treated	$1,17^{Ab}$	$4,33^{Ba}$	$6,73^{ABa}$
Residual		$3,18^{Ba}$	$4,10^{Ba}$

In the columns, values with the same capital letters, do not differ statistically from each other (P<0,05). In the lines, values with the same lower cases are equivalent (P>0,05) (Kruskal-Wallis test).

Table 2. Average values of the apoptotic index, according to the treatment and to the experimental period.

	Period (days)		
Groups	**1-7**	**1-14**	**1-21**
Control	$0,14^{Ab}$	$0,11^{Bb}$	$0,17^{Aa}$
Treated	$0,17^{Aa}$	$0,28^{Aab}$	$0,22^{Aa}$
Residual		$0,25^{Aa}$	$0,29^{Aa}$

In the columns, values with the same capital letters do not differ statistically from each other (P<0,05). In the lines, values with the same lower cases are equivalent (P>0,05) (Kruskal-Wallis test).

HISTORY OF THE WESTERN POULTRY DISEASE CONFERENCE 1952-2001

Carol Cardona[A, B], Arnold Rosenwald[A, B], Bryan Mayeda[A]

[A]American Association of Avian Pathologists' History of Avian Medicine committee, Western group
[B]Veterinary Medicine Extension, Surge III, Rm. 1383, University of California, Davis, Davis, CA 95616

The Western Poultry Disease Conference (WPDC) had its humble beginnings at the Rosemont Grill in Sacramento in the summer of 1951. Three veterinarians, Arnold Rosenwald from the University of California, Davis (UCD), Paul DeLay from the Animal Pathology Laboratory in Sacramento, and Don Stover of the California Department of Agriculture in Sacramento met and decided to organize a meeting

which would attract veterinarians interested in poultry diseases from the Western United States. They were frustrated because Western poultry disease workers were not able to discuss and interact with people making discoveries in other parts of the world. They were inspired to begin a regional meeting by their colleague, Dr. Bill Hinshaw, one of the founders of the Northeastern Conference on Avian Diseases (NECAD) who had come from Massachusetts to UCD in the early 1930s. They named the meeting they organized the Western States Poultry Disease Worker's Conference, which was changed to the Western Poultry Disease Conference at the 6th meeting in 1957 (3).

The first conference, like the next 16 (with one exception) was held in conjunction with the annual Midwinter conference of the California Veterinary Medical Association (CVMA). Forty-two veterinarians attended and Dr. "Rosy" Rosenwald served as the first program chairman. The single session ran from 9:00 am to 12 noon and consisted of a general discussion following each of three formal presentations. The group met in the Veterinary Science Building (Haring Hall) on the UCD campus (3).

The second meeting had a full day's program and more than 50 veterinarians attended. There were no formal presentations but nine discussion leaders were selected in advance of the meeting. Scheduled discussions on presentations and current topics were regular and important parts of the early conferences. An innovation was added to the program of the 6th WPDC when a second speaker was asked to comment on the presentation of a principal speaker to further topical discussion (3). As the program was filled with more and more scientific papers, the formally scheduled periods of discussion decreased in length and frequency. Discussion and comment remained an important part of the WPDC but the tone and level of the commentary changed when the publication of the proceedings in advance of the conference began in 1986 (4).

Dr. Osteen from the United States Department of Agriculture, Washington, D.C. who spoke at the 3rd conference was the first WPDC speaker from outside the Western United States. He was the first of many non-Western speakers who have come to the WPDC over the years. The names of the first speakers from outside the United States are lost to history. But, they have been followed by a continuous flow of international presenters from all over the world who have added to the quality and scope of the conference.

The 7th WPDC was held in Pullman, Washington. It was the first of several meetings held outside of Davis, California and the first not held with the CVMA meeting. The next WPDC held outside of Davis was the joint WPDC and Asociación Nacional de Especialistas en Ciencias Avícolas (ANECA) conference in Acapulco, Guerrero, Mexico in 1980. Since then, three meetings have been held jointly with ANECA in 1986 (Puerto Vallarta, Jalisco), 1991 (Acapulco, Guerrero), and again in 1996 (Cancún, Quintana Roo). The only other meeting held in the United States outside of California since the 7th WPDC was the 38th conference held at Arizona State University in Tempe, Arizona. The first Canadian meeting was held in Vancouver, British Columbia in 1999. From 1990 to 2000, the home meeting site of the WPDC became Sacramento, California rather than Davis. The 37th WPDC in 1988 was the last held on the UCD campus until its return this year for the 50th anniversary of it's founding, 2001.

The 1986 meeting in Puerto Vallarta, Guerrero, Mexico was probably the most memorable for the attendees. At about midnight on the first night of the conference, a 7.0 magnitude earthquake hit Puerto Vallarta. The official hotel of the WPDC was not seriously damaged but reportedly shook and swayed alarmingly during the quake. Many of the participants, fearing aftershocks, spent the remainder of the night, sleeping on the beach outside the hotel, in truck beds in the parking lot, or in cars on the sand. Other conference attendees enjoying the Mexican nightlife in the area never felt the quake.

Proceedings. The first proceedings appeared in 1956 at the 5th WPDC. They were mimeographed and distributed after the meeting by Rosy and the UCD Agricultural Extension Service (AES) secretaries (3, 4). The proceedings for the 6th, 7th, and 8th meetings were all similarly created. In 1961, Rosy succeeded Dr. P.P. Levine as editor of *Avian Diseases* (AD) (2), the WPDC program was printed in the journal, and no proceedings were produced. WPDC presenters were encouraged to submit manuscripts for publication in AD after the meeting. The proceedings were revived for the 15th meeting in 1966, when Rosy turned the editorship of AD over to Dr. Leland Grumbles (2). The UCD AES secretaries typed the 16th proceedings, as in previous years, but for the first time, the AES publications at the University of California, Berkeley printed them. When AES publications discontinued printing the proceedings, the publication and mailing costs became conference expenses and a charge for WPDC proceedings was instituted in 1969 to defray the costs. In 1987, Dr. Marcus Jensen of Brigham Young University (BYU) became the proceedings editor and the printing of the proceedings was transferred. Dr. Jensen brought the WPDC proceedings into the digital age by being the first to encourage the submission of papers on a computer diskette. The appearance of the cover gracing modern WPDC proceedings first appeared in 1987 at the 36th meeting. Dr. Craig Riddell was the proceedings editor from 1997 through 1999 and was the first editor to

accept papers for the proceedings submitted by Email. Dr. David Frame is the editor of the 2001 WPDC proceedings.

Financial matters. The WPDC began as a small, regional meeting and had little need for an income or budget. The conference grew quickly and in 1960, the announcement was made that "contributions of $2.00 per person to cover the cost of mailings, speakers' expenses, etc. would be accepted" (3). In 1961, the contributions from attendees were bolstered by a $300 contribution from Merck and Co., courtesy of Dr. Otto Siegmund, the first corporate donation to the WPDC (3). Corporate contributions "in kind" became and have remained an important source of support for the WPDC. In 1968, a committee was formed to look more seriously at fundraising activities and in 1969 a fundraising structure was initiated by Dr. McCapes and permanently adopted. The WPDC program was free until 1968 when registrants were charged $10 to attend the meeting. Student and speaker registrations remained free until a separate fee schedule was begun in 1992.

Organization of the WPDC. The WPDC has had a rather casual organization over the years, although discussions of a constitution and formalized organizational plans have transpired. The first constitutional discussion occurred in 1962 at the 11th WPDC. The question was addressed at the business meeting when Dr. Harold Chute of the University of Maine related to the group that the NECAD had never adopted a constitution in its 34-year history and had always operated informally. The group voted to continue to without a formal constitution. In 1984, however, the question arose again and a constitution was adopted. The constitution requires that an annual conference called the "Western Poultry Disease Conference" be held in the Western region. An ad hoc committee was appointed in 1984 to develop by-laws but has remained inactive.

The officers of the WPDC began as a program chairman (Rosenwald) at the first meeting and at the second meeting expanded to include a president (DeLay), program chairman (Rosenwald), and secretary/treasurer (Rosenwald) (3). The president and program chairmen were elected annually at the business meeting and in 1964 the practice whereby the past-program chairman became the president began. Today, only the program chairman-elect is selected annually at the WPDC business meeting. In 1960, Dr. Walter Sadler from UCD became the permanent treasurer of the WPDC (3), an office he retained until his retirement in 1978. Dr. Sadler's influence on the WPDC was immense and the account structures and financial interactions with UCD, which he created, remain largely unchanged today. Rosy succeeded Dr. Sadler combining the offices of executive secretary and treasurer for the first time. Rosy remained the treasurer of the WPDC until 1996 when Dr. Richard Chin of the California Animal Health and Food Safety Laboratory (CAHFS) succeeded him.

In 1963, Rosy was selected to become the WPDC's permanent secretary. It was decided that as long as a permanent secretary was retained, the informal basis of the WPDC could continue. Rosy was succeeded as secretary by Dr. Chalquest from the University of Arizona in 1987. Dr. Chalquest was secretary 1988-1989 and was succeeded by Dr. Richard Yamamoto from UCD. Dr. Yamamoto was the WPDC executive secretary from 1990 until 1993 when Rosy returned as the secretary/treasurer, an office he held until 1996 when Dr. Richard Chin succeeded him.

A long-range organizational plan was recommended in 1988. An ad hoc committee led by Dr. Art Bickford recommended the election of eight WPDC officers. The existing slate of officers was to be expanded to include a local arrangements chairperson, local arrangements chairperson-elect, and program chairman-elect. The committee also recommended that a support committee with a representative from the 1) Northwest and Canada, 2) California, 3) Mexico, and 4) other Western states be formed. By 1989, a representative from the Northwest and Canada had been selected but none others. Once again, the informal character of the WPDC came through and no additional action was taken on the formation of a support committee. At the 41st WPDC, the local arrangements election schedule was changed when Dr. Bryan Mayeda became the permanent local arrangements chairman. Dr. Mayeda handled local arrangements for the 41st, 42nd and 43rd WPDCs, all held in Sacramento, California. In 1994, Dr. Leslie Woods (CAHFS) joined Dr. Mayeda as local arrangements co-chairman and became chairman in 1995. Dr. Woods remained local arrangements chairman until 2000 when Dr. Carol Cardona (UCD) succeeded her.

Workshops. The WPDC began a tradition of holding a workshop in conjunction with its annual meeting in 1968 (17th WPDC). The first workshop was on the subject Poultry Disease Control Programs and Drs. McCapes and Rosenwald were the coordinators. The proceedings of the workshop and a photograph of the attendees are printed together with the 17th WPDC proceedings.

At the 1992 business meeting, Dr. Art Bickford mentioned the possibility of holding continuing education programs for members of the newly formed American College of Poultry Veterinarians (ACPV) prior to the WPDC. Dr. Hugo Medina and Dr. Bruce Charlton organized the first of several highly successful ACPV continuing education programs. Ninety-two people took part in the first ACPV

continuing education program, the *Poultry Mycoplasma workshop* held in 1994 on the UCD campus.

Poultry Health Symposium. The California Poultry Health Symposium (PHS) was organized by Rosy to immediately follow the WPDC. The PHS was an effort to directly extend the information of the WPDC to the poultry industry of California. This meeting was open to poultry producers, farm managers, and members of allied industries. The first PHS was held in 1967, following the 16th WPDC and continued until 1982. The proceedings from the symposia were published separately for its first three years. But, the 19th – 31st WPDC proceedings were published jointly with the proceedings for the 4th to the 16th PH symposia.

The PHS name was resurrected in 1996 when Dr. Joan Jeffrey, poultry extension veterinarian at UCD once again began an annual meeting on poultry health topics for producers. The 2001 PHS will be convened immediately following the 50th WPDC in Davis and will be the first to follow the original model of the PHS since it was discontinued in 1982.

People. There have been many people whose efforts over the years on behalf of the WPDC have given it quality, longevity, and its own individual character. Many of those individuals have been honored in a variety of ways over the years for their contributions and service. In 1992, a Special Service award was instituted to honor persons who have made a significant contribution to avian medicine in the West in teaching, research, extension, or conference activities. The first honorees were Dr. Henry Adler from UCD (posthumously), Dr. Raymond Bankowski from UCD, and Dr. Charles Whiteman from Colorado State University. All of the individuals who have been honored with Special Service awards are listed on the front pages of the conference proceedings.

Many people who have made special contributions to the WPDC have been honored with the dedication of the proceedings. The first person honored with a dedication was Dr. Hector Bravo in 1977 (26th WPDC), a well respected and well-known veterinary management expert from Mexico who was honored after his tragic death in an airplane crash (4). The 37th and 40th proceedings were dedicated to Rosy, fittingly the only double dedicatee. All persons honored with a dedication are listed on the front pages of the conference proceedings.

The WPDC has embraced its Canadian and Mexican participants and they have been an integral part of the conference. Dr. Craig Riddell became the WPDC's first Canadian program chairman in 1974 and it's president in 1975. The next Canadian to be WPDC program chairman and president was Dr. Keith MacMillan in 1999 and 2000 respectively. Dr. Gabriel Galvan was the conference's first and only Mexican program chairman and president for the 25th and 26th WPDCs. There have been ANECA program chairmen in 1980, 1986, 1991, and 1996 who worked closely with the program chairmen of the WPDC but never another WPDC program chairman or president from Mexico.

The WPDC has been an international meeting almost since its inception and one innovation in particular deserves mention for the role it played in furthering the accessibility of the conference to international attendees. The WPDC became the first and the only regional poultry veterinary conference to offer simultaneous translation of its sessions into Spanish. In 1975, Dr. Dean Young convinced Elanco Animal Health to donate the use of their translating equipment to the WPDC. This equipment was difficult to manage and required almost constant battery changes, a task Dr. Young took on himself during the conferences. Dr. Ben Lucio-Martinez, Dr. Victor Mireles, and Dr. Mariano Salem generously provided translation services at the 24th through the 47th WPDCs. Dr. Pedro Villegas joined them as a translator for the joint 45th WPDC and 21st ANECA conference in 1996.

Among the many loyal and constant attendees of the WPDC, Dr. Don Zander and Dr. Bryan Mayeda had a running contest as to who would miss the first conference. These two renowned Western poultry veterinarians were both present at the 1st meeting and attended every WPDC until the 7th in Pullman, Washington when Dr. Mayeda failed to attend. Dr. Zander attended every WPDC until 1998 when an illness prevented him from attending the 47th conference. The attendees of the meeting signed a copy of the proceedings and sent it to this friend of the WPDC. Dr. Mayeda continues his tradition of attending all Sacramento and Davis meetings and will attend again in 2001. Dr. Mayeda reminds us that his own record of attendance pales in comparison to that of Dr. Zander who had to travel from the state of Washington to attend the WPDC while Dr. Mayeda had only 15 miles to travel from Sacramento to attend the Davis meetings. Dr. Zander was honored with the dedication of the 45th proceedings and the 46th proceedings were dedicated to Dr. Mayeda.

WPDC traditions. In 1952, Davis regulations prohibited the sale of alcoholic beverages within three miles of the campus. This meant few restaurants and no bars in the city limits. And the beginning of the welcome reception was really an outgrowth of very early conferences when the last and only northbound train, left at 7:30 pm. Participants heading home to Oregon, Washington, and western Canada were left with nowhere to get a meal in Davis. The answer was New Mexico chili and beans and a Scotch drink

(Bobby Burns) at someone's home. As the number of people attending the WPDC grew, the receptions grew into the infamous wall-to-wall affairs with drinks, snacks, and the famous "turkey fries" in great supply. Thus, what is today known as the welcome reception really began as a farewell party.

A final word. The WPDC has changed and grown over its 50-year history as we have chronicled here. Over the years, one aspect of the conference has remained constant, its focus on poultry health. The proceedings of the meetings accurately reflect the changing disease concerns and approaches to management and treatment over this 50-year period (1). In the early and mid-fifties, *Salmonella pullorum*-contaminated vaccines caused severe losses for the poultry industry. Discussions about the problem and its solution dominated the early meetings (4). Unfortunately, since proceedings were not made for those early meetings no written record remains of those discussions. Marek's disease (MD) devastated the poultry industry in the sixties. The number and tone of the papers in the proceedings of the 16th through the 25th WPDCs reflect the intense concern and later the triumph of controlling MD. In the early 1970's the poultry industry in the West was challenged with an outbreak of exotic Newcastle disease, then called viscerotropic, velogenic Newcastle disease. Exotic Newcastle disease (eND) was diagnosed in chickens in Fontana, California in the fall of 1971 and its control changed the face of the Western poultry industry. The 1972 and 1973 proceedings (21st and 22nd) contain a series of papers describing the outbreak, reviewing the disease, outlining vaccination protocols, and evaluating the economic impact of the outbreak. In 1974, a special workshop on *Exotic Avian Disease—Prevention, Control, and Eradication* was held in conjunction with the 23rd WPDC and its papers are included in the proceedings. That workshop provided an opportunity to learn from the practical and philosophical lessons of the eND outbreak in California. In the decade of the nineties, a steady stream of papers on the epidemiology and control of *Salmonella enteritidis* reflect the emerging importance of this agent in issues facing the poultry industry. Throughout the history of the WPDC, poultry veterinarians have sought answers to the disease problems of the day. The pages of the compiled proceedings are filled with innovations in treatment such as antibiotics, vaccines, coccidiostats, methods and agents for pest control and regulatory programs. Some of these innovations have thankfully disappeared but some of them have contributed greatly to poultry medicine. The proceedings are now fully available in digital format, thanks to Dr. Rich Chin, representing another advance for the conference. The rise and fall of diseases, the effects of vaccines, medications and changing management systems are reflected in the pages of the WPDC proceedings. We encourage you to explore these pages both for their scientific content and for the history they embody.

REFERENCES

1. Bell, D. Western Poultry Disease Conference Index 1966 - 1999, http://animalscience.ucdavis.edu/extension/avian/wpdc1a.htm
2. Grumbles, L.C., and C.F. Hall. The history of avian medicine in the United States. VIII. The American association of avian pathologists. A thirty-year history. Avian Dis. 32:175-194. 1988.
3. Stover, Don. History of the Western Poultry Disease Conference-1951-63. University of California Agricultural Extension Service. 19 pgs. 1964.
4. Unpublished observation, Dr. A.S. Rosenwald.

As an aside, Rosy has not attended all of the WPDCs. He missed the 3rd meeting when he was on sabbatical from UCD at the University of Wisconsin to complete his PhD degree. He also missed the 7th and 12th conferences.

EARLY DAYS OF MYCOPLASMA TESTING IN CALIFORNIA TURKEYS

B. A. McCrea[B], J. S. Jeffrey[AB], J. C. Voris[C]

[A]Departments of Veterinary Extension and Population Health and Reproduction
[B]Poultry Health and Food Safety Laboratory, University of California-Veterinary Medicine Teaching and Research Center, Tulare, CA 93274
[C]University of California-Kearney Agricultural Center, Parlier, CA 93648

Today's commercial turkey industry owes a great deal to individuals and organizations dedicated to controlling vertically transmitted diseases caused by the *Mycoplasma* species. *Mycoplasma* infection in

turkeys was first described in 1905 (3) and was commonly known as "swellhead", "sinusitis", or "roup". Later, in 1938, the name "infectious sinusitis" was given by Dickinson and Hinshaw (2). The recognition of the infectious nature of the disease condition opened the door to increased scientific inquiry of the causative agent. *M. gallisepticum*, *M. synoviae*, and *M. meleagridis* subsequently became targets of disease control programs (6). Hallmark organizations such as the National Poultry Improvement Plan (NPIP) and National Turkey Improvement Plan (NTIP) were organized to provide funding and to create uniformity in testing procedures. The NPIP, founded in 1933 as a voluntary program to control Pullorum Disease, grew to include the testing of turkeys and other species. The NTIP was established in 1943 with the aim of applying NPIP testing methods to turkey flocks (5). The development of the tube agglutination test by 1927 (1), the rapid whole-blood stained-antigen test in 1931, and the rapid plate serum test in 1927 (4) were essential to identifying "reactor" birds and establishing *M. gallisepticum*, *M. synoviae*, or *M. meleagridis* free flocks. Serologic testing combined with antibiotic egg dipping allowed for the attainment and maintenance of breeding flocks that were "Mycoplasma-Clean". The California breeding program, established by 1947 under the NTIP, benefited from multiple contributions by veterinarians such as Dr. Henry E. Adler, Dr. Richard Yamamoto, and Dr. Walter W. Sadler in mycoplasma research (1).

REFERENCES

1. Biddle, G. 1989. Western Poultry History. In: Pacific Egg and Poultry Association, pp. 341-349.

2. Dickinson, E. M., and W. R. Hinshaw. 1938. Treatment of infectious sinusitis of turkeys with argyrols and silver nitrate. J. Am. Vet. Med. Assoc. 93:151-156.

3. Dodd, S. 1905. Epizootic pneumo-enteritis of the turkey. J. Comp. Pathol. Ther. 18:239-245.

4. Graham, R., F. Thorp, Jr., J. P. Torrey. 1933. A study of Pullorum Disease diagnostic tests. Poultry Science. 12:112-119.

5. Hanke, O. A., J. L. Skinner, J.H. Florea. 1974. In: American Poultry History 1823-1973. American Poultry Historical Society, Inc., pp. 285-288.

6. Kleven, S. H. 1997. Mycoplasmosis. In: Diseases of Poultry 10th ed. B.W. Calnek, H.J Barnes, C. W. Beard, L. R. McDougald and Y. M. Saif, eds. Iowa State Univ. Press. Ames, Iowa.

ERRATA: WAS IT ONLY PSEUDOTUBERCULOSIS (P.ps.) IN TURKEYS?

A.S. Rosenwald, C.J. Cardona

Veterinary Medicine Extension, Surge III, Rm. 1383, University of California, Davis, Davis, CA 95616

In 1940 Beaudette (1) reported a case of *Pasteurella pseudotuberculosis* (P.ps.) in a blackbird along with a comprehensive international review. He indicated that the organism had never before been positively identified from any US outbreaks of disease in birds and was almost ostracized by the AVMA poultry committee for a lengthy report on such an unimportant subject.

Rosenwald and Dickinson (4) reported on the occurrence of a disease in turkeys, which appeared in the fall of 1940, and again in the fall of 1941. Based on known facts discussed by Beaudette (1), bacterial isolation, and observation, they made a diagnosis of P.ps. (pseudotuberculosis).

Several outbreaks of a disease marked by anorexia, diarrhea, droopiness, lameness in a few, and sudden death of some affected birds occurred in a few turkey flocks in Oregon during the fall of 1940. Postmortem findings were not constant, although miliary, light-colored areas of necrosis and infiltration in the liver and spleen and catarrhal enteritis were the lesions most regularly noted.

--Rosenwald and Dickinson, 1944

Several of the dead and ill birds were examined and P.ps. was recovered from some of the affected birds. Guinea pigs were inoculated with pure P.ps. from the turkeys and subsequently died with gross lesions consistent with those described by Moss and Battle (2). The organism was recovered from them postmortem. One white leghorn pullet was inoculated intravenously with live P.ps. culture, died and P.ps. was recovered postmortem. A turkey fed the liver of an infected turkey became sick, but survived, and surprisingly no P.ps. could be recovered. Neither histopathological studies nor virus isolation was attempted on any of the affected turkeys. Thus, although P.ps. was involved in the turkey disease outbreaks, Koch's postulates could only be partially fulfilled.

Years after this report was published, the senior author of this paper read a review of reticuloendotheliosis virus (REV) strain T by Robinson and Twiehaus (3). That paper sparked a recollection of this case of disease outbreaks in turkeys published 30 years earlier. Based on the gross appearance of affected turkeys, the author is convinced a mistaken diagnosis was made that he would like to correct. As late as 1984 the senior author begged the editor of *Diseases of Poultry* to correct references in the text to the 1940-42 turkey problem. Though there is no way to prove the true causes of these disease outbreaks, the appearance of the gross lesions, the course of the disease, the inability to reproduce the lesions seen and the lack of signs seen in turkeys inoculated with pure cultures of P.ps. strongly indicate that the 1940-42 outbreaks reported, were due to P.ps. and REV.

REFERENCES

1. Beaudette, F.R. A case of pseudotuberculosis in a blackbird. J.A.V.M.A. 98: 151-157. 1940.

2. Moss, Emma S., and John D. Battle, Jr. Infection with Pasteurella pseudotuberculosis rodentium of Pfeiffer. Am. J. Clin. Path. 11:677-699. 1941.

3. Robinson, F.R. and M.J. Twiehaus. Isolation of the avian endothelial virus (strain T). Avian Dis. 18:278-288. 1974.

4. Rosenwald, A.S. and E.M. Dickinson. A report on Pasteurella pseudotuberculosis infection in turkeys. A.J.V. R. 16:246-249. 1944.