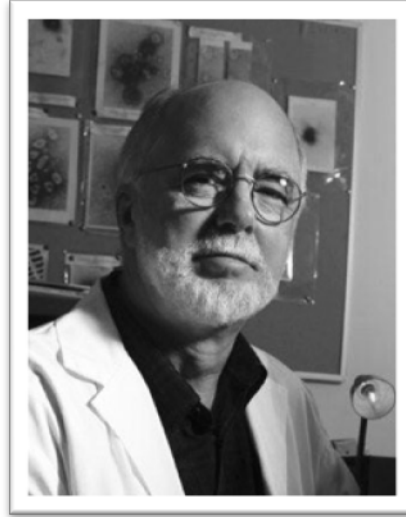# PROCEEDINGS OF THE FIFTY-NINTH WESTERN POULTRY DISEASE CONFERENCE

April 19-21, 2010 Vancouver, BC, Canada

59TH WPDC SPECIAL RECOGNITION AWARD

JOHN ROBINSON

The Western Poultry Disease Conference (WPDC) is pleased to present the 59th WPDC Special Recognition Award to Dr. John Robinson.

With humble beginnings as a farm boy on the golden slopes of Eastern Washington, John Robinson ("JR") has always maintained his innate inquisitiveness and deep appreciation for the beauty and mystery of the natural world. His personal dedication to science was inevitable.

JR received his DVM from Washington State University in 1968 directly followed by an MSc (1970) and PhD (1973) from the University of Wisconsin. His graduate work focused on avian influenza, an experience which firmly set the direction of his career.

After a brief tenure as an assistant professor at Oregon State University, JR followed his heart north to British Columbia where he began his illustrious 35+ year career as Head of the Virology Section of the Provincial Veterinary Diagnostic Lab in Abbotsford.

JR is a dynamic scientist whose enthusiasm and passion has overseen the development of an internationally recognized diagnostic virology service for BC livestock, pets, and aquaculture. From turkeys to frogs to shellfish, to John the host species really doesn't matter; it is the viruses that will always intrigue and challenge him.

JR is the silent hero of the BC Poultry Industry. His efforts over the last 35 years have assisted the commercial poultry industry in achieving an unprecedented positive health status through the development and application of state of the art diagnostics. In 2004 John breached the pinnacle of his career by detecting and sequencing the H7N3 viruses of the HPAI outbreak in BC commercial poultry. He has managed the Virology Section through three outbreaks of Notifiable Avian Influenza with unfaltering endurance and selfless commitment.

Through his tenacity, approachability and sincerity he has earned the admiration and respect of his colleagues, his co-workers and his clients. In 2006 he was selected as the "Laboratorian of the Year" by the Canadian Animal Health Lab Network. He has also been awarded the BC Premier's "Award of Excellence" in recognition of his extraordinary efforts during the HPAI outbreak.

An inveterate scientist, he is an infinite resource for technical information relating to animal viruses. He is a committed professional whose level of dedication and compassion is an inspiration to all those who know him.

His only comment when hearing of his nomination for this award was "I'm just a guy doing his job." And that says it all.

SPECIAL ACKNOWLEDGMENTS

59th WPDC CONTRIBUTORS LIST

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

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

PROGRAM CHAIR-ELECT
Dr. Larry Allen
Animal Health Branch
California Dept. of Food & Agriculture
1220 N Street, A-102
Sacramento, CA 95814

CONTRIBTIONS CHAIR
Dr. Yan Ghazikhanian

PROCEEDINGS EDITOR
Dr. David Frame
Utah Veterinary Diagnostic Laboratory,
Central Utah Branch
1451 South Main
Nephi, UT 84648
Office: (435) 283-7586
Cell: (435) 851-2233
david.frame@usu.edu

SECRETARY-TREASURER
Dr. Richard P. Chin
California Animal Health & Food Safety
 Laboratory System - Tulare
18830 Road 112
Tulare, CA 93274-9042
rpchin@ucdavis.edu

LOCAL ARRANGEMENTS
Dr. Stewart Ritchie

59th WPDC PROCEEDINGS

The Proceedings of the 59th Western Poultry Disease Conference are <u>not</u> refereed, but are presented as a service and a source of information to those attending the conference and to others who wish to gain some insight as to the information presented. Copies of the Proceedings are available in either book or electronic formats.

Copies of these Proceedings are available from: Dr. R. P. Chin
CAHFS-Tulare
University of California, DaviS
18830 Road 112
Tulare, CA   93274-9042
rpchin@ucdavis.edu

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WESTERN POULTRY DISEASE CONFERENCE (WPDC) HISTORY

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

YEAR	PRESIDENT	PROGRAM CHAIR	DEDICATION	RECOGNITION
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36th WPDC – 1987	D. A. McMartin	Marcus M. Jensen		
37th WPDC – 1988	M. M. Jensen	Barry Kelly	A. S. Rosenwald	
38th WPDC – 1989	B. Kelly	Masakazu Matsumoto		Louise Williams
39th WPDC – 1990	M. Matsumoto	Jeanne M. Smith		Dean Young
40th WPDC – 1991 16th ANECA	J. M. Smith Martha Silva M.	Richard P. Chin David Sarfati M.	A. S. Rosenwald A. S. Rosenwald	
41st WPDC – 1992	R. P. Chin	Rocky J. Terry	Marcus Jensen	Henry E. Adler * *(posthumous) R. A. Bankowski C. E. Whiteman
42nd WPDC – 1993	R. J. Terry	A. S. Dhillon	W. W. Sadler	Royal A. Bagley
43rd WPDC – 1994	A. S. Dhillon	Hugo A. Medina		G. B. E. West
44th WPDC – 1995	H. A. Medina	David D. Frame	W. M. Dungan* *(posthumous)	A. J. DaMassa Gabriel Galvan Walter F. Hughes W. D. Woodward R. Yamamoto
45th WPDC – 1996 21st ANECA	D. D. Frame R. Salado C.	Mark Bland G. Tellez I.	Don Zander M. A. Marquez	Pedro Villegas Ben Lucio M. Mariano Salem Victor Mireles Craig Riddell
46th WPDC – 1997	Mark Bland	James Andreasen, Jr.	Bryan Mayeda	Roscoe Balch Paul DeLay J. W. Dunsing Don Helfer D. E. Stover
47th WPDC – 1998	J. Andreasen, Jr.	H. L. Shivaprasad	W. J. Mathey	Marcus Jensen Duncan Martin
48th WPDC – 1999	H. L. Shivaprasad	R. Keith McMillan		
49th WPDC – 2000	R. K. McMillan	Patricia Wakenell	R. P. Chin	Ralph Cooper Robert Tarbell
50th WPDC – 2001	P. Wakenell	Ken Takeshita		Don Bell Art Bickford
51st WPDC – 2002 27 ANECA	K. Takeshita J. Carillo V.	Barbara Daft Ernesto P. Soto	Hiram Lasher	Bachoco S.A. de C.V. Productos Toledano S.A.
52nd WPDC – 2003	B. Daft	David H. Willoughby		Roland C. Hartman
53rd WPDC – 2004	D. H. Willoughby	Joan Schrader		G. Yan Ghazikhanian
54th WPDC – 2005	J. Schrader	Stewart J. Ritchie	W.D. Woodward	R. Keith McMillan
55th WPDC – 2006	S. J. Ritchie	Peter R. Woolcock		M. Hammarlund
56th WPDC – 2007	P.R. Woolcock	Bruce Charlton	R. Keith McMillan	M. Matsumoto
57th WPDC – 2008 33rd ANECA	B. Charlton M. A. Rebollo F.	Rocio Crespo Maritza Tamayo S.	A. S. Rosenwald* *(posthumous) A. S. Rosenwald*	B. Daft Ernesto Ávila G.
58th WPDC – 2009	R. Crespo	Victoria Bowes		G.L. Cooper
59th WPDC - 2010	V. Bowes	Nancy Reimers		John Robinson
60th WPDC - 2011	N. Reimers	Larry Allen		

MINUTES OF THE 58TH WPDC ANNUAL BUSINESS MEETING

President Crespo called the meeting to order on Tuesday, 24th March 2009, at 3:20 PM, at the Holiday Inn Capitol Plaza, Sacramento, CA. There were 24 people in attendance.

APPROVAL OF 57th WPDC BUSINESS MEETING MINUTES

The minutes from the 57th WPDC business meeting were reviewed and a motion was carried to approve them as printed in the Proceedings of the 58th WPDC.

ANNOUNCEMENTS

President Crespo acknowledged all the contributors; in particular, those contributing at the Benefactor level, which included the American Association of Avian Pathologists. She also thanked all the contributors for their generous donations. President Crespo acknowledged the efforts of the current WPDC officers for their work and participation in the organization of this year's meeting. President Crespo asked that we remember Dr. Bryan Mayeda, who passed away in July 2008.

REPORT OF THE SECRETARY-TREASURER

Dr. R.P. Chin presented the Secretary-Treasurer report. There were approximately 600 registrants for the joint meeting of the 57th WPDC and XXXIII ANECA held at the Sheraton Buganvilias, Puerto Vallarta, Jalilsco, Mexico, April 9-12, 2008. While working on completion of the budget prior to this meeting, Dr. Chin discovered additional expenses that he did not report to ANECA. Hence, he did not have a complete report. Contributions to WPDC for the 57th WPDC were $25,575, with a total income of $214,419.18. Total expenses were $173,471.52 for the meeting. Hence, there appears to be a net gain of $40,947.66. However, since WPDC kept their contributions and made expenses from of their own budget, Dr. Chin still needed to determine exactly WPDC's final accounting.

The current balance in the WPDC account was $74,638.70.

Contributions for this year's meeting (58th WPDC) were very good, considering the current economic situation. Unfortunately, as of the business meeting, we had only 182 registrants. This is down approximately 10% from the previous meeting in Sacramento. Estimated expenses for this year are approximately $71,000 due to increase cost in travel and hotel expenses. Dr. Chin expressed his concerns regarding the increased costs. A motion was made and approved to change from two social events (welcome reception and awards banquet) to only one event (to be determined). Dr. Chin will work with the Fairmont Hotel Vancouver regarding this change to the WPDC banquet order. Dr. Chin also noted that the Executive Committee recommended increasing registration fees to $125 for speakers and $175 for early regular registration. A motion was made, seconded and approved to increase registration fees at next year's meeting.

REPORT OF THE PROCEEDINGS EDITOR

Dr. D. Frame presented the Proceedings Editor report. He was pleased to report that all papers made it into this year's proceedings. Omnipress printed the Proceedings hard copy at an approximate cost of $7.00 per book; up $1/book from 2004 (the last WPDC held in Sacramento). The CD's were duplicated by AAAP at a cost of approximately $635.

OLD BUSINESS

None discussed.

NEW BUSINESS

President Crespo reported that the WPDC Executive Committee nominated Dr. Larry Allen (California Department of Food and Agriculture) for Program Chair-elect of the 60th WPDC in 2011. There were no other nominations and Dr. Allen was elected unanimously as program chair-elect. President Crespo nominated the following officers for 2009-2010:

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

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

In 2010, the 59th WPDC will be in Vancouver, BC, Canada, at the Fairmont Hotel Vancouver.

At last year's business meeting, it was approved that WPDC would rotate every-other year to Sacramento. Hence, in 2011, the 60th WPDC will be in Sacramento, CA. The Holiday Inn Capitol Plaza has been reserved for March 20-23, 2011.

The Executive Committee voted to hold the 61st WPDC (in 2012) in either Salt Lake City, UT, or Phoenix, AZ. Following a brief discussion, no other cities were suggested. A vote was taken and Phoenix, AZ was approved.

Dr. Reimers discussed the current situation on CE credits. She said that the newly formed Association of California Veterinarians (ACV) tried to get approval to provide CE credits for this year, but was unable to do so in time. Hence, the American College of Poultry Veterinarians will provide the CE credits this year. Dr. Reimers will again see about obtaining future CE credits from ACV.

President Crespo passed the presidency to Dr. Victoria Bowes who thanked those involved in the organization of the meeting. President Bowes adjourned the meeting at 4:20 PM.

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PROCEEDINGS OF THE FIFTY-NINTH
WESTERN POULTRY DISEASE CONFERENCE

CHALLENGES FOR THE POULTRY INDUSTRY: ANTIMICROBIAL USE AND FOOD BORNE DISEASES

Dennis P. Wages

College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina

INTRODUCTION

There are numerous continuous challenges that the poultry industry faces on a daily basis. One could argue that one challenge is as important as another. Welfare, global trade, grain prices, and environmental impacts are just a few of the issues that are of major concern for the industry. However, the continuing challenges facing the industry regarding antimicrobial use and the relationship (or lack thereof) of antimicrobial use in animal agriculture with food borne illness resistance issues in humans continues to plague the industry. Antimicrobial use and food borne disease issues will be time consuming for the industry in the coming months if not years. The question is, will antimicrobial use as we know it continue? This talk is designed to provide more food for thought and ideas for debate rather than answers to the antimicrobial use and food borne disease issues. However, the answers we seek will not be found doing the same thing we have always done because that is the way we have always done it!

ANTIMICROBIAL CHALLENGE

The 1969 Swann committee issued a report from the United Kingdom that reviewed scientific information regarding antimicrobial use in animal feed. That report initiated controversy regarding the safety and use of feed grade antimicrobials in livestock feeds. The controversy continued regarding the use of antibiotics in food producing animals with the "*Salmonella* Smoking Gun" report in 1984. This report linked the feeding of low level chlortetracycline in South Dakota beef cattle to a drug resistant *Salmonella* Newport infection in humans. Since that time, National Academy of Sciences, National Research Council (9), the Heidelberg Appeal Nederland (HAN) Foundation (2), the Government Accounting Office (GAO) (5), and countless other bodies of scientists and governments have reviewed the use of antibiotics in food animals. The results of these studies have been inconclusive at best and controversial in that the scientific evidence to date does not support the link between antibiotic use in food production and human antibiotic resistance. However, we still are in jeopardy of losing the ability to use antimicrobials effectively.

Since the original "Salmonella Smoking Gun" report, animal agriculture and, especially poultry production, has been in a constant battle to retain the use of antimicrobials for disease treatment, prevention, control and growth promotion. In the 1990s, the American Veterinary Medical Association recognized the importance of the antibiotic issues and in conjunction with the American Association of Avian Pathologist, National Chicken Council, and National Turkey Federation and other food animal groups approved Principles for Judicious Therapeutic Antimicrobial Use in 2000 (7). Poultry guidelines were then drafted by the American Association of Avian Pathologists based on the AVMA approved principles (1). These guidelines were to aid the poultry veterinarian in their decision-making process prior to, during, and after antibiotic therapy. These guidelines actually reinforced procedures that were already ongoing in the poultry industry.

Antimicrobial use in the poultry industry has been a fundamental intervention strategy since the late 1950s. Even though preventative disease management is the primary focus of the industry's disease control, disease outbreaks do occur that require antimicrobial therapy. Adequate therapeutic interventions are minimal at best and have been used in the industry for the past 50 years. Most all therapeutics are administered through the drinking water and are limited in efficacy, spectrum, and total number of products available. Many of the products that demonstrated efficacy over time have been removed from the market and include: enrofloxacin, nitrofurazone, neomycin-oxytetracycline, dimetridazole, and triple sulfas. Most would argue that the removal of these products lacked scientific validation. Reaffirming that to many of us the antimicrobial use issues have become the foundation for decisions to be made based on hidden agendas and emotion and not on sound science.

The most controversial antimicrobial use has been the feed grade application. Low level feed grade antimicrobials have been used for years with good success and mechanisms for the "growth promoting effects" with low level antibiotics been studied not only in poultry but in other food animals (10). These "growth promoting" antimicrobials are added to the feed primarily as control measures for common enteric bacterial diseases, specifically clostridial infections. Hence, one major way of maintaining growth rate and

optimal feed conversion (growth promotion) is by preventing intestinal disease. History has determined the "growth promotion" use to be "sub therapeutic"; a term that has been used against all food animal industries for years, and a term that is inappropriate. The reason the sub therapeutic antibiotic use phrase was adopted years ago to describe growth promotion can be explained. Because coccidia challenges in commercially raised flocks predispose birds to necrotic enteritis, antibiotics such as virginiamycin, bacitracin, and lincomycin, etc. were added to the feed to prevent necrotic enteritis infections due to *Clostridium perfringens*. These antibiotics were needed because historic coccidiosis prevention programs were aimed at interrupting the coccida life cycle and were static and not cidal. Clinical coccidiosis was prevented but subclinical infection was not. It is the subclinical infection and an unhealthy intestine that leads to increased susceptibility to necrotic enteritis. Since the levels of antibiotics used in the feed to prevent and control necrotic enteritis were lower than levels used to treat active, acute outbreaks the term sub therapeutic was adopted. As an example, bacitracin could prevent necrotic enteritis at a level of 25-50 grams per ton of feed, however, if an outbreak of necrotic enteritis occurred, a level of 100-200 grams per ton was needed to treat the active infection. As a result, bacitracin at 200 grams per ton was appropriately referred to as therapeutic and 25-50 grams per ton was referred to as sub therapeutic, and the industry has suffered considerable damage because of this term.

Besides disease control, antibiotics have other modes of growth promotion, some known and some unknown (10). From a non disease aspect, theories of growth promotion from antibiotics include reducing bacterial populations that produce toxins; maintaining a stable micro flora intestinal environment; and reduced toxin formation. In stabilizing the micro flora intestinal environment, this results in minimizing the frequency and intensity of mucosal immune responses that occur often in the intestines (8). Other review papers have detailed how antibiotics affect micro flora in the intestines (11). Certain antibiotics used in chickens increase chilled and hot carcass weights; improve breast meat yield, and increase leg quarter weights (6). Studies also indicate that antibiotics alter key enzymes in the intestinal tract that may result in a growth promotion effect (3).

Intestinal health and tensile strength is important not only for the overall health of birds and growth promotion but also is an advantage at the time of processing to prevent bacterial contamination. Antibiotics, such as bacitracin, increase tensile strength and intestinal integrity that prevents the tearing of intestines during the automated evisceration process. Antibiotics that increase overall health of the intestines helps prevent contamination from intestinal breakage at processing. Besides overall disease reduction and cost benefits, growth promotion decreases the amount of feed required per pound of gain and thus reduces fecal nitrogen and phosphorous excretion in litter. This is an environmental advantage when applying litter to pastures and crops.

FACING THE ANTIMICROBIAL USE CHALLENGE

There is considerable research performed to prove the benefit of antimicrobials at all levels in the feed and very few would argue for the need for therapeutic antibiotics in feed or water. However, the low-level feed use continues to be controversial. Groups that are anti-animal agriculture are gaining public support not only to remove low level antimicrobial use but also remove all classes of antimicrobials from animal feed that are also used in humans. The Preservation of Antibiotics for Medical Treatment Act (PAMPTA) was introduced in Congress last year that gained momentum early but did not pass. This type of political pressure is not going away and will continue to be supported by those that hide behind "Public Health" as a way to sway uniformed politicians and our customers that removal of antimicrobials in animal feed will save human lives.

Questions will still be raised and concerns will be identified and addressed regarding antimicrobial use. The industry needs to continue to put science at the forefront of our decision making. Risk analysis like those performed for virginiamycin, penicillin, and ampicillin and others should continue to be put forward to those that legislate and are involved in the regulatory aspects of drug use and approval. Science should determine the outcome of this debate and not emotions and hidden agendas.

The antibiotic controversy has raised questions that need to be answered or at least debated. Should veterinarians control all aspects of antimicrobial use including feed? Should over the counter antibiotics be eliminated? Should there be an easier way to prescribe antibiotics in the feed? Can there be a mechanism put in place to allow antimicrobials to be used in feed that is not in accordance with the Feed Additive Compendium (4)? Is the pipeline to new drugs closed for poultry? Can the veterinary feed directive be improved? What will replace antimicrobials? What role will "antibiotic free" and organic production play in the industry's future? Can customers and consumers be re-educated on how and why we do what we do? The AVMA has established the Antimicrobial Use Task Force to determine how judicious use of antibiotics will be defined and may answer some of the aforementioned questions but many of the questions

need to be answered by a trusted authority. Identifying a trusted authority that all may agree with may be the most difficult challenge.

FOOD BORNE DISEASE CHALLENGE

For years when food born diseases were discussed it was apparent in that discussion that we were dealing with a contaminated food product with a pathogen that resulted in disease in humans. Now we need to include commensal organisms that "may" carry with them resistance that "may" be transferred to human commensal organisms that "may" lead to treatment failures in humans. In poultry, the two major species of interest are *Salmonella* spp*.,* and *Campylobacter* spp. and the major commensal organisms are *Enterococcus* spp.

Multiple control strategies have been utilized to decrease the incidence of bacterial contamination in fresh poultry. These strategies involve both the procession plant and live production. Theses strategies include: pre and post-chill processing plant strategies, biosecurity, vaccination, competitive exclusion, organic acid treatments, cleaning/disinfection, testing and depopulation, mannanoligosaccharides, antimicrobials, feed quality control, and experimental use of bacteriophages. Each of the above approaches alone or in combination has had varying degrees of success. Some poultry integrators have had very good success in eliminating *Salmonella* spp. from breeders using multiple control strategies. According to Food Net, the incidence of *Campylobacter* infections in humans has dropped from 23.59 cases per 100,000 in 1996 to 12.68 in 2008. Human *Salmonella* infections have basically remained relatively constant over time from 14.46 per 100,000 in 1996 to 16.20. However, in spite of the poultry industry's best efforts, food borne illness remains a top concern of integrators, public health officials, and consumers worldwide. With Russia following the European Union and restricting fresh poultry imports that have been treated with chlorine, reducing bacterial contamination on fresh product is vital.

Currently in the Department of Agriculture holds processing plants accountable for a *Salmonella* contamination standard. Plants are placed into categories based on percent contamination of post chill poultry carcasses. The categorization policy is currently being discussed to be changed. If the US *Salmonella* spp. standards change to zero tolerance like what is being proposed in Europe, then prevention strategies need to be improved, innovative and most of all, consistent. It will take an integrated, multifaceted approach to consistently reduce/eliminate *Salmonella* in fresh product. There is also great concern in the US that after *Salmonella* is addressed the same type of standards will be used for *Campylobacter* spp. contamination in fresh product.

ADDRESSING THE FOOD BORNE DISEASE CHALLENGE

Unfortunately, I have much more questions to this challenge rather than answers. Food safety issues will be a continuing challenge for the poultry industry. Will current combination food safety strategies involving live production and the processing plant be enough? Will it be addressed by irradiation of product? Do we need to change chilling methodology of fresh product? Will control of food borne pathogens at both the breeder and grow-out level be mandated? Will the role of the HAACP program be expanded? Will other antibacterial rinses be identified to "sterilize" the carcass? Will zero tolerance for both *Salmonella* and *Campylobacter* be initiated? Will *Campylobacter* tolerances at the plant level be established? At what point are the end users of our product responsible to handle food correctly and minimize food borne illness? Overall, the food safety record for poultry is excellent and we continue to provide a high quality safe product to consumers.

SUMMARY

Antibiotic intervention in poultry is one tool used in a total disease prevention and control program that emphasizes preventative disease management. Our industry does not encourage nor endorses indiscriminant use of antibiotics in our flocks. The American Association of Avian Pathologists Committee on Drugs and Therapeutics has drafted specific guidelines to promote judicious use of antimicrobials in poultry to preserve the efficacy of all antimicrobials in both poultry and human medicine (1). This effort, supported by the American Veterinary Medical Association, is endorsed by the National Chicken Council. These guidelines provide the front-line poultry veterinarian to continue to provide informed decisions regarding poultry intervention strategies. Our antibiotic arsenal is small, but when the need for antibiotic use is warranted, we need to have access to the appropriate therapeutic avenue. The low level use of antibiotics is also an important tool to prevent intestinal disease that results in growth promotion. With respect to food borne diseases, the poultry industry produces a high quality safe product and has for many years. However, the poultry industry must continue the effort to reduce the bacterial contamination from fresh poultry and is a priority.

The relationship of antimicrobial use in poultry to human antimicrobial resistance is still up for debate. Unfortunately there are those that hide behind public

health to continue to push their anti animal agriculture agenda and will not be satisfied until either the bacteria or antibiotics are taken out of the antimicrobial resistance equation.

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PERFORMANCE OF ABF TURKEY ANTIBIOTIC-FREE COMMERCIAL TURKEY BROILERS: PERFORMANCES FROM TWO COMMERCIAL FARMS IN ONTARIO

Eng H. Lee, Majed Al-Attar, and Tom Cosstick

Vetech Laboratories Inc.131 Malcolm Road, Guelph, Ontario, Canada N1K 1A8

SUMMARY

Mortality rates averaged body weights and feed conversion of 21 consecutive flocks of ABF turkeys were reported here. They were all vaccinated for coccidiosis control since January 2005. The first eight flocks were on antibiotics but, from January 2007 onward, only Bio Mos (Alltech) was added to their feed. In other words, they had been antibiotic-free (ABF) for nearly three years. They were raised to about 11 weeks of age and averaged around 6 kg each with livability over 96% and a feed conversion of slightly less than two. There were four flocks of heavy toms grown along side of these hens. There were no incidences of coccidiosis and necrotic enteritis reported in all five years.

Comparison of performance between these ABF with the non ABF turkey broilers, as well as toms of the same farm, as well as with those of another Ontario farm will be made. Possible reasons for the success of preventing coccidiosis and necrotic enteritis during the periods of raising these ABF turkey broilers with or without Bio Mos will be reported.

Antimicrobials are added to commercial turkey diets partly to improve performance, but mainly to control or to prevent necrotic enteritis (4). This use of antimicrobials is well established practice and has long been used and considered to be safe. However, the persistence of some antibiotic resistant bacteria recovered from poultry, has led to regulatory changes some as severe as the prohibition of their use in Europe (3). All this has led to more production and increasing demand by consumers for drug free poultry (5).

However, the main reason cited for the reluctance to meet this demand. ABF poultry production necessitates the use of vaccination for coccidiosis control. However, it is well established that coccidiosis can be one of the predisposing factors to necrotic enteritis (1,6). Therefore, in any ABF production, vaccination against coccidiosis have to be applied and managed properly to prevent or reduce the occurrence of necrotic enteritis, a disease that can incur additional increase in production cost (2).

Here performances of ABF and non-ABF turkeys are compared and their inferences discussed.

MATERIALS AND METHODS

Data collection. Livability, condemnation, feed consumption, average daily gain body weight, and feed conversion rates of 13 ABF and 41 non ABF flocks were provided by two commercial growers in Ontario: Farm 1 (TD) provided data on 13 flocks of ABF and eight flocks of Non-ABF turkeys over a period from January 2005 to April 2009 on a total of 76,387 turkeys; and data of 512,415 of Non-ABF turkeys were provided by Farm 2 (SC) collected from January 2008 to January 2009. These are for comparison purposes. In general, hens were raised to ages of 75 to 81 days and toms from 118 to 122 days. Farm 2 was selected mainly because it provided sufficient numbers of flocks for meaningful comparison; and is representative of most commercial turkeys grown in Ontario, which is they are relying equally on either vaccination or medication for coccidiosis control but mainly on antibiotics for the control of necrotic enteritis.

Feed additives for bacterial control. All ABF birds were fed standard turkey pre starter ration containing 2 kg of Bio Mos/tonne of feed for 10 to15 days, thereafter 1 kg of Bio Mos was added to the grower and finisher feed. Non-ABF turkeys were raised on ration containing BMD or virginiamycin (Stafac) in feed.

Acid- Pak 4-Way was also added to the drinking water of toms from day one to four and later when poults were moved from the brooding barn to the growing barn as an anti stress product.

Coccidiosis control. All poults in Farm 1 were vaccinated against coccidiosis with Immucox® gel pucks (Vetech Laboratories Inc. Guelph Canada) containing *Eimeria meleagrimitis* and *E. adenoeides* at one day of age. Each puck of 100 doses was divided into two halves; and four plates placed in each brooder ring. Turkeys that were not vaccinated against coccidiosis were on monensin in their ration (100 ppm).

Post vaccination management. Two hundred vaccinated poults were kept in each brooder ring or one half square foot per poult for seven days before being set free in the brooder barn. The poults were allowed one square foot or less each, until four to five weeks of age. This proximity between poults will allow proper recycling of oocysts.

Profit Calculation. The following prices were used for calculation**:** Feed cost 400 CDN \$/ton; Immucox 3.5¢/dose; anticoccidial 9 CDN\$/ton; Bio Mos 4way 5 CDN \$/ ton; and BMF- virginiamycin 4 CDN \$/tonne.

RESULTS and DISCUSSION

Necrotic enteritis. No incidence of necrotic enteritis was observed in Farm 1 over the five years. This includes all ABF and Non-ABF birds over the five years. This is also true for all the Non-ABF birds of Farm 2 whether they were vaccinated or they were medicated for coccidiosis control. This absence of necrotic enteritis is evidenced by the high rates of livability, particularly among the broilers, as reported in Table 1.

In addition to these higher rates of livability, the turkeys appeared heavier in body weight and lower in the adjusted feed conversion as well as lower in the rate of condemnation (Table 1).

Also in Farm 2, among the non-ABF broilers, those with vaccination for coccidiosis control appeared heavier in body weight and lower in adjusted feed conversion when compared to their counterparts that were medicated with monensin in the feed (Table 1).

Among heavy toms, these differences between the ABF and Non-ABF toms hold (Table 1). However, the number of ABF heavy tom is still small in number and not very insignificant. Interestingly, the disparity in feed conversion between heavy toms of Farm 2 that were vaccinated and those medicated for coccidiosis control have the same magnitude of differences as previously reported (Lee and Cosstick ,Gobbles 2007).

Profit calculations. When comparing the profit of weight (kg) sold of ABF turkey versus non-ABF of Farm 1 they were 0.753 and 0.739 respectively. This represents a difference of 1.4 cents per kg in favor of the ABF turkeys.

This little or no difference in cost of raising ABF versus Non-ABF turkeys has been reported previously (2), from a farm without either antibiotics or Bio Mos being added to the feed. Limited access to this farm which is located at far corner of Ontario and from other turkey farms; and repeated tilling of the litter allow this farm to raise turkeys with just coccidiosis vaccination; and with no incidence of necrotic enteritis. The same practice has continued for over six years

Here, however, Farm 1 is not that isolated from other turkey farms nor has the advantage of repeated tilling; therefore, it may need the feed additives such as Bio Mos to grow turkeys.

A common feature among these two farms and the one reported previously (2) is the use of vaccination for coccidiosis control and the practice of proper post vaccination management of the litter. This appears to be adequate for now in growing ABF turkeys.

In conclusion, although in performance differences between ABF and non-ABF birds were not statistically significant, all parameters appeared to be the same or slightly in favor of the ABF birds. All these results suggest that ABF turkeys can be grown like any other commercial turkeys in Ontario.

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Table 1. Comparison of performance between ABF and non- ABF turkeys.

Types of turkeys	Farm #	Age (days)	% Livability	Weight (Kg)	F/C	Condem.	Total #	# Consecutive Flocks	Immucox®	Anti-Coccidial
ABF broiler	1	**77**	**97.39**	**5.92**	**2.01**	**0.18**	**38,900**	**9**	**+**	**-**
Non-ABF broilers	1	77.5	97.37	6.00	1.98	0.14	29,650	8	+	-
Non-ABF broiler	2	75.6	95.09	5.55	1.97	0.35	150,778	9	+	-
Non-ABF broiler	2	77.5	95.12	5.495	2.01	0.37	65,773	4	-	+
ABF heavy toms	1	**121.5**	**89.9**	**15.78**	**2.40**	**4.12**	**4,750**	**4**	**+**	**-**
Non ABF Heavy toms	2	122	88.63	15.10	2.32	1.83	139,023	14	+	-
Non-ABF heavy toms	2	118.7	88.22	15.05	2.33 (2.46)	2.19	69,197	6	-	+

LIVE PRODUCTION AND PROCESSING PERFORMANCE RESPONSES OF TURKEY HENS FED DIETS SUPPLEMENTED WITH VIRGINIAMYCIN

Hector M. Cervantes[A], K. W. Bafundo[A], M. Y. Shim[B], S. Hooper[B], and G. M. Pesti[B]

[A]Phibro Animal Health, Ridgefield Park, NJ,
[B]Poultry Science Department, University of Georgia, Athens, GA

INTRODUCTION

By the United Nations Food and Agriculture Organization's own estimates, there are nearly one billion hungry people around the world and in order to prevent an even larger number of hungry people the world must double its production of food by the year 2050 with essentially the same area of land dedicated to the production of food (1,2,3). The Food and Agriculture Organization also points out that at least 70% of this 100% increase in food production must come from improved production efficiency. Probably only second to production efficiency gains obtained through genetic selection, the antibiotic feed additives classed as "the growth promoters" have been for over half a century the most effective and reliable tool available to food animal producers to enhance production efficiency. However, following the European Union's ban on antibiotic feed additives classed as growth promoters and fueled by activist groups opposed to animal agriculture in the USA, there is a continued debate in this country regarding the use of antibiotic feed additives in poultry and livestock.

It has frequently been said by activist groups opposed to conventional animal agriculture that the efficiency gains achieved by the use of the antibiotic feed additives classed as growth promoters are too small, ranging from 0.5 to 3% and that they are not worth the potential risk of creating antibiotic resistance that theoretically could eventually impact the efficacy of human antibiotics. Since the scientific risk assessments conducted to date (both in the EU and in the USA) have not produced scientific data to support the bans, the EU resorted to the use of the "precautionary principle" in order to implement the bans, while in the USA legislative proposals to severely restrict or end the use of the antibiotic feed additives in poultry and livestock production have been introduced in Congress every year for more than a decade.

It is the belief of the authors that even today there are significant benefits derived from the use of antibiotic feed additives, so in order to demonstrate the live and processing performance benefits derived from feeding diets supplemented with virginiamycin (20 g/ton) to turkey hens, an experiment was conducted in collaboration with researchers of the University of Georgia Poultry Science Department.

MATERIALS AND METHODS

In this experiment, 744 day-old Nicholas 85 x 700 female poults were randomly assigned according to treatment to 24 pens. On the day of placement 31 poults were placed in each pen. The pens measured 11.5 ft by 7.5 ft or 86.25 ft^2, this provided 2.78 ft^2/bird. Each pen contained two hanging bucket-type feeders and one water line with 10 nipple drinkers. Target live weight for processing was no less than 14 lbs (~6.4 kg).

There were two treatments that were identical except that virginiamycin was added at 20 g/ton to all diets offered to the turkeys in treatment #2. Therefore, there were 12 replicates of 31 birds in each treatment.

All turkeys were fed a crumbled pre-starter (0 to 21 days) and starter diet (21 to 42 days), and a pelleted grower (42 to 63 days) and finisher diet (63 to 77 days). The pre-starter, starter and grower diets were supplemented with 60 g/ton of monensin for coccidiosis prevention. Diet formulation and nutrient specifications were in line with industry standards. Calculated nutrient content for each diet is shown in Table 1.

At the end of each feeding phase, all the birds and feeds were weighed in order to calculate average body weight, feed consumption and feed conversion. At the final weighing eight turkeys selected randomly and tagged at day of placement for yield evaluation were placed back in their respective pens and fed their corresponding diets, these turkeys were slaughtered the next day for the processing evaluation.

RESULTS

Live performance. The final average body weight of the turkey fed diets supplemented with virginiamycin was significantly ($P<0.05$) heavier than that of the control turkeys. Likewise, the average adjusted feed conversion of the turkeys fed diets supplemented with virginiamycin was significantly ($P<0.05$) lower than that of the control turkeys. In

addition, the calculated amount of feed required to reach an average 14 lb. body weight was significantly ($P<0.05$) less for the turkeys fed diets supplemented with virginiamycin (Table 2).

Processing performance. There was a total of 96 (8 x 12) turkeys processed for each treatment. The average live body weight of the turkeys fed diets supplemented with virginiamycin was significantly ($P<0.05$) heavier than that of the control turkeys and correlated nicely with the live performance results. Likewise, the advantage in live body weight of the turkeys fed diets supplemented with virginiamycin was reflected by significantly ($P<0.05$) heavier average hot and chilled carcass, and breast meat weights (table 3).

CONCLUSIONS

Nicholas turkey hens raised from 0 to 77 days in floor pens with feeding, watering, lighting and ventilating equipment, stocking density and diets similar to those used in commercial turkey production, showed the following advantages when virginiamycin was included in their diets at 20 g/ton:

1. Significantly heavier (+0.294 lb) final average body weight.

2. Significantly lower (-0.05) adjusted feed conversion ratio.

3. Significantly less (-0.878 lb) calculated feed consumed/turkey to reach 14 lbs.

4. Significantly heavier hot and chilled carcass, and breast meat weights (+0.337, 0.298, and +0.08 lb, respectively).

COMMERCIAL IMPLICATIONS

A production complex raising five million turkey hens/year is expected to derive the following benefits from the dietary inclusion of virginiamycin at 20 g/ton:

1. An additional 1,474,000 pounds of live weight.

2. An estimated 1,990 less tons of feed produced and delivered.

3. An additional 400,000 pounds of breast meat.

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Table 1. Calculated nutrient content of turkey diets.

	Pre-starter	Starter	Grower	Finisher
M.E. Kcal/lb.	1300	1350	1425	1475
Crude protein (%)	27.50	25.50	22	18
Lysine (%)	1.75	1.60	1.40	1.15
Methionine (%)	0.75	0.67	0.57	0.45
TSAA (%)	1.20	1.10	0.95	0.78
Calcium (%)	1.40	1.30	1.20	1.00
A. phosphorus (%)	0.75	0.70	0.65	0.55

Table 2. Final live performance results of Nicholas turkey hens fed diets with and without virginiamycin.

Treatment		Body weight (lbs)	Adjusted F.C.R.	Mortality (%)	Feed to 14 lbs.
1 (Control)	Mean	14.371	1.907	3.226	26.367
	S.E.	0.284	0.059	3.890	0.926
2 (VM*)	Mean	14.665	1.857	4.106	25.489
	S.E.	0.319	0.064	3.395	1.001
Difference		0.294	-0.050	1.344	0.878
Sig. prob.		0.047	0.032	0.602	0.047

*VM = Virginiamycin.

Table 3. Processing performance results of Nicholas turkey hens fed diets with and without virginiamycin.

Treatment		Live weight (lbs)	Hot carcass weight (lbs)	Chilled carcass weight (lbs)	Breast meat weight (lbs)
1 (Control)	Mean	14.472	11.402	9.211	2.291
	S.E.	0.097	0.085	0.081	0.030
2 (VM*)	Mean	14.846	11.739	9.509	2.371
	S.E.	0.097	0.082	0.075	0.017
Difference		-0.374	-0.337	-0.298	-0.080
Sig. prob.		0.012	0.009	0.013	0.029

*VM = Virginiamycin.

CHANGES IN ANTIBIOTIC RESISTANCE OF *ORNITHOBACTERIUM RHINOTRACHEALE* ISOLATES

Kakambi V. Nagaraja and Anil J. Thachil

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

Antibiotics are designed to fight bacteria by targeting specific parts of the bacteria's structure or cellular machinery. However, over time, bacteria can defeat antibiotics in many ways. *Ornithobacterium rhinotracheale* (ORT) has been identified as an emerging respiratory pathogen in turkeys and chickens (1,2). *Ornithobacterium rhinotracheale* is a Gram negative, non-sporulating, non-motile, rod-shaped and pleomorphic bacterium of the rRNA superfamily V. *Ornithobacterium rhinotracheale* has a separate generic status and it is distantly related to the members of the subgroup Flavobacter from Flavobacter-bacterioides phylum (1,3,4).

The pathogen has been reported worldwide and it has been incriminated as an infectious respiratory agent in poultry. The organism has also been isolated from wild birds. In turkeys and chickens, ORT infection causes tracheitis, sinusitis, airsacculitis and pneumonia.

Clinically, the respiratory infection with this agent causes relatively mild signs, which are usually accompanied by increased mortality and growth retardation (4). However, adverse housing conditions, virulent strains, susceptible age and secondary infections may exacerbate the clinical signs of ORT infection (4,5). Bacteriological and biochemical characteristics of ORT have been shown to be uniform among different strains. The existence of genetic diversity in ORT strains have been examined using ribotyping, random-amplified polymorphic DNA (RAPD) techniques, and rep-PCR. They all showed a diversity among strains and reproducible fingerprints and a good level of discrimination (6,12).

The differences in serotypic relationships between isolates have been found among ORT isolates (7,8,9,10,11,12). The agar gel precipitation technique using boiled extract antigens was used to distinguish

serotypes (10,13). There have been 15 serotypes identified (A through O) (4). *Ornithobacterium rhinotracheale* serotype A is the most prevalent, reaching 95% of the chicken strains (48). Turkey isolates are more heterogeneous, however serotype A is still the most prevalent (5,48,49,54).

In turkeys and chickens, the severity of clinical signs of ORT infection is variable depending upon strain, flock management, field conditions and pre-existing infections (11,15,16,17). The clinical signs of ORT infection in both species include: Weakness, dyspnea, sneezing, nasal discharge, coughing, and cyanosis of head (15,16,17). These symptoms are similar to those found in other respiratory diseases in poultry. Therefore, differential diagnosis is recommended to properly identify the organism.

The modern techniques in the poultry industry use high-density confinement to rear chickens and turkeys. Even though these practices are economically beneficial, they facilitate the spreading of diseases and results in difficulties in the control of diseases. Several attempts to control ORT have been reported and involve primarily antibiotic therapy, vaccination and biosecurity measures.

The antimicrobial sensitivity profiles for ORT isolates have been described to be inconsistent and highly variable depending upon strain and geographic origin (18). The successful treatment of an infected flock has been described to show varying results depending upon choice of antimicrobial drug, concentration and application method (19).

We examined the susceptibility of *Ornithobacterium rhinotracheale* isolates from Minnesota to various antimicrobial agents using an agar disk diffusion method. More than one hundred isolates of *Ornithobacterium rhinotracheale* isolated between 1996 through 2010 from turkeys in the Midwest states including Minnesota were examined in this study. Our results did show a significant increase in the resistance to enrofloxacin from 4% in 1996 to 80% in 2006 (*P*<0.01). A significant increase was also noticed in the tetracycline resistance from 2% in 1996 to 50% in 2006 (*P*<0.001). All the isolates tested were found to be sensitive to tilmicosin, erythromycin, ceftiofur, and chloramphenicol. The resistance to streptomycin, gentamicin and neomycin remained consistent in most of the isolates tested. The resistance to ampicillin decreased and there was a moderate increase in the sensitivity to penicillin G sodium over these years. These changes reflect the trends of using different antibiotics in poultry over time.

In the United States, ORT isolates have been investigated by region. In a study in Delmarva Peninsula, eleven isolates from broiler chickens were found sensitive to tetracycline, erythromycin, sarafloxacin, novobiocin, nalidixic acid, lincomycin, and bacitracin and resistant to penicillin, gentamycin, and sulfadimethoxine/thrimethoprim (20). Isolates from North Carolina, Minnesota, Iowa and Wisconsin were also investigated. All 68 isolates from theses places were sensitive to tylosin, chloramphenicol, ampicillin, penicillin, erythromycin, and spectinomycin, but 7.9% of isolates were resistant to tetracycline, neomycin, and spectinomycin; 40% resistant to sulfadimethoxine/ thrimethoprim and 95% resistant to gentamycin (17). Isolates from Virginia have been found to be resistant to tetracycline and apramycin, but sensitive to other antibiotics (18). In Delaware, most of the isolates were sensitive to the common antimicrobials and were shown to be resistant to neomycin, streptomycin, and gentamycin (21). Californian isolates from three turkey outbreaks were sensitive to erythromycin and resistant to neomycin, gentamycin, streptomycin, and sulfadimethoxine/thrimethoprim (22).

In the United Kingdom, outbreaks have been treated with tetracycline with satisfactory results (18). In Germany and Netherlands, isolates were found to be highly sensitive to amoxycillin, chloramphenicol, erythromycin, and chlortetracycline, but resistant to apramycin, neomycin, gentamycin, enrofloxacin, and sulfonamide/thrimethoprim (9,23,24). In Belgium, fourteen isolates from turkeys, chickens and wild birds were examined and showed sensitivity to tylosin and resistance to enrofloxacin, tetracycline doxicycline, and sulfadimethoxine/thrimethoprim (25). Canadian isolates from an outbreak in turkeys were found to be sensitive to enrofloxacin and resistant to neomycin (26). The combination of colistine-spectinomycin has been shown to be effective in 96% of the isolates in France (27). In Egypt, isolates were 100% sensitive to chloramphenicol and amoxycillin, 75% were sensitive to danofloxacin and 25% sensitive to gentamycin, neomycin, and sulfadimethoxine/thrimethoprim (19). Isolates from outbreaks in Slovenia were sensitive to ampicillin, amoxycillin, spectinomycin, oxytetracycline, penicillin, but resistant to enrofloxacin, flumequin, gentamycin, kanamycin, neomycin, and streptomycin (28). In Spain, three isolates from outbreaks were resistant to erythromycin, colistine, sulfadimethoxine/thrimethoprim, nalidixic acid, and flumequin and sensitive to tetracycline and furazolidone (29).

Our study also showed an increase in the quinolone and tetracycline resistance in spite of its declined use in the turkey farms. The use of appropriate drug of choice is very important for future therapeutic and preventive strategies. Methods to prevent ORT infection also have included the implementation of biosecurity. Biosecurity measures intend to protect flocks from transmission of infectious agents by using good management and sanitation practices, such as climate control and chlorinated

drinking water to prevent the introduction of infectious pathogens (19). The effective cleaning of the water system and disinfection of equipment between flocks has been proven to be efficient in avoiding perpetuation of diseases in poultry (19). The application of biosecurity procedures improves and enhances the quality production of poultry products, maintaining good animal welfare and economically viable operations in commercial systems of production.

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BACTERIOCINS TO CONTROL *CAMPYLOBACTER* IN POULTRY

Norman J. Stern

USDA-ARS, Poultry Microbiological Safety Research Unit, Athens, GA

ABSTRACT

Enhanced biosecurity, fly screen controls, competitive exclusion, immunization, and phage therapies have yet to be demonstrated as commercially plausible or effective to control *Campylobacter* in poultry production. Bacteriocins (BCN; antimicrobial peptides) secreted by selected bacteria have been cultured and purified for delivery through broiler drinking waters. This farm-friendly approach has resulted in four BCN being demonstrated as highly effective in therapeutic oral treatment of both chicken and turkeys. *Campylobacter*-colonized broilers more than five weeks old (simulating ANY source of intestinal colonization) were treated with BCN three days prior to slaughter had consistently five to six log reductions of cecal colonization. The simplicity of providing BCN to flocks via medicators is a simple approach to deliver poultry to the processing plants with greatly reduced *Campylobacter*. The goal of producing raw chicken carcasses consistently containing $<10^{2.7}$ cfu/carcass is potentially available.

DEVELOPMENT OF BACTERIOCINS (BCN) TO CONTROL *CAMPYLOBACTER* SPP. IN BROILERS

The association of campylobacteriosis and mishandling of contaminated poultry meat accounts for an unacceptably high association with the disease. As demonstrated by Callicott *et al*. (1), levels of *Campylobacter* in excess of $10^{3.56}$ cfu/processed broiler carcasses were implicated in transmission of the organism to humans, resulting in human gastroenteritis. As a corollary, levels of $10^{2.72}$ cfu/processed broiler carcasses were not implicated in disease transmission. There is a need for, and responsibility by the poultry industry to apply an intervention which predictably brings control in the levels of *Campylobacter* on processed poultry meats. Monitoring the levels of *Campylobacter* on poultry flocks is the responsibility of the regulatory agencies, as is enforcing the consequences for exceeding health compromising levels produced by the industry.

We began our cooperative studies almost ten years ago with the central purpose being to identify and use enteric bacteria that would inhibit or compete with *Campylobacter jejuni* within the intestinal tract of commercial broiler chickens. Each of our *C. jejuni* antagonistic enteric isolates came from healthy, commercially-reared broiler chickens. Although we were never able to identify such antagonists which would successfully compete within the GI tract and control *Campylobacter*, these same enterics were able to produce bacteriocins (BCN) which could be produced *in vitro* and used to dramatically control the high levels of gut colonization of the target pathogen in the birds (7). Consistently, *per os* therapeutic administration of BCN in chickens colonized under commercial settings by *C. jejuni* has reduced >5 logs of the organism within the GI tract of chickens.

Unlike antibiotics, BCN are low-molecular weight antimicrobial polypeptides which are synthesized within the ribosome of bacteria and are subsequently secreted into the extracellular environment. BCN typically target bacterial cell walls

and disrupt the structural integrity of this membrane. Oftentimes antibiotics will target sub-cellular bacterial functions. When the BCN molecule comes into contact with a susceptible bacterium, ionic membrane perturbation occurs and a pore is created in the cell walls with cytolysis resulting in the target organism (4). Another significant differentiation from antibiotics is that individual target cells may survive BCN by expressing a variety of cell membrane variants, whereas treatments with an antibiotic typically result in progeny bacteria containing genetically transmissible determinants (resistance genes).

Our first published study on anti-*C. jejuni* peptides described several class IIa BCN produced by *Bacillus circulans* and *Paenibacillus polymyxa* isolates (8). The complete amino acid sequences of the antimicrobial peptides (AMP) were described in that publication and they contained the conserved concensus pediocin-like N-terminal YGNGV residues. The molecules ranged in weights from 3,214 to 3,864 daltons, and contained 30 to 39 amino acid residues in each BCN. The pI of these BCN ranged from 4.8 to 7.8. The application of BCN (B 602) to control *C. jejuni* in chickens documented use of the BCN produced by *Paenibacillus polymyxa* NRRL B-30509 (5). In replicate experiments, high doses (10^8 cfu) of four *C. jejuni* previously isolated from US poultry production operations were orally gavaged into day-of-hatch individual chicks contained in eight isolation units (IU), with two IU per *C. jejuni* isolate for each experiment. In half of the IU's, the birds received BCN amended (250 mg/K) feed from day seven through day nine post-hatch and the corresponding second half of the IU birds served as positive controls. Untreated control groups of birds were colonized at typically observed levels of $10^{6.6-8.3}$ cfu *C. jejuni*/g of cecal materials. None of the 80 BCN treated birds were detected as colonized by *C. jejuni*. These data were highly significant in demonstrating the reduction of colonization mediated by the BCN therapeutic treatment.

Subsequently, a *Lactobacillus salivarius* (NRRL B-30514) isolate originating from the cecum of a commercial broiler was used for BCN production (6). The isolate was used to ferment, purify and characterize the associated BCN (OR 7). The BCN was comprised of 54 amino acid residues, had a molecular mass of 5,123 Da, and a pI of 9.5. The BCN was susceptible to digestion by a variety of protease enzymes but was resistant to pH extremes, lysozyme, lipase or elevated temperatures of 90°C for 15 min. Using this stable material the BCN was microencapsulated and incorporated into chicken feed at levels of 250 mg/kg. Eight experimental bird trials consisting of 10 chickens in each isolation unit were conducted to test four poultry colonizing isolates of *C.*

jejuni. In these trials the untreated control birds were colonized at normally high levels of *C. jejuni*/g cecal materials. The paired OR 7 BCN treated chickens had colonization cultured at undetectable levels in three of the eight treated groups or, contained levels of no greater than a mean of $10^{1.3}$ cfu/g cecal materials. Again, the reductions in *C. jejuni* colonization were analyzed and the BCN treatment significantly reduced the target within the chicken gut.

Similar BCN treatment efficacy trials have been conducted with turkey poults (2). Using 10 poults per treatment per trial, three trials of birds challenged and colonized by *C. coli* were conducted in isolated floor pens. One pen per trial contained turkeys provided untreated feed (positive control) while the other two pens contained similarly colonized birds given feed amended with 250 mg/kg microencapsulated BCN B 602 or BCN OR 7, respectively. In the three trials the positive control birds yielded an average of $10^{7.30}$, $10^{4.91}$ or $10^{7.11}$ cfu *C. coli*/g cecal materials, respectively. The corresponding B 602 and OR 7 treated birds had undetectable levels of *C. coli*/g cecal materials with the cultural detection limit of $10^{2.00}$ cfu/g.

The next BCN producing isolate we studied was identified as *Enterococcus durans/faecium/hirae* (NRRL B-30745), which produced BCN E 760 (3). This BCN was susceptible to a variety of protease treatments, resistant to lysozyme and lipase and, was stable after exposure to a heat treatment of five min at 100°C and to pH extremes of 3.0 to 9.5. The polypeptide consisted of 62 amino acid residues and had a molecular weight of 5,362 Da with an isoelectric point of 9.5. Per-os BCN E 760 treatment of chicks infected with two isolates of *C. jejuni* significantly reduced the colonization by more than 8 $\log_{10}$ cfu/g. The same BCN E 760 also controlled colonization by commercially acquired *Campylobacter* in market aged broilers from a mean of $10^{6.16}$ cfu/g cecal materials in the untreated animals to non-detectable levels ($<10^2$ cfu/g) when administered in feed four days before analysis.

The final BCN producing isolate that we have reported on was identified as *Enterococcus faecium* (NRRL B-30746), which produced BCN E 50-52 (9). The polypeptide E 50-52 bio-chemical traits were consistent with its classification as a class IIa BCN containing a consensus N-terminal sequence of YGNGV. The BCN had a molecular weight of 3,340 Da, was 39 amino acid residues in length, and had a pI of ~8.1. A portion of the molecule was predicted to be highly hydrophilic and the remaining portion was predicted to be hydrophobic. These characteristics are consistent with the hypothesized mechanism by which BCN are thought to perturb bacterial cell walls, penetrating both the hydrophobic and hydrophilic

portions of the target cell wall. In comparative *in-vivo* studies young birds were challenged and colonized with two *C. jejuni* isolates. From day four to seven the chicks were given 31 mg of BCN per kg of feed. The cecal content of the birds from the untreated isolation units was measured at 15 days of age and contained $10^{8.47 \pm 0.47}$ cfu/g of *C. jejuni*, while the birds within the treated group did not contain detectable levels of the target organism. Similarly, commercially *C. jejuni* colonized market aged birds (41 d old; ~ 2 kg) were provided 10.8 mg BCN E 50-52 per bird in the drinking water over a three day treatment while the control group was not given therapeutic treatment. The control group of broilers contained $10^{8.0+0.90}$ cfu/g of *C. jejuni* while the ceca of the birds within the treated group contained $10^{2.90+0.23}$ cfu/g of *C. jejuni*. These results were statistically significant.

Worldwide and consistently, poultry has both been repeatedly implicated as associated with human campylobacteriosis and, farmers/producers have been unable to methodically control commercial broiler flock infections. Numerous epidemiological studies have pointed to a large number of factors that are associated with flock colonization. Manipulating these putative interventions has not resulted in a marked or consistent decrease in *Campylobacter* colonization frequency among commercial broiler flocks. Dramatically enhanced biosecurity, enhanced fly screen control, competitive exclusion, immunization, and phage therapies may hold suggestions to experimentally control flock infections but have yet to be demonstrated as commercially plausible or effective. Alternatively, BCN which are non-toxic ribosomal produced antimicrobial peptides secreted by bacteria, have been identified, cultured, and purified for delivery through broiler drinking waters. Using this farm-friendly applied approach has resulted in at least four different BCN being demonstrated as highly efficacious in therapeutic oral treatment of both chicken and turkeys. Initially, microencapsulated BCN were incorporated into feeds provided to *Campylobacter*-colonized poultry and resulted in dramatic colonization control. Subsequently, we determined that BCN provided in the bird drinking waters was equivalently effective. Mature *Campylobacter*-colonized broilers (>5 weeks old, simulating ANY potential source of intestinal colonization) provided BCN over the course of the three days prior to slaughter and processing consistently reduced five to six logs of cecal colonization. The simplicity of adding BCN to flock medicators over the last days of poultry production makes such interventions very easy for farmers and delivers flocks of poultry to the processing plants with greatly reduced potentials for transmitting *Campylobacter* for human disease. The goal of producing raw chicken carcasses consistently containing $<10^{2.7}$ cfu *C. jejuni*/carcass at a modest cost is at hand. Large-scale commercial studies to demonstrate efficacy are planned for the near future. Reduced human campylobacteriosis is anticipated through BCN treatment of poultry on the farm.

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EVALUATION OF CANDIDATE *BACILLUS* PROBIOTICS (DIRECT-FED MICROBIALS) DURING COMMERCIAL TURKEY BROODING

R. E. Wolfenden[A,B], N. R. Pumford[A], M. J. Morgan[A], S. Shivaramaiah[A], A. D. Wolfenden[A], C. M. Pixley[B], J. Green[B], G. Tellez[A], and B. M Hargis[A]

[A]Department of Poultry Science, University of Arkansas, Fayetteville, AR
[B]Pacific Vet Group USA, Fayetteville, AR

SUMMARY

Since the 1940s subtherapeutic levels of antibiotics have been used in animal agriculture, including commercial poultry production, to increase growth rate and improve feed efficiency (1,2). While these antibiotic growth promoters are still widely used, increasing pressure from both consumers and government regulatory agencies to decrease or limit the use of these compounds has led to the need for viable alternatives. One potential alternative may be *Bacillus*-based direct-fed microbials, as this type of direct-fed microbial has been shown to increase body weight as well as to decrease foodborne pathogens in commercial poultry (3,4).

Candidate *Bacillus* isolates were selected from environmental samples using a defined set of selection criteria. All candidate isolates were evaluated *in vitro* for antimicrobial activity against selected bacterial pathogens including *Salmonella* Enteritidis, *Clostridium perfringens*, and *Campylobacter jejuni*. Additionally, *Bacillus* direct-fed microbial candidate isolates were evaluated for their ability to produce high numbers of heat tolerant spores. All candidates were speciated, and only nonpathogenic species were selected for further evaluation.

In experiment 1, isolates evaluated using the above criteria were mixed with turkey starter to achieve 10^6 cfu/g of feed. Day-of-hatch turkey poults were orally gavaged with 10^5 cfu of *Salmonella* Typhimurium, randomly placed into groups, tagged, weighed, and fed control or spore-containing rations. At day 11, all poults were weighed, humanely killed, and cultured for *Salmonella* from the crop and ceca. Isolates PHL-NP122, -JH33, -MM65, -NP119B, and -NP117B were significantly heavier than negative controls ($P \leq 0.05$). Additionally, *Salmonella* was recovered significantly less frequently ($P \leq 0.5$) from ceca and crops of poults treated with PHL-MM65 and PHL-NP122 as compared to negative controls.

In experiment 2 isolates PHL-MM65 and PHL-NP122 (a *Brevibacillus laterosporus* and *Bacillus subtilis*, respectively) were further evaluated using poults raised under commercial conditions. After 7 d, 480 poults from within the house were tagged, weighted, and placed into one of four replicate pens for each treatment group (negative control, nitarsone, PHL-MM65 at 10^6 spores/g feed, or PHL-NP122 at 10^6 spores/g feed). After 30 days the poults were weighed and body weight gain calculated. PHL-NP122 (852 g), and nitarsone (853 g) were found to be heavier ($P \leq 0.05$) than the negative control (784 g), while PHL-MM65 (794 g) was not significantly heavier. Additionally, *Salmonella* was recovered less frequently ($P \leq 0.05$) from the cecal tonsils of the groups treated with PHL-NP122 (17.5%) and PHL-MM65 (23.3%) as compared to the negative control (48%). Nitarsone was not different ($P \geq 0.05$) (33.3%) from any other group.

While further study is warranted, isolate PHL-NP122 has been shown to increase body weight as well as reduce the frequency of *Salmonella* incidence in turkey poults in both commercial and laboratory conditions. Evidence presented here indicates that isolate PHL-NP122 may potentially be useful as a direct-fed microbial in commercial turkeys.

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FIELD STUDIES WITH SELECTED LACTIC ACID BACTERIAL PROBIOTICS FOR POULTRY

G. Tellez, A. Wolfenden, J. Higgins, S. Higgins, A. Torres, J. Vicente, and B. Hargis

Poultry Health Laboratory, Department of Poultry Science, University of Arkansas, Fayetteville, AR 72701

SUMMARY

Bacterial antimicrobial resistance in both the medical and agricultural fields has become a serious problem worldwide. During the last eight years, our laboratory has worked toward the identification of probiotic candidates for poultry which can actually displace *Salmonella* and other enteric pathogens which have colonized the gastrointestinal tract of chicks and turkeys. Laboratory and field data is presented, indicating that selection of therapeutically efficacious probiotic cultures with marked performance benefits in poultry is possible, and that defined cultures can sometimes provide an attractive alternative to conventional antimicrobial therapy. Data from multiple studies, focused on specific pathogen reduction, performance under commercial conditions, and effects on idiopathic enteritis is summarized.

Research conducted in our laboratory has elucidated an effective *in vitro* screening technique for identification of candidate probiotic organisms (1), and developed a defined LAB culture. These probiotic cultures have been shown, in both laboratory and field studies, to accelerate development of normal microflora in chicks and turkeys, providing increased resistance to *Salmonella* spp. infections (2,3,4,5,6,7,8, 11,12,13,15,16,17,18,19). Published experimental and commercial studies have shown that these selected probiotic organisms are able to reduce idiopathic diarrhea in commercial turkey brooding houses (7). Large scale commercial trials have indicated that appropriate administration of this probiotic mixture to turkeys and chickens increased performance and reduce costs of production (10,11,15). These data indicate that selection of therapeutically efficacious probiotic cultures with marked performance benefits in poultry is possible, and that defined cultures can sometimes provide an attractive alternative to conventional antimicrobial therapy. This LAB probiotic (FloraMax[TM]) has been licensed to a University startup company and has become one of the more successful antemortem intervention strategies used by the U.S. poultry industry to date.

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DETERMINING THE EFFICACY OF A COCCIDIOSIS CONTROL PROGRAM

Marshall Putnam

Director of Health, Wayne Farms LLC, 4110 Continental Drive, Oakwood, Georgia
marshall.putnam@waynefarms.com

Determining the efficacy of a coccidiosis control program is one of the most subjective opinion driven decisions made in live production. Decisions to change are often made on poorly supported opinions, short term data and a need to "do something" to deflect attention. Determining the efficacy of your control program should be based on as much objective data as you can gather and follow your long term objective to maximize performance at the best cost. Effective control of coccidiosis should be based on understanding bird immunity, what gives you the best long term performance, and what is most cost effective. Producing early bird immunity by promoting early oocyst cycling prevents performance impacts and minimizes the challenge on the subsequent flock.

Objectively you should understand the products available for control, how the products work and how house management and conditions affect the product's mode of action. Documenting the oocyst cycling patterns through regular posting sessions provides you with objective data you can use to judge the efficacy of your program against the objective. Cycling patterns should be evaluated not only during the usual expected time but also through the entire growing cycle to ensure cycling is not occurring in the later stages of the grow cycle when performance impacts are much more

pronounced. Oocyst cycling should be over by 35 to 40 DOA for leakage programs, 28 to 35 days for vaccine programs and low to non-existent for shut down chemical programs. Any program that removes the bird's immune system from participation carries a higher failure risk and will not work long term. Cycling late in the grow-out cycle not only affects current flock performance but also puts pressure on the next flock by elevating early challenge above normal levels.

Immune system status of the flock and the grow-out must be considered when evaluating the efficacy of the coccidiosis control program. Performance loss due to repeated failure to control cycling beyond the 35 day mark should suggest the immune system may be involved as one of the possibilities. For the success of any grow-out program an intact functioning immune system is critical.

One of the ways to judge if you are keeping the oocyst cycling in a pattern that will produce early immunity, minimize negative live performance impacts, and not impact the next flock is seeing what happens to performance when you change programs. Often operations change programs and expect to get a "bump" in performance. That usually means you have been suffering performance loss on the previous flocks and not that you are getting performance improvements from the change. For most operations the long term view of controlling oocyst challenge to produce early flock immunity will minimize cycling beyond 35 days, produces the best performance results, and improves the longevity of products available to control coccidiosis challenge.

MANAGEMENT OF COCCIVAC-D IN CAGE-REARED PULLETS TO MAXIMIZE IMMUNITY

Mike Petrik[A], Linnea Newman[B], John Barta[C], and Steve Fitz-Coy[B]

[A]St. Mary's, ON N4X 1C2
[B]Intervet/Schering-Plough Animal Health
[C]University of Guelph, Parasitology Laboratory, Guelph, ON

SUMMARY

In this Ontario study, cage-reared pullets were vaccinated with Coccivac-D via spray cabinet at the hatchery. The birds were placed in cages containing paper or fiber trays to capture feces to enhance vaccine recycling. Oocyst counts were taken from high and low cages from both recycling methods. Birds were challenged with a high dose of coccidia to determine the level of protection at the time of moving from the pullet facility to the laying house. The more durable fiber trays, lasting approximately five weeks, facilitated better immunity than the shorter-lived papers. But lower cage rows, with access to the cleaned manure belt returning overhead, developed better immunity regardless of whether paper or trays were initially used.

INTRODUCTION

Coccidiosis vaccines depend upon repeated recycling of oocysts to induce complete immunity. Full immunity to all common chicken species of coccidia (including *E. necatrix)* requires at least four to five coccidial life cycles (approximately five weeks).

Maintenance of immunity, once developed, requires periodic re-exposure to the coccidia.

Recycling occurs predictably in floor-reared birds, but not in cage-reared pullets which lack constant and uniform access to feces. Cage-reared layers in Ontario have had problems with either coccidiosis or necrotic enteritis breaks between 19 and 26 weeks of age. This may be the result of insufficient coccidiosis immunity prior to the onset of lay. This study investigated two methods to enhance recycling of oocysts for cage-reared pullets: Paper on the cage floors (lasting approximately two weeks) or a more durable fiber tray (lasting approximately five weeks).

MATERIALS AND METHODS

Commercial Bovans White and Shaver White pullets were reared in 38" x 26" cages in four tiered rows (Hellman cage system). Each row consisted of four tiers of cages. A belt system under each tier of cages served to carry manure away from the birds. Manure was scraped off with an automatic rubber scraper at the end of the row, and the cleaned belt returned over the heads of the tier below.

A full dose of Coccivac-D vaccine was applied via spray cabinet at the hatchery to all pullets. Two

methods of vaccine oocyst recycling were used: Paper lining the cage bottoms and lasting 10 days vs. paper lining plus two heavy fiber vegetable trays per cage (SHO-PAK 5.75" by 8.25") covering 10% of the cage floor and lasting five weeks. A row using paper only for recycling was compared to a row using fiber trays for recycling. Second from top and second from bottom tier cages were sampled from each row.

Sequential pooled fecal samples were collected every three days from the high tier (trays - Group 1), low tier (trays - Group 2), high tier (paper - Group 3) and low tier (paper - Group 4). The samples were split and oocysts per gram of feces were counted by Faculté de Médecine Vétérinaire, Université de Montreal ("St. Hyacinthe Lab") and Dr. Greg Mathis (Southern Poultry Research – Georgia).

RESULTS

Shedding oocysts indicated an initial vaccination take on all tiers, but oocyst numbers were low compared to typical floor-reared broilers. Periodic low-grade shedding occurred throughout the 19-week growing period in the low tiers, with a secondary shedding peak at 14 to 15 weeks, when the pullets were tall enough to reach the returning belt overhead. The high tiers did not demonstrate periodic recycling, and only one of two labs detected a minimal shedding at 14 to 15 weeks.

Fifteen birds from each of the four treatment groups were challenged with pathogenic, mixed-species challenge material. The challenge was severe: Mortality in negative control birds was 91.67%. The highest mortality came from the high tiers (paper - 75%, trays - 60%), where less recycling occurred. Lower mortality came from both low tiers (paper - 25%, trays - 0%). Tier position p = 0.0005. Birds from the tray-vaccinated rows (high and low) had lower mortality than their paper counterparts (p = 0.098). The only birds that completely withstood mortality from challenge were those that came from the low tier and used trays to induce recycling for five weeks. All groups demonstrated substantial oocyst shedding post-challenge.

Pullets from the tray-vaccinated rows were transferred to a laying house that had experienced necrotic enteritis/coccidiosis at 23 to 26 weeks of age in the previous four flock placements. Some of the prior placements had been vaccinated with Coccivac-D using paper to enhance recycling. At the time of placement of the test pullets, necrotic enteritis was a common problem for broilers in the province. Nevertheless, the pullets vaccinated using fiber trays for recycling did not break with necrotic enteritis through the end of the observation period at 26 weeks of age. Subclinical oocyst shedding occurred primarily in high tier cages and revealed a predominance of *E. necatrix*-like oocysts.

DISCUSSION

The sequential oocyst counts revealed several important points about coccidiosis in cage-reared pullets:

1) Coccidiosis vaccine will initiate recycling using either paper or fiber tray method.
2) The longer the exposure to recycling oocysts, the better the protection will be.
3) Coccidiosis exposure via the "clean" returning manure belt does occur and is important to immunity development.
4) Naïve pullets will be exposed to coccidial challenge when placed in a contaminated laying house.

The shedding of oocysts in a tiered cage system results in an infection that is amplified with each successive cage row below the top cage: Top-tier birds defecate on the belt below, contaminating it with a few oocysts. The next tier picks up those oocysts in addition to their initial infection…they shed comparatively more oocysts to the belt below, and so on. The bottom row gets the largest dose of oocysts.

This trial was a clinical success: The layer flock derived from pullets using fiber trays for coccidial recycling did not break with coccidiosis or necrotic enteritis for the first time in years.

But protection was not 100% in the upper cage tiers. Evidence of oocyst shedding was present in samples taken from the upper tier of cages after placement in the layer house.

Successful vaccination with Coccivac-D in cage-reared pullets requires a durable surface to enable coccidial recycling to occur for at least five weeks. Coverage of 10% of the cage floor (in addition to the short-duration paper lining of the full floor) did not produce adequate immunity in the face of a strong laboratory challenge, but did prevent a clinical coccidiosis outbreak under field conditions.

Based upon the relatively weaker immunity developed in the top tiers, management of pullet movement to ensure that top-tier pullets are transferred to the top tier of the layer house is recommended.

Future experimental work will examine whether a booster vaccination might enhance protection for all tiers, and provide complete protection to cages in the upper tier.

Figure 1. Fecal oocyst counts paper + fiber tray High vs. Low cage tiers.

→ Age at moving from pullet house to laying house
★ Initial Coccivac-D vaccination response
◇ Pullet house coccidiosis cycling prior to movement.
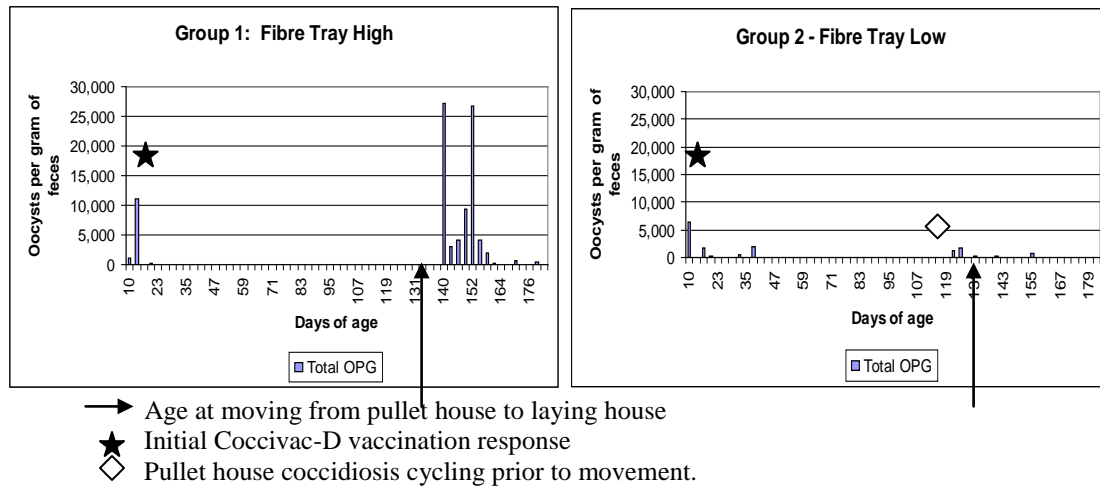
IDENTIFICATION AND CHARACTERIZATION OF RNA VIRUSES IN THE TURKEY GUT USING METAGENOMICS: AN ABUNDANCE OF PICORNAVIRUSES AND OTHER "SMALL ROUND VIRUSES"

J. Michael Day and Laszlo Zsak

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

INTRODUCTION

Enteric disease syndromes are a continual economic burden for the United States poultry industry. Poultry enteric disease is marked by diarrhea, stunting, and increased time to market; the more severe forms of enteric disease are characterized by immune dysfunction (3) and increased mortality (1,2). Numerous viruses have been detected in or isolated from the intestinal tract of poultry (both diseased and healthy), and have subsequently been implicated in poultry enteric disease. Despite the isolation and characterization of many of these suspect viruses, the etiology of the poultry enteric disease syndromes remains elusive, and many enteric viruses can be detected in otherwise healthy turkey and chicken flocks (6,7). Regional and national enteric virus surveys have revealed the ongoing presence of avian reoviruses, rotaviruses, and astroviruses in turkey and chicken flocks, with combinations of viruses often present in the poultry gut. A recent study utilizing a sequence-independent molecular screen of virus particle associated nucleic acid (PAN) in chicken enteric samples identified a novel chicken parvovirus (ChPV).

This parvovirus is a member of the *Parvovirinae* sub-family within the *Parvoviridae*, and a PCR-based diagnostic test has been developed that targets the ChPV non-structural (NS) gene (9,10). An important tool in ongoing research into poultry enteric disease would be knowledge of the complete viral intestinal flora present in the normal poultry gut and in poultry experiencing enteric signs. This would facilitate the development of updated molecular diagnostic tests, plus a more thorough knowledge of the viral constituency in the poultry gut would lead to a better understanding of the role viruses play in enteric disease and in the performance of poultry flocks in general. This study aimed to identify and assemble an unbiased library representing the RNA virus community present in the turkey gut—an RNA virus metagenome.

MATERIALS AND METHODS

With the cooperation of industry stakeholders, intestinal tracts from turkey farms in North Carolina, U.S.A. with histories of enteric disease problems were received at the Southeast Poultry Research Laboratory (SEPRL), USDA/ARS in Athens, GA, USA in October

2008 and were pooled and processed promptly into ~20% homogenates in sterile PBS. Samples representing five farms ranging in age from seven to 34 days were pooled into a single 20% homogenate for the present analysis. After an initial 5000 rpm centrifugation step to clarify the sample, a stepwise filtration process involving 0.8µm, 0.45µm, and 0.2µm cutoff filters was used minimize the presence of large particles and bacteria. This was followed by ultracentrifugation (5 h, 113,000 X G, 4°C) to pellet virus-sized particles. The pellet was resuspended in Tris-HCl buffer and treated with RNAse and DNAse to remove unencapsidated (non-viral) RNA and DNA. The Qiagen QIAmp Minelute Virus Spin Kit was used to extract RNA from the treated pellet. cDNA was generated via random hexamer priming using the Invitrogen SuperScript Choice System. This cDNA was utilized in high-throughput nucleic acid sequencing using Genome Sequencer FLX Titanium pyrosequencing technology and reagents (Roche) (5). The initial pyrosequencing runs produced in excess of 139,000,000 bases of high quality sequence with an average read length of 362. Contigs were assembled using the gsAssembler software (454 Life Sciences) using stringent parameters (50bp overlap with 95% identity). Using the assembled contigs as query sequences, the BLAST non-redundant (nr) protein database (GenBank) was searched using the blastx program. The blastx output was analyzed and contigs were assigned to taxa using the software package MEGAN.

RESULTS AND DISCUSSION

The initial pyrosequencing runs were used to assemble 6526 contigs ranging in size from 97 to 2578bp. 4563 contigs produced no hits in the nr protein database using the blastx search parameters. 724 contigs had similarity to sequences from cellular organisms, including bacteria, fungi, and vertebrates. 788 contigs had similarity to RNA viral sequences, including sequences from the dsRNA viruses (*Reoviridae* and Picobirnaviruses), and the ssRNA viruses (*Caliciviridae*, *Leviviridae*, *Picornavirales*, and *Astroviridae*). The majority of the assigned viral contigs (620) showed similarity to database sequences from the *Picornavirales* order and other picorna-like viruses, viruses that, as a group, contain a positive sense single-stranded RNA genome and a virion approximately 30nm in diameter (4). Recently, a retrospective study of electron micrographs of enteric viruses from California turkeys experiencing poult enteritis revealed a large number of "small round viruses" ranging in size from 15 to 30nm (8). These small round viruses are present in turkeys across a range of ages, but they have only been identified

morphologically, making specific identification difficult. The results of this metagenomic analysis are a step toward identifying some of these undescribed, small enteric viruses, and validate this metagenomic approach to identifying known and novel RNA viruses in the poultry gut. The sequence data generated via this approach will prove useful in the molecular characterization of the viral constituency of the poultry gut and will inform the selection of molecular diagnostic tests for enteric viruses.

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PARVOVIRUS-INDUCED ENTERITIS IN YOUNG TURKEYS

Laszlo Zsak and J. Michael Day

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

SUMMARY

Previously we identified a novel parvovirus from enteric content of turkeys that were affected by enteric diseases (1). Comparative sequence analysis showed that the turkey parvovirus (TuPV) represented a new member in the Parvovirus family and it is closely related to the chicken parvovirus. Here, we describe some of the pathogenic characteristics of TuPV in young turkeys. Following TuPV infection, two-day-old SPF turkeys showed characteristic signs of enteric disease. Viral growth in gut and shedding was detected at early times post infection (PI), which was followed by viremia and generalization of infection. TuPV could be detected in most of the major tissues for at least three weeks PI. At the end of the study (35 days PI), the virus was still detectable in the intestine, spleen, and bursa of Fabricius. Our data indicate that turkey parvovirus induces enteric disease in turkeys and may be involved in additional syndromes as well.

ACKNOWLEDGMENTS

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FILLING THE GAP: PET POULTRY FOR VETERINARY PRACTITIONERS

R. Crespo[A], D. Moore[B], S. A. Dhillon[A], R. Newberry[C], and C. Faux[D]

[A]Animal Health and Food Safety Laboratory, Washington Animal Disease Diagnostic Laboratory,
Washington State University, 2607 W Pioneer, Puyallup, WA 98371
[B]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University,
P. O. Box 646610, Pullman, WA 99164
[C]Department of Animal Sciences, 116 Clark Hall, Washington State University, Pullman, WA 99164
[D]Washington State Department of Agriculture, Animal Health Program, P. O. Box 42560, Olympia, WA 98504

Traditionally, poultry has been classified as a production animal and small animal practitioners have limited experience or knowledge of this species. In recent years however, the practice of keeping urban poultry is burgeoning in the United States. Urban poultry may be presented to local private practitioners for veterinary services. Because these small flocks interact with wild birds, they are at higher risk to succumb to foreign or emerging diseases, including influenza. Furthermore, these pet poultry present a potential interface of zoonotic disease with humans.

In response to the need for trained veterinarians and to encourage practitioners to accept poultry in their practices, Washington State Department of Agriculture and Washington State University developed a short course for veterinary practitioners to teach about poultry. In contrast to other programs this course focused on small poultry flocks and pet poultry, rather than traditional commercial poultry. The agenda covered the following areas:

1. Introduction to poultry: types of chickens, breeds, where people buy chickens, chicken behavior, and other facts about chickens;

2. Basic husbandry and nutrition: What do chickens eat, how much, variety of needs based on the purpose of the bird, types of drinkers and feeders, housing, predator protection, and principles of biosecurity;

3. Anatomy and physiology, with emphasis in normal values, weight, and normal growth and development;

4. Handling, physical examination, blood sampling, and swab sampling;

5. Common medical ailments (for example: mites, cannibalism, bumblefoot, egg binding, egg peritonitis, molting, omphalophlebitis, broodiness, coccidiosis, avian influenza, pullorum disease, infectious rhinotracheitis, exotic Newcastle disease, blood in eggs, toxins, cuts and bruises, and spurs on roosters);

6. Zoonotic concerns: avian influenza, *Salmonella*, campylobacteriosis, chlamydiosis, erysipeloid, dust allergies, etc.;

7. Medicating chickens, including euthanasia;

9. Wet lab (handling, physical examination, blood and swab collection, simple necropsy techniques and common findings); and

10. Where to turn for help for diagnostic assistance, regulatory medicine, etc.

The course was offered two times in 2009 and one time in 2010, in two different locations of Washington State. As anticipated, the majority of the participants had little knowledge on husbandry, nutrition, and even disease conditions of poultry. On the other hand, and to the organizers surprise, about 70% of the participants owned poultry. In the evaluations, 97% of the participants reported an increase in knowledge and competency with most aspects of poultry; only 3% indicated that the course was too basic and did not increase their knowledge significantly. The participants also reported that because of the course they would be more likely to accept pet poultry and that they felt more capable to provide basic care for these animals. Participants would like to continue learning about poultry and suggested more hands-on experience and case-solving training. Organizers are planning for future more specialized courses as suggested by the evaluations and similar basic training course on pet poultry for veterinary technicians.

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

USDA FSIS IN POULTRY PLANTS – A VETERINARY STUDENT INTERN'S PERSPECTIVE

Travis Schaal

College of Veterinary Medicine, Oregon State University, Corvallis, OR 97333

The Veterinary Student Employment Program (VSEP) through the USDA Food Safety Inspection Service (FSIS) offers first and second year veterinary students the opportunity to work for the agency on school breaks under the mentorship by experienced Public Health Veterinarians. The VSEP internship provides professional growth and a deeper understanding of the agency while serving as a recruitment tool to introduce veterinary students to careers in the federal government directly related to their interests and career goals. The unique opportunity is an incredible chance to tour slaughter and meat processing facilities while learning about the regulatory role of the federal government in regards to food safety and risk assessment.

Most of the paid internship includes traveling to a variety of facilities as available in the district that the student has been stationed. Visits to plants can include: turkey, chicken, and multi-species slaughter plants, liquid and processed egg facilities, red meat slaughter plants, and further processing facilities (producing ready-to-eat foods). Time spent in slaughter plants provides the student with practical, hands-on pathology training that compliments first and second year veterinary courses. Additionally, exposure to the field operations of FSIS provides insight to the safety of our nation's food supply and the hard work inspectors and

plant employees put forth daily to provide wholesome food for consumers.

A highlight of the internship includes a trip to Washington, D.C. to learn about the history, legislation, data integration, and risk assessment activities that determine the actions of the agency. The opportunity to meet with veterinary epidemiologists, risk analysts, executive associates, and program directors provides a glimpse into the organization and the methodology of a government regulatory agency that works under legislation enacted by congress.

Veterinary students and faculty advisors should be made aware of the FSIS VSEP, and the ability to gain experience through this unique program. Program information and the application instructions can be obtained online at the following website:

http://www.fsis.usda.gov/careers/Veterinary_Stud ent_Employment_Program/index.asp

Modern poultry production began after World War II and has seen great transformations throughout the last fifty years. As such, the USDA FSIS has had to adapt to the needs of the industry to continue to ensure the wholesomeness of meat, poultry and egg products on behalf of consumers. The agency employs approximately 1,100 veterinarians across the nation, many of whom work in slaughter plants as Supervisory Public Health Veterinarians overseeing the inspection of meat and poultry products. It is through inspection and monitoring activities that the agency serves to protect the safety of meat products for consumers. It is also very important to remember that our elected representatives introduce bills and enact legislation that govern the operations of the agency.

The Acts, Regulations, and Directives governing the operations of the FSIS are available for viewing online:

- The main website for FSIS: http://www.fsis.usda.gov/

- The Poultry Product Inspection Act and Egg Product Inspection Act which are the basis for FSIS presence in poultry plants: http://www.fsis.usda.gov/regulations_&_policies/acts_&_authorizing_statutes/index.asp
- FSIS Regulations and Policies that further explain the role of inspectors in plants: http://www.fsis.usda.gov/regulations/index.asp
- Directives related to slaughter and post-mortem inspection (includes "6100.3 Anti-Mortem and Post-Mortem Poultry Inspection - Revision 1"): http://www.fsis.usda. gov/regulations_&_policies/6000_Series-Slaughter_Inspection/ index.asp

The role of FSIS in poultry slaughter plants includes: ante-mortem inspection of birds presented for slaughter (assurance the plant is following "good commercial practices"), online post mortem inspection, offline pre and post-chill finished product standards inspection, examining/approving product labels, and monitoring of all critical control points and records as per the plant's written HACCP and SSOP guidelines (examples: Product/cooler/oven temperatures, water retention, packaged product weights, etc.). Residues and bacterial limits may also be monitored based on random selection of a plant to participate in a baseline study, due to quantity of birds processed, or type of finished (ready to eat) product the plant produces.

From small, single-line traditional slaughter facilities to large multiple-line poultry plants, the presence of FSIS employees and overall job functions remain basically the same. Plants of all sizes pose similar and distinct challenges to inspection. It is clear the presence of FSIS ensures that plants follow their own HACCP and SSOP standards; however, FSIS and plant employees must work synergistically with one another to maintain production of unadulterated, wholesome, and affordable products for consumption.

AVIAN INFLUENZA REAL-TIME RT-PCR TRAINING OF SCIENTISTS IN SOUTHEAST ASIA

Lloyd H. Lauerman

The United States Department of Agriculture (USDA) Foreign Agricultural Service (FAS) and the Animal and Plant Inspection Service (APHIS) have been jointly coordinating the delivery of USDA international outreach and technical assistance to partner countries to combat highly pathogenic avian influenza. In line with the National Strategy for Avian and Pandemic Influenza, the USDA's international technical assistance covers preparedness and com-munication, surveillance and detection, and emergency response and containment. USDA's partners include other U.S. Government (USG) agencies, the U.S. poultry industry and land-grant universities, the Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (OIE), World Bank, as well as the public and private

sectors in the countries. In order to help facilitate USDA participation and leadership in the global effort to combat Avian Influenza (AI), under the supervision of the APHIS Associate Deputy Administrator for International Services (IC), APHIS and the University of Maryland were collaborating to support positions for Specialists in polymerase chain reaction (PCR) technology. In 2006 at 73 years of age Lloyd Lauerman applied to be a PCR Specialist with USDA, APHIS, IC.

The Australian Animal Health Laboratory (AAHL), an OIE regional laboratory for work with Southeast Asian (SA) AI viruses, was also in the process of training scientists from SA at AAHL in Geelong, Australia. A collaborative project was developed between AAHL and USDA to prevent duplication of work in the area. Two USDA PCR Specialists (Chris Groocock and Lloyd Lauerman) were trained as trainers at the AAHL in Geelong (November 20 through December 6, 2006) using their Real-Time Reverse-Transcription (rRT) PCR techniques and equipment since the Australian primers were more sensitive and specific for the SA AI virus isolates than the National Veterinary Services Laboratory (NVSL) primers. The objectives established for the PCR Specialists were to give additional training to SA scientists that had received two weeks of laboratory training at NVSL in Ames, Iowa or AAHL at Geelong. The additional training would be given to the SA scientists in their home country laboratory work place with their equipment, facilities and supplies to expand their experience and knowledge of AI rRT- PCR and standard laboratory diagnostic techniques.

Indonesia was the first country to receive training for their scientists (English interpretation was necessary in many situations) in-country and this training began in February, 2007. There were seven regional laboratories and one central laboratory in Indonesia that asked for AI rRT-PCR training for the staff. The two USDA PCR Specialists were each assigned four Indonesian laboratories to give additional training concerning AI rRT-PCR and standard laboratory diagnostic techniques during this February period. There were two teams of AAHL scientists that were each assigned four Indonesian laboratories to perform follow up training later. During the last two weeks of April, 2007, the USDA PCR Specialists returned and presented additional information concerning biosafety, sample handling, quality control, serological assays, virus detection and identification, recording and reporting results. The two teams of AAHL scientists returned in May and June, 2007, to complete all the training in the Indonesian laboratories. Semiannual visits were planned to be made by the AAHL scientists to check the Indonesian scientists progress.

During the first trip to Indonesia I met a scientist that had obtained his Masters Degree in Molecular Microbiology from one of the Universities in Indonesia. That encouraged me to determine how many Universities had Masters Level of training in Molecular Microbiology and inquire about the approximate overall cost for the degree. At least four Universities in Indonesia gave Master Degrees in Molecular Micro-biology and a two year course cost about $5,000 total. The USDA picked up on this information and developed a scholarship program for a number of Indonesian scientists to be trained in Molecular Microbiology in Masters Degree programs to increase the level of knowledge in Indonesia.

In order to prepare US scientists to be qualified to train scientists in developing countries concerning the Australian rRT-PCR procedures for detection and characterization of avian influenza virus, a one and one-half day session was arranged to train trainers that had already participated in the NVSL training course at Ames, Iowa. Nine US scientists (Laura Austgen, Alejandro Banda, Erdol Erol, Cristina Gerhard, Jose Linares, Morgan McCoy, Sonya Mora, Kristy Pabilonia, and Yan Zhang) were available and these participants were presented with a CD disc containing the complete material of the CSIRO Manual of Avian Influenza Diagnostic Techniques originally presented as a Training Workshop on Diagnostic Techniques for Avian Influenza at the AAHL, Geelong, Australia, October 9 through 20, 2006. Dr. Lauerman presented selected portions of the NVSL and AAHL rRT-PCR protocols, virus isolation techniques and serology techniques emphasizing differences and similarities. Various power point presentations from the CSIRO Manual were presented with discussion.

In March the USDA APHIS, CI began looking for an agency with a laboratory in SA to develop a collaborative training session for scientists in Southeast Asia. The Animal Health Research Institute (AHRI) in Taiwan offered their full assistance in the project. Dr. Lauerman was assigned the responsibility of coordinating the logistics of organizing and setting up the laboratory training as lead trainer for the USDA primarily by e-mail with key scientists in Taiwan and one week prior to the Training Course to set up equipment, materials and supplies in three designated rooms for the course with the full cooperation of the Director and staff of the Taiwan AHRI. Wet and dry laboratory exercises were run to confirm that all equipment, supplies and reagents were in place and functioning properly. The Training Course was designed to teach the trainees (SA scientists) techniques for diagnosis of Avian Influenza using Virologic, Serologic and Molecular procedures as described in the laboratory manual entitled "Manual of Avian Influenza Diagnostic Techniques: Training

Workshop on Diagnostic Laboratory Techniques for Avian Influenza" held at the AHRI, Taiwan, May 7 through 18, 2007. The Training Course was sponsored by the USDA; AHRI, COA, Taiwan; AAHL, CSIRO; and Australian Agency for International Development. The trainers for the course were Lloyd Lauerman (Lead Trainer and Virology/Serology), Morgan McCoy (Virology/Serology), Ian Pritchard (Molecular), Erdal Erol (Molecular), and Yan Zhang (Molecular). Twenty-five scientists from four countries (Cambodia – 7, Indonesia – 11, Sri Lanka – 5, and Taiwan – 2) were trained during the two week Avian Influenza Diagnostic Techniques Training Course. English translation was necessary for some of the Cambodian and Indonesian scientists. One Indonesian scientist was being trained as a trainer. Due to the success of this first Training Course held at the AHRI in Taiwan a second course was promptly organized.

The second Avian Influenza Diagnostic Techniques Training Course was held at the AHRI in Taiwan June 4 through 15, 2007. The trainers for the course were Lloyd Lauerman (Lead Trainer & Virology/Serology) Jose Linares (Virology/Serology) Ian Pritchard (Molecular) Chris Groocock (Molecular) and Robert Heckert (Biosafety). Twenty-five scientists from six countries (India – 2, Indonesia – 2, Philippines – 6, Taiwan – 11, Thailand – 2 and Vietnam – 2) were trained during the course. Two Vietnamese doctors and one Indonesian scientist were being trained as future trainers for their own countries. The second Avian Influenza Laboratory Diagnostic Training Course in Taiwan was considered a successful event by all participants.

In country training was the next assignment requested by the National Animal Health and Production Investigation Center (NAHPIC) in Phnom Penh, Cambodia. Eight Cambodian scientists were to be trained in their laboratory with their equipment concerning Diagnosis of Avian influenza using rRT-PCR techniques. Dr Tom Chang assisted in the training. Complications were encountered on this trip in that the Real-Time PCR thermal cycler to be purchased by FAO had not arrived and arrangements had to be made to obtain a loaner machine (BioRad iQ5 High Throughput 96-well thermal cycler), which would need to be returned right after completion of the training. A controlling computer was not supplied with the thermal cycler and so my personal laptop computer was used and thus these critical pieces of equipment would not be available for the Cambodian scientists to use immediately after the training session. The BioRad technician set up and calibrated the iQ5 machine and gave us very preliminary information on the use of the machine and left. Neither Dr. Chang nor I had any previous training with the BioRad iQ5 machine and we found that the instruction manual was not user friendly

so we were able to obtain cooperation of a scientist from the Institute Pasteur to give us proper training with the machine. Instruction was then given to the Cambodian scientists individually to set up the thermal cycling programs and template preparation to properly run the assays. Positive AI H5 RNA and Type A RNA were obtained from the Institute Pasteur. The first day in the laboratory in Cambodia, nine-day-old embryonating chicken eggs were inoculated with swab samples collected from wild birds. Lectures were presented (interpretation necessary) concerning biosafety, diagnosis of AI using rRT-PCR, RNA extraction procedures, primer and probe calculations for reconstitution and preparation of working solutions, preparation of master mix, and then running the rRT-PCR assay. The data were analyzed from the previous days run. The Cambodian scientists harvested the allontoic fluids from the embyronating eggs that had been inoculated with the swab samples from the wild birds and the RNA extracted from the allontoic fluids. Ten-fold dilutions were made of the AI Type A positive RNA obtained from the Institute Pasteur. The scientists prepared a new rRT-PCR template on the computer, prepared the master mix and dispensed it into the plate. The RNA samples were inoculated into the master mix in the proper wells of the plate by the scientists. The rRT-PCR plate was run on the thermal cycler. The results were read and analyzed. Both the Type A and H5 positive controls were good and the dilutions of the positive controls were well spaced. The wild bird samples were negative. The Cambodian scientists will need additional experience with the thermal cycler and rRT-PCR technique to develop expertise and confidence since the thermal cycler and computer were only in the laboratory for the training session. A new laboratory building is being constructed and so the additional training will occur after they have moved into this new facility.

The next in country training request came from Veterinary Research Institute (VRI) in Kandy, Sri Lanka and occurred November 12 through 24, 2007. Fifteen scientists were available for the Training Course concerning Diagnostic Techniques for Avian Influenza and Dr. Tom Chang assisted in the training. We had been informed prior to departure that the rRT-PCR thermal cycler would not be available until sometime after the course was completed so we went ahead with training using standard RT-PCR techniques since the necessary equipment was available. The first week was scheduled for training in molecular techniques. Power point presentations were given concerning Diagnostic Nucleic Acid Amplification Techniques, Basic principles of Laboratory Biosafety, Viral RNA Extraction, Primer and Probe Dilution, and Master Mix preparation. We moved into the laboratory and gave a demonstration of an AI RT-PCR master mix

using the VRI molecular reagents since the supplies we had shipped had not arrived. The master mix was inoculated with AI viral RNA extracts and ran on the standard thermal cycler. Set up of an agarose gel for electrophoresis was demonstrated with the use of a DNA ladder and application of the DNA samples mixed with tracking dye on parafilm for inoculation into the wells of the agarose gel and electrophoresis. The results of the electrophoresis were photographed on a UV transilluminator. We discussed the results, which were negative due to storage of their reagents for a long period of time in -20ºC. The significance of determination of melting temperature of primers and annealing temperature for use in PCR amplification were discussed. A demonstration was also given concerning how to find the GenBank on the Internet and performing a BLAST procedure for evaluation of primers. Supplies began to arrive Friday that were sent by NVSL and USDA for the Training Course, which allowed us to divide the scientists into two groups during the second week with one group continuing to be trained in molecular techniques and the other group trained in chick embryo tissue culture and other diagnostic techniques under Dr Chang's direction.

During the second week the molecular group performed all of the techniques described above using the Sri Lanka reagents. On Thursday of this second week the Customs released the order containing a new set of primers for H5, H7, H9, and N1, as well as the SuperScript III One-Step RT-PCR reagents so the method of reconstituting the lyophilized primers (H5), calculate the proper dilution of the stock primers to working solutions and preparation of a master mix with all fresh reagents was demonstrated. We extracted four 10-fold dilutions of AI viral RNA from the NVSL AGID antigen (prepared from AI H5 virus stock) and we also had an H5 positive RNA control. We worked into the evening in order to get the assay on the thermal cycler. We performed the electrophoresis on the amplicons produced from the H5 primers and positive RNA controls. All four dilutions of the viral RNA extracts of AGID antigen exhibited a decreasing intensity of one size band, with the greatest dilution showing the weakest band. The H5 RNA positive control exhibited a strong band at the proper base pair size and the negative control was negative as well as the wild bird RNA samples. The other group of scientists was instructed from Dr Chang through lectures concerning the technique of chick embryo fibroblast tissue culture and he demonstrated the procedure during the week with success. Dr Chang also gave training in immunofluorescence of NewCastle Disease infected tissue culture cells at the Virology Laboratory.

The second trip to the Veterinary Research Institute, Kandy, Sri Lanka, was scheduled for September 22 through 26, 2008. The Applied Biosystems (AB) Real-Time PCR machine arrived in country the first part of September, 2008. Thirty-one scientists attended the molecular techniques lectures and ten scientists participated in the laboratory training. Dr. Chang participated in the teaching again on this trip. Lectures were presented concerning Real-Time PCR versus Standard PCR; Amplicon size, Primer and Probe selection and dilution; rRT-PCR; Nucleic Acid Extraction procedures; Good Laboratory procedures; Aspects of Quality Assurance and Quality Control and Biosafety. The AB technician arrived the second day and set up and calibrated the AB Real-Time PCR machine and then gave good instructions in the use of the machine. He assisted in additional training during the rest of the week. The trainers supervised the scientists in RNA extraction of swab samples using the Qiagen RNeasy Mini Kit and showed types of records to be kept. The trainers taught the scientists how to dilute the primes and probe and prepare the master mix for the rRT-PCR assay for AI. Principals of quality assurance and quality control were discussed during the process, as well as record keeping and reporting was discussed. We were able to make a run on the thermal cycler Thursday afternoon which was successful. Friday the 26th the trainers taught the scientists how to use the AB dry beads kit for AI that was supplied with the rRT-PCR machine and we had a successful run using this procedure also.

The second trip to the National Veterinary Research Institute (NaVRI), Department of Animal Health and Production, Phnom Penh, Cambodia, occurred April 20 through 30, 2009. Dr. Chang assisted again as a trainer. Sixteen scientists were trained in the use of rRT-PCR assays for diagnosis of avian influenza including biosafety principles. The scientists were also scheduled to be trained in virologic and serologic techniques for diagnosis of AI but due to frequent delays from power outages and the additional time required for English translation this area was not presented. The scientists moved into the new laboratory building a few months before our arrival. However, intermittent power outages have been occurring because they built the building in a new area of the city that does not have adequate electrical supply. The government is working to resolve the problem. The FAO had purchased a Qiagen Roto-Gene 6000 thermal cycler for the laboratory, installed it and gave the scientists training on the use of the machine prior to our arrival. Lectures were presented concerning diagnostic nucleic acid amplification techniques, primer and probe selection and dilution, viral RNA extraction using a Nucleospin RNA II extraction kit and Qiagen RNeasy Mini Kit, rRT-PCR assays and principles of laboratory biosafety and biosecurity. Laboratory exercises were supervised that allowed

participants to have hands on practice with the various procedural steps leading up to placing the final product, master mix with sample RNA inoculated, into the rRT-PCR machine. All lectures and exercises were translated into the Cambodian language to be sure the participants understood the material. A short test was given at the end of the course to evaluate their comprehension of the material and the results were very good.

The combined efforts of the International Agencies to develop an effective Avian Influenza diagnostic network of laboratories throughout South Eastern Asia has been demonstrating major progress in combination with effective control measures. The AI H5N1 virus is still active throughout the world but the reduction of human deaths from 79 in 2006 to 32 human deaths in 2009 is remarkable. Many other parameters of AI control in domestic birds also point to major steps of positive progress.

A POULTRY PRODUCER'S ON-FARM BIOSECURITY SELF-ASSESSMENT GUIDE

Victoria A. Bowes

British Columbia Ministry of Agriculture & Lands, Animal Health Centre
1767 Angus Campbell Rd, Abbotsford, BC V4X 1R5, CANADA
Victoria.Bowes@gov.bc.ca

SUMMARY

Following the outbreak of highly pathogenic avian influenza (HPI) in Fraser Valley commercial poultry in 2004, the BC poultry industry recognized the immediate need for improved on-farm biosecurity. With federal and provincial government support the BC Poultry Industry Advisory Management Committee (IAMC) was formed and identified three key initiatives to mitigate the financial risks of another outbreak of Notifiable Avian Influenza (NAI) in BC (1). Those initiatives are mandatory on-farm biosecurity standards for poultry producers, emergency disease response planning and industry risk identification/mitigation. The first step in the BC Poultry Industry Enhanced Biosecurity Initiative was the development of an on-farm biosecurity self-assessment guide for poultry producers (2).

The Guide is an 11-page document that, through a series of questions with gradated answers, illustrates common biosecurity risks and the steps that can be taken for immediate improvement. Answering the questionnaire takes the producer less than one hour and is intended to be private, non-judgemental and thought-provoking. By answering the questions the producer is able to gauge the current level of biosecurity practices against the industry ideal and is given some guidance for improvements.

The Introduction to the Guide describes the general principles of biosecurity and its basic components (Prevention, Containment, and Bird Health). Here biosecurity is defined as "an overall program that uses a combination of physical barriers (things) and directed actions (people) in a specific way that should prevent the introduction of, or limit the spread of , infectious disease causing agents (bacteria & viruses) into a group of susceptible poultry." The basic working concept of enhanced biosecurity is to 1. IDENTIFY the risk and then 2. MANAGE that risk.

The producer is prompted to simply read the question and circle the response that best fits the current biosecurity practice. There are four possible responses: Unacceptable, Questionable, Adequate, or Ideal. Biosecurity measures that score "Unacceptable" are considered to fall below the minimum standards defined by the industry and require immediate corrective action. Optimal or "Ideal" practices as defined by the Guide are considered to be the industry "gold standard" that producers should work toward. The Guide encourages the producer to take ultimate responsibility for enforcing biosecurity on the farm.

The 15 sections of the Guide are as follows:
- Farm location
- Barn layout
- Access deterrents
- Building Entryways
- Pest control programs
- Truck Traffic
- Deliveries
- Visitor concerns
- Tools and equipment
- Cleaning and disinfection
- Water sanitation
- Employee concerns
- Multi-farm management
- Flock health management

☐ Carcass/manure management

Figures 1, 2, and 3 provide examples of the different questions with their responses and illustrate the educational nature of the Guide.

It's anticipated that this guide would be useful for delivery to poultry producers through private veterinarians and other extension services. A Word copy is available from the author that would enable customizing the details to a specific client group.

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Figure 1. An example of the questions from the Access Deterrents section of the Guide.

Access deterrents

a) No biosecurity or information signs at entrance _____**Unacceptable**
b) Biosecurity sign is readable from the road _____**Adequate**
c) Biosecurity signs are posted at the gates of all access points onto the farm _____**Ideal**
d) There is no vehicle disinfection station on the farm _____**Unacceptable**
e) There is a freshly stocked vehicle disinfection station at the gate _____**Ideal**
f) There is no clearly identified designated visitor parking area _____ **Unacceptable**
g) Visitor parking area <30 meters from barn(s) _____**Questionable**
h) Visitor parking is outside the farm perimeter or "control area" _____**Ideal**
i) Driveways are designed for one-way traffic flow _____**Ideal**
j) Driveways are
   i) dirt _____**Unacceptable**
   ii) gravel _____**Adequate**
   iii) paved, no potholes _____**Ideal**

Figure 2. An example of the questions from the Employee Concerns section of the Guide.

Employee concerns

a) Employees have routine contact with other poultry species without following a written biosecurity protocol_____**Unacceptable**
b) Following contact with other poultry species, employees have a minimum 24 hour "away time" requirement before re-entering the farm _____**Ideal**
c) The consequences of coming in contact with off-farm poultry is explained to employees _____**Adequate**
d) Employees do repair work on other farms without following a written biosecurity protocol _____**Unacceptable**
e) Contractual employment agreement outlines biosecurity expectations _____**Ideal**
f) Biosecurity training is provided for all employees _____**Adequate**
g) Annual biosecurity training is a mandatory condition of employment _____**Ideal**
h) Catching crews and vaccinators are greeted at the gate, directed to a designated parking area and provided with protective clothing and footwear _____**Ideal**
i) There is a designated clean and comfortable area for catching and vaccinating crews to assemble, take breaks and avoid returning to vehicles_____**Ideal**

Figure 3. An example of the questions from the Flock Health Management section of the Guide.

Flock Health Management

a)   Each barn is capable of all-in all-out management _____**Adequate**
b)   All-in all-out management is strictly practiced _____**Ideal**
c)   There are more than one species of bird on the farm _____**Questionable**
d)   Mortality is collected and recorded daily _____**Adequate**
e)   Excess mortality or unexplained clinical signs are investigated within 24 hours __**Ideal**
f)   Excess mortality or unexplained production changes are not routinely investigated
_____**Unacceptable**
g)   There is active flock health monitoring through serology _____**Adequate**
h)   Vaccination protocols are:
    i)   "routine" and unwritten _____**Unacceptable**
    ii)   administered or overseen by the producer _____**Adequate**
i)   Medication or vaccine brand name, serial number, date, storage conditions, application
and expiry date are part of the written health record _____**Ideal**
j)   I do not have an emergency after-hours phone number contact list _____**Unacceptable**
k)   I have immediate access to the advice of a poultry health professional _____**Adequate**
l)   I do not have a written vaccination or disease prevention program _____**Unacceptable**
m)   My vaccination and disease prevention programs are a result of veterinary consultation
_____**Adequate**
n)   The written vaccination and disease prevention program is continually being updated as
new information becomes available _____**Ideal**

LAYING HEN WELFARE – IN DEFENSE OF CAGES

Mike Petrik

65 Munroe Cres., Guelph, ON, N1G 5E4
mpetrik@gmail.com

Recently there has been a great deal of public debate regarding the appropriate manner in which to produce eggs for human consumption. This is a complicated and emotional issue, and the discussion encompasses animal welfare, production, public health, and the environment. To date, the bulk of information presented to the public regarding laying hen housing has supported the position that hens should be housed loosely. This information has furthermore indicated that housing hens in cages represents an unacceptable method of housing and production due to animal welfare concerns. Indeed, several regions have effectively banned all cage systems for egg production. Because the current scientific evidence is ambiguous, any discussion on the future of egg production should involve unbiased evaluation of various caged housing systems, as well as loose housing systems. Very little consideration has been given to the positive aspects of cage systems, or the reasons that cage systems were

originally implemented. Some of the reason there has been little public defense of cage housing systems is that it is often difficult to present the issues to people who are not familiar with the laying hen industry. This presentation is designed to give interested parties some insight into the complexities of laying hen welfare and how to effectively present the issue of laying hen housing systems to people unfamiliar with poultry production. This discussion will hopefully lead to a situation where future developments in laying hen housing will be directed by people who understand the needs of the hens, understand the demands of the industry, and understand the implications of the changes that they recommend. Failure to proceed in this manner will result in a situation where the industry suffers; the consumer suffers; and ironically, the hen suffers.

Animal welfare is often held to the standard of the Five Freedoms that were developed to describe the

different aspects of animal welfare. These freedoms are defined as the Freedom from Hunger and Thirst; Freedom from Discomfort; Freedom from Pain, Injury and Disease; Freedom from Fear and Distress; and Freedom to Express Normal Behavior. Poultry housing impacts all five of these freedoms. Conventional cages perform very well in providing the first four of these freedoms. Accessibility of feed and water, low inter-bird aggression, exceptional environmental control, protection from predation, low incidence of in-lay injury and unparalleled disease control make conventional cages arguably superior to other housing systems in these areas. Conventional cages are routinely criticized for their deficiencies in the freedom to express normal behavior. Lack of nest boxes, perches, dust baths, room to forage, and the barrenness of the environment are evident even to those who are not familiar with the poultry industry. A question worth asking is why one of the five freedoms should supersede the successful provision of the other four.

Despite providing superior behavior and movement opportunities, non-cage systems have deficiencies when evaluated in other areas. Suffering is much more likely in these flocks than conventionally caged birds due to bird injury including keel fractures, which are surprisingly common in hens kept in non-cage housing. Inter-bird aggression is more of a risk in cage free housing, due to larger group size. This can result in mortality and morbidity due to cannibalism and which often requires more aggressive beak trimming as a preventative measure. Increased prevalence and re-emergence of diseases including bumblefoot, coccidiosis, and red fowl mites are seen in cage free systems, and not seen in conventional cage houses. Because bird body heat effectively heats poultry barns, the relatively few number of birds housed in cage free systems means that there is decreased ability to control the bird's environment. The decreased volume of air that can be ventilated without chilling the hens, along with the presence of litter results in a significant risk of suffering due to increased levels of dust and ammonia in the barn.

There has been a recent trend for governments, under pressure from animal activist groups to enact legislation which imposes restrictions upon the poultry industry with regards to the manner in which they can house laying hens. These regulations have resulted in relatively short time lines in which poultry producers must alter their methods of egg production. The result of these regulations is unfailingly to restrict the use of cages. Unfortunately, these short timelines make it impossible to continue the systematic, science-based and gradual improvement to laying hen welfare that has been occurring over the past several decades. Regulations furthermore have impeded the incorporation of furnished cages into the industry in any significant amount, because enriched cage housing unfailingly has been categorized as a cage system, undifferentiated from conventional cage systems. Producers are faced with the dilemma of adopting furnished cages with no premium paid for their eggs, or adopting cage-free systems for which they receive financial incentive. The increased capital cost of furnished cages cannot be recouped, while the cage-free system is relatively more profitable. An increasing amount of scientific evidence demonstrates that furnished cages maintain many of the welfare benefits of cage systems, while providing nest areas, dust baths, perches, and improved room for movement and expression of natural behaviors. Enriched cage systems maintain small group sizes, separation of birds and manure, excellent availability of feed and water, and superb environmental control. By heavy-handed and misinformed imposition of cage restrictions, jurisdictions are ultimately decreasing the number of tools available to the industry to improve laying hen welfare. The implementation of these legislations may ultimately result in decreasing laying hen welfare by banning what is arguably the best current technology for laying hen housing. A more effective solution would be to scientifically identify the needs of hens, and develop strategies to meet these needs in the most complete way possible. In order to do this, it is crucial to include caged housing systems in the discussion, and not dismiss them out of hand.

FIELD EVALUATION OF A RECOMBINANT RHVT/NDV VACCINE IN BROILERS IN A VNDV REGION IN MEXICO

J. F. Ríos-Cambre and R. G. Hein

Intervet/Schering-Plough Animal Health, Mexico
Intervet/Schering-Plough Animal Health, USA

ABSTRACT

Broilers chicks were vaccinated with a recombinant rHVT/NDV vaccine at the hatchery at one day of age, followed by a primer vaccination with a B1 type vaccine by spray at the hatchery; and two Clone 30 additional doses at 12 and 30 days of age, plus an AI killed shot at 10 to 12 days of age by the subcutaneous route. Another group of chickens were vaccinated at one day of age twice with an AI/ND killed vaccine at one day of age and at 10 to 12 days subcutaneously, according to the accustomed vaccination schedule in the region. The live vaccinations were similar to those of the first group. Serum samples were extracted at 21, 28, and 42 days of age for HI and ELISA. At the same dates, birds were extracted from both flocks and put in isolation for virus challenge using the Mexican official velogenic viscerotropic Chimalhuacán strain by the intra-ocular route and kept in observation for fourteen days for ND lesions, clinical signs and mortality scoring. In all cases a complete protection against ND was observed in both groups, demonstrating that a vaccination schedule using the recombinant rHVT/NDV vaccine with no killed ND vaccine fully protected the birds against a very virulent challenge.

INTRODUCTION

Newcastle disease (ND) is a poultry disease caused by an agent classified as Newcastle disease virus (NDV), Paramyxovirus type 1, order *Mononegavirales*, family *Paramyxoviridae*, subfamily *Paramyxovirinae*, and genus *Avulavirus* (2,3,4). It is recognized as one of the most important poultry diseases because of its economic impact. Due to its importance, NDV is subject to epidemiological surveillance and the emergence of the most virulent strains is considered a matter of reporting by OIE subscribing countries (1). Mexican poultry industry has been suffering from the impact of ND viruses since its initial report in 1946.

In addition to obvious biosecurity measures, vaccination has been the only way to control ND outbreaks. Since the 1950s, live lentogenic vaccines, such as B1 type, B1 strain, and/or B1Type, La Sota strain based live vaccines have been used worldwide.

Since the 1970s inactivated, oil-adjuvanted vaccines were introduced in the country, and for decades they seemed to control the disease effectively. However, since 2000 the emergence of virulent isolates has been keeping both authorities and producers on high alert (5).

As reported, all these isolates have been reported as considerably more virulent than the US official reference strain Texas GB (ICPI 1.75). Ever since 1992, results were published showing the effect of inserting the F gene, which codes for the Fusion (F) protein of ND virus. Given the fact that all challenge studies for this vaccine have conducted using the US reference strain, it was considered important to show its effectiveness against more virulent strains. In 2008, results from challenge studies using the official Mexican standard challenge velogenic, viscerotropic strain Chimalhuacán (ICPI 1.89) were presented, showing that recombinant HVT vectored-vaccines were capable of protecting broilers against much more virulent ND virus strains.

Since 1994, low pathogenic avian influenza (LPAI) virus has been endemic in the Mexican poultry industry. This situation has forced the Mexican poultry producers to apply oil-adjuvanted killed vaccines using the standard individual-injection method for both AI and ND. However, given the economic importance ND has in the country, it was considered a priority if a new development, such a recombinant rHVT/ND, was able to protect the birds in the field against Mexican virulent strains, being the birds denied any dose of a killed vaccine, as it is used in Mexico for years, with the consequent decrease of production parameters because of the use of an individual injection, and the risks of biosecurity breaks due to the transit of vaccination crews consisting of several people between farms.

The objective of this study was to show if a recombinant rHVT/ND vaccine, once applied in commercial conditions, was able to protect broilers subjected to Mexican standard rearing conditions, and placed in farms located as known of virulent ND virus circulation.

MATERIALS AND METHODS

At the hatchery, 77,300 Ross 308 day-old broilers were subcutaneously vaccinated with a recombinant rHVT/ND+SB-1. In addition, all birds were vaccinated using a B1 type, C2 strain ND vaccine by course spray using a custom-made spray cabinet. All birds were vaccinated on the same day and placed in a farm located at 6,500 feet above sea level in the Central Mexican state of Queretaro, where about 25 million broilers belonging to different producers are located at any given time of the year; and with a known history of virulent ND virus circulation. The birds were placed in this area in mid-July, during summer, in a semi-dry climate, but due to the enormous amount of different age-broilers virulent ND viruses can be isolated year-long. These birds were identified as Farm 1.

In an adjacent farm, 137,700 broilers of the same age and breed were placed. All birds from this second group, identified as Farm 2, were vaccinated with a commercially available, combined AI+ND killed vaccine by the subcutaneous route, in addiction to the B1 type, C2 strain vaccine, applied by course spray using the same custom-made spry-cabinet. The addition of a combination of one killed and at least two live vaccinations is considered standard in Mexican broilers.

At 12 to 14 days of age all birds were vaccinated by course spray with a Clone 30 strain live vaccine and an AI killed vaccine by the subcutaneous route.

Due to a standard procedure, both flocks were re-vaccinated with a sprayed Clone 30 at 25 days of age. At 21, 28, and 42 days of age, 10 birds form each farm were sent to the lab, bled for serum samples, and placed in Horsfall-Bauer-type isolator chambers.

All serum samples were analyzed by ELISA for ND-antibody detection using an IDEXX kit (Westbrook, MA, USA). After isolation, all birds were left to rest for 24 hours. Afterwards, all birds were challenged with the official Mexican Chimalhuacán strain with a titer of $10^{6.0}EID/_{50}$/bird by eye-drop. Protection was measured as a percentage of birds showing mortality and/or clinical signs/lesions attributable to ND. All dead birds were necropsied for ND lesions. At the same time, similarly-aged SPF birds were challenged using the same method. Both the serology test and the challenge trials were conducted at the Laboratorio de Biología of Investigación Aplicada (IASA, Tehuacán, Puebla, México) a third-party reference lab.

RESULTS

Serology results were as follows. At 21 days of age, birds from Farm 1 had a Geometric Mean Titer (GMT) of 431, with a Coefficient of Variation (CV) of 158.5%. At 28 days of age birds from Farm 1 showed a GMT of 196, with a CV of 230.8%. For birds from Farm 2, at 21 days of age, the ELISA titer was 471, with a CV of 196.9%; at 28 days the ELISA GMT was 521, CV of 101.4%. As for the birds from Farm 1, at 42 days of age the ELISA titer was 4,513 with a CV of 43.4; meanwhile the birds from Farm 2 the GMT was 1,448, with a CV of 47.0%. All SPF birds showed negative results at all ages. For the challenge trial results, both groups showed 100% protection.

DISCUSSION

Serology from the conventionally vaccinated birds was considered normal, taking into account that the only killed ND vaccine was applied at one day of age. On the other hand, considering that according to the literature rHVT/ND+SB-1 vaccinated birds, while being protected from challenge, should not seroconvert due to expression of only the (F) protein, not the H/N antigen, antibodies from these birds should not be significant. However, both the GMT and the CV from these birds were much higher than those of the birds vaccinated with a combination of killed and live vaccines. This implies that the birds might have been exposed to a field virus. Nevertheless, according to field production data from the poultry company (data not shown), all parameters were not affected and were similar in both farms. At the same time, in all three challenge trials, full protection was accomplished regardless of the serological titers. These data show that in this field trial, birds vaccinated with an rHVT/ND+SB-1 recombinant vaccine were as fully protected as those vaccinated with a conventional vaccination program that included a killed vaccine. Since the objective of the use of the recombinant vaccine is to get rid of the individual handling of the birds, field trials in which birds vaccinated with a concentrated AI killed vaccine applied at the hatchery simultaneously with the recombinant vaccine are in process.

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A KILLED RECOMBINANT NEWCASTLE DISEASE VIRUS-AVIAN INFLUENZA VIRUS H5 VACCINE

Ernesto Soto-Priante, Manuel Gay-Gutiérrez, Felipa Castro-Peralta, David Sarfati-Mizrahi, and Bernardo Lozano-Dubernard

Laboratorio Avi-Mex SA de CV, J. I. Bartolache 1862, Colonia del Valle, 03100, México, D.F. México

SUMMARY

The vaccine was tested in SPF birds. Birds were vaccinated once at 12 days of age. At 21 days PV, one group was challenged with a Mexican HPAIV (H5N2) and a second group with a Mexican viscerotropic velogenic Newcastle disease virus (VVNDV). Recovery of both pathogenic viruses was performed after the challenge. Sera samples were obtained at different times for serological tests. Results indicate that vaccinated birds were properly protected against signs and mortality with both viruses and no challenged virus was recovered from internal organs. Also, a good antibody response against both viruses was detected by the HI tests but not by ELISA. It can be concluded that this killed vaccine can be useful for commercial birds with the aid of the DIVA factor.

INTRODUCTION

Inactivated whole avian influenza (AI) virus vaccines (K-AIV) were first used in 1978 to control low pathogenicity AI virus (LPAIV) in Minnesota (H4N8) and California (H6N2) turkeys. Then, they were used in Italy for breeders, layers, and turkeys as a polyvalent vaccine against three different subtypes of AI viruses (H5N2, H6N2, and H10N2). In 1995, K-AIV vaccines were used in Mexico (H5N2) and Pakistan (H7N3) in broilers, breeders, layers, turkeys, and fighting cocks. In 1997, K-AIV were used in some countries of the Middle East and Asia to control a mildly pathogenic avian influenza virus (MPAIV) causing mortality in broilers, commercial layers, and turkeys (H9N2). Since the beginning of the Asiatic outbreak in 2004 (H5N1), K-AIV vaccines have been used extensively in all kind of domestic birds, including chickens, turkeys, fighting cocks, guinea fowl, partridges, pheasants, quail, and different species of domestic ducks and geese. At the present time, some countries used a polyvalent vaccine against three different subtypes of AI viruses (H5N1, H9N2, and H5N2).

Inactivated whole virus vaccines are administered individually by the subcutaneous (SQ) or the intramuscular (IM) routes at one week of age and on, and are used with the intention to protect birds against death and clinical disease, but also to stop or diminish field virus replication and excretion. Killed vaccines are preferred for long life birds.

An inactivated recombinant Newcastle disease virus-avian influenza H5 vaccine (K-rNDV/AI-H5) was developed to protect commercial birds with the advantage of a DIVA system. This kind of vectored virus allows the production of safety vaccines containing any subtype of AIV HA gene, without the need of an active AIV, eliminating any kind of risks.

This study was done to determine the protection to challenge and the antibody response, but also to verify the effect of differentiating infected from vaccinated animals (DIVA) of SPF chickens after one single SQ injection of a K-rNDV/AI-H5 (K-NewH5, Laboratorio Avi-Mex SA de CV, México).

MATERIALS AND METHODS

Birds. Newly hatched SPF white leghorn chicks were obtained directly from the hatchery and housed in isolators (Controlled Isolation Systems, Inc., San Diego, CA, USA) at CENID-Microbiología high-biosecurity facilities (BSL-3).

Vaccine. The recombinant virus containing the HA insertion of an AIV was prepared to contain a viral titer of 109.3 mean chicken embryo infective doses (CEID50)/mL with a minimum 520 HA units (HAU)/mL. Inactivation was performed using formaldehyde to a final concentration of 0.15%. The vaccine was prepared as water-in-oil emulsion.

Experimental procedure. Birds were bled and vaccinated once at 12 days of age with 0.5 mL of the K-rNDV/AI-H5 vaccine. One group remained

unvaccinated as control group. At 21 days PV, birds were bled and then divided in two groups containing 25 birds each. One group was challenged with a Mexican HPAIV (H5N2) strain A/Chicken/Queretaro/14588-19/95 (H5N2), using $10^{6.5}$ CEID$_{50}$ by the ocular route and a second group with a Mexican viscerotropic velogenic Newcastle disease virus (VVNDV) strain Chimalhuacán using $10^{6.5}$ CEID$_{50}$ also by the ocular route. In both cases, birds were observed for 10 days PC. At this time, survival birds were bled again before humanely euthanized and incinerated. Recovery of both pathogenic viruses was performed after the challenge in five birds per group.

RESULTS

For the VVNDV or the HPAIV challenges, morbidity and mortality were 0% for vaccinated groups in both cases. In the control group challenged with a VVNDV, the mortality was 100% within five days PC showing all signs and lesions characteristic of the virus. For control group challenged with a HPAIV, all birds died within seven days showing all signs and lesions characterized of the virus.

Virus recovered from trachea at three days PC was negative for both challenged viruses in vaccinated birds, while in un-vaccinated birds the VVNDV was recovered in the dilution 10^{-6} and for HPAIV it was recovered at the dilution 10^{-5}.

Hemagglutination-inhibition (HI) test was performed using 8 HA units of inactivated antigen (LaSota) or 4 HA units of inactivated H5N2 antigen (LPAIV A/Chicken/Mexico/435/2005) homologous to the gene used in the recombinant vaccine. K-rNDV/AI-H5 vaccine induced antibodies in all vaccinated birds as shown in Table 1.

ELISA commercial kit (IDEXX Laboratories, Inc., Westbrook, Maine, USA) was used for the detection of AIV antibodies. Positive antibodies were found in the vaccinated group only after the AIV challenge, as shown in Table 1.

DISCUSSION

Results indicate that SPF birds vaccinated with the K-rNDV/AI-H5 vaccine were properly protected against the effects of both challenged viruses since no morbidity, mortality or virus recovery was seen or obtained, as expected with any inactivated whole virus vaccine. A good antibody response against both viruses was detected in vaccinated birds by the HI tests but not by ELISA. Positive antibodies were detected in the vaccinated group by ELISA only after birds were challenged with an active AIV, resulting in an excellent DIVA. It can be concluded that this killed vaccine can be useful for commercial birds with the aid of the DIVA factor.

Table 1. Serology response in SPF birds vaccinated with the inactivated recombinant Newcastle disease virus-avian influenza H5 vaccine (K-rNDV/AI-H5) before vaccination, at 21 days after vaccination and at 10 days post-challenge with a VVNDV or with a HPAIV H5N2.

Time of sampling	12 days of age		21 days PV		10 days PC	
	Vaccinated	Control	Vaccinated	Control	Vaccinated	Control
HI-NDV	0[A]	0	6.1 (SD[D] 1.3511)	0	8.6 (SD 1.0211)	ND[B]
HI-AIV	0[C]	0	8.8 (SD 1.1521)	0	12 (SD 0.0000)	ND
ELISA-AIV[E]	Negative	Negative	Negative	Negative	Positive	ND

[A]Geometric mean titer of the reciprocal Log2 of the highest dilution to inhibit 8 HA units.
[B]Samples from un-vaccinated group were not collected, as all chickens died before 10 days PC.
[C]Geometric mean titer of the reciprocal Log2 of the highest dilution to inhibit 4 HA units.
[D]Standard deviation.
[E]Values >0.5 cut-off values were considered positive with regards to the presence of AIV NP-specific antibodies (optical density sample / optical density of positive control).

GENETIC CHARACTERIZATION AND EVOLUTIONARY ANALYSIS OF FOUR FULL-GENOME NEWCASTLE DISEASE VIRUS ISOLATES FROM SOUTH CHINA

Shaoxin Cai*[A], Peirong Jiao*[A], Li Jun*[B], Mabel Ting Wong*[B], Huiying Fan[A], Dawei Liu[A], Ming Liao[A], Tao Ren[A], Jingwei Jiang[B], Mang Shi[B], Tommy Tsan-Yuk Lam[B], and Frederick Chi-Ching Leung[B]

[A]College of Veterinary Medicine, South China Agricultural University, Guangzhou, 510640, China
[B]School of Biological Sciences, The University of Hong Kong, Hong Kong, China
*These authors contributed equally to this work

ABSTRACT

We have isolated and characterized four complete genomes of four Newcastle disease virus (NDV) strains from ducks and wild birds in Guangdong province of China from 2003 to 2007. Pathogenicity tests in chicken embryos and chickens illustrate that D3, R8, W4, and P4 belong to lentogenic, lentogenic, mesogenic, and mesogenic strains, respectively. The phylogenomic tree constructed with all six genes provides a high resolution profile for genotype designation as genotype I (D3 and R8) and genotype VI (W4 and P4). In addition, the molecular dating based on different genes suggests that D3 and R8 diverged from their common ancestor at around 1998; W4 and P4 diverged from their common ancestor at round 1999. Subsequent selective pressure analysis displayed some specific traits of genes evolution in four strains since they diverged from the recent common ancestor. Furthermore, the geographic origins of four strains were deduced to be from Europe through parsimony phylogeographical analysis. This provides insights to the potential influence of waterfowl migration on NDV epidemiology.

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PATHOGENICITY OF TWO VARIANT INFECTIOUS BRONCHITIS VIRUS ISOLATES FROM GEORGIA

Holly S. Sellers, Erich G. Linnemann, Susan M. Williams, and Mark W. Jackwood

Poultry Diagnostic & Research Center, Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA, USA

Two variant infectious bronchitis viruses were isolated from commercial broilers in 2007-2008 and were designated as GA/60173/07 and GA/64513/08. The variant virus GA/60173/07 was isolated in the summer of 2007 from 28 to 35 day old broilers with clinical presentation of severe flushing and significant mortality. Houses on the farm were described as slicking over. IBV was isolated from the kidneys submitted and propagated in SPF chicken embryos. Embryo lesions included mortality, stunting and significant ureate deposits in the kidneys. In early 2008, another variant virus, designated GA-08, was isolated from the same flock with clinical signs characteristic of an upper respiratory tract infection. Upon necropsy, the most significant finding was sudsy airsacculitis. IBV was isolated from the tracheas and kidneys submitted and propagated in SPF chicken embryos. Embryo lesions included stunting, and clubbed down. Both viruses were characterized using reverse transcriptase-polymerase chain reaction of the S1 gene followed by sequence analysis to determine the relatedness of the new viruses with other strains of IBV. Sequence analysis determined that both viruses were in fact different from each other despite being isolated from the same farm within a six month period of time. The closest isolates related to the GA/60173/07 virus at 95% relatedness were isolates DMV/5642/06 and CA/1737/04 as determined by BLASTn search analysis (www.ncbi.nlm.nih.gov/ BLAST). The GA/64513/08 was found to be different from all other IBV types sequenced to date. The closest match to the GA/64513/08 was an isolate from NY designated CU8034 at 84%. It is important to note that both GA variants were <80% similar at the nucleotide

level to the commercial vaccines ARK, MASS, Conn, and De072.

Independent pathogenicity studies were performed in one week old SPF leghorns and commercial broilers housed in Horsfal isolation units. In both studies, commercial broilers were more significantly affected than the SPF leghorns and thus data obtained from the broiler study will be presented. In each study, 10 birds were challenged via eyedrop/choanal cleft with $10^{4.0}$ EID50. A negative control group was also included. At five days post challenge birds were euthanized, trachea swabs collected for IBV real time RT-PCR, and tracheas and/or kidneys harvested for histopathology. In birds challenged with GA/60173/07, clinical signs observed at five days post challenge included a mild snick in 5/10 birds and mild airsacculitis in 8/10 birds. No macroscopic lesions were observed in the kidney. IBV was detected in all of the tracheas and kidneys by real time RT-PCR. Tracheal scoring (on a scale of one to four) was performed on the fixed tracheal sections. The mean tracheal lesion score for the GA/60173/07 was 2.0 compared with 1.5 in the negative control group. Moderate levels of edema and mild to moderate lymphocytic infiltration of the medullary cones were observed in the kidneys.

In the GA/64513/08 study, clinical signs observed in birds at five days post challenge included 8/10 with conjunctivitis, 9/10 with tracheal rales and 10/10 with airsacculitis. IBV was detected in 10/10 tracheas by real time RT-PCR. The mean tracheal lesion score for the GA/64513/08 3.1 compared with 1.0 in the negative control group. In each of the studies, the negative control groups remained negative as determined by real time RT-PCR and no clinical signs or macroscopic lesions were observed. In addition, no mortality was observed in any of the experimental groups.

In summary, the two GA variant viruses do not cause severe disease or mortality under experimental conditions. The GA/60173/07 isolate is molecularly similar to a previously reported isolate from California, while the GA/64513/08 isolate was found to be different from all other IBV isolates examined to date. Based on the sequence differences and neutralization studies performed in embryos (data not shown), these viruses are not serologically related to any of the commercial vaccines. Since the original isolations of these viruses, the GA/60173/07 virus was sporadically isolated from additional broiler farms within the same company in geographically distinct locations, as well as a layer farm during 2008. This virus has not been isolated from any clinical submissions in the past year. In contrast, the GA/64513/08 virus did persist in broiler flocks during 2008 into early 2009. Results from a field survey of broiler companies in Georgia indicated that the virus was present on multiple farms in each of the five companies. In addition, clinical submissions of respiratory cases where GA/64513/08 was isolated suggests it became well established in broiler flocks in this area. While the GA/64513/08 by itself is clinically mild, the economic impact of this virus culminates in secondary airsacculitis and severe condemnations at the processing plant. An attenuated live vaccine for this virus was produced.

(The full length article will be published.)

AN OUTBREAK OF ILT IN THE FRASER VALLEY OF BRITISH COLUMBIA, 2009

Nancy de With and William Cox

BACKGROUND

Infectious laryngotracheitis (ILT) is a highly contagious disease of poultry, primarily chickens and pheasants, caused by a herpesvirus. The virus causes severe upper respiratory disease, resulting in dyspnea, rales, tracheitis, and conjunctivitis. Death is usually due to suffocation as exudates collect in the trachea. The virus may be transmitted by direct bird contact, contaminated equipment or clothing, or windborne particles.

An outbreak of infectious laryngotracheitis occurred in the Fraser Valley of British Columbia between May and October of 2009. A few cases of ILT are generally seen each year in BC, and previous smaller outbreaks have occurred, most recently in 2006 and 2007. In each outbreak, the first case was seen in spring and the last case observed in the late fall. The ILT cases occurred in a relatively confined geographic area, with occasional cases occurring outside that area, related to vaccine "reactions" or catcher contacts.

Risk factors or suspected risk factors for all outbreaks include movement of people between farms, movement of birds, sharing of equipment between farms, manure spreading, and dry and windy weather

conditions. The source of virus for the index case in each outbreak could not be confirmed.

Prior to, and during the initial months of the 2009 outbreak, vaccination for ILT was used in long-lived flocks (layers and broiler breeders). There are currently five vaccines licensed for use in Canada; all are modified live virus vaccines. Four of those are chick embryo origin (CEO) and one is tissue culture origin (TCO).

The CEO vaccines result in an excellent level of protection for vaccinated birds by causing an active infection; however, as the vaccine passes through birds, it tends to gain virulence and can spread to other flocks. Presently, the only alternative available in Canada is a TCO vaccine which has lower virulence and minimal spread. The TCO vaccine must be given by eye drop, a very labour intensive procedure, whereas the CEO vaccines can be given via drinking water or spray, in addition to intraocular or intranasal (depending on the formulations).

In all three outbreaks, genetic evaluation of the viruses isolated from cases showed that they were indistinguishable from the chick embryo origin (CEO) viruses used in vaccine products.

2009 OUTBREAK SUMMARY

The first case in the 2009 ILT outbreak was diagnosed on May 1st, and the last case on October 30th. A total of 54 cases were diagnosed. Six farms had repeat infections - after an infected flock was removed and the farm repopulated, a subsequent flock was also diagnosed with ILT.

Of particular interest is the number of broiler and specialty meat bird cases that occurred. In broiler birds, the mean age of diagnoses was 33 days of age (range 24-38 days), while in the specialty meat birds the mean was 11 weeks (range 7-18 weeks). The disease was often diagnosed just prior to the birds being scheduled to be shipped to slaughter. These birds could not be vaccinated, as the withdrawal period of the vaccines is 21 days. The birds had to be shipped as the barn capacity, and market requirements as to bird size, would not allow for the birds to remain in place for an additional three week time period. Consequently, the flocks were transported at a time when it can be assumed that a significant amount of virus was being shed. Due to the density of poultry farming in the Fraser Valley, it was surmised that downwind were exposed to virus from infected flocks. The broiler and specialty meat chicken cases were considered to be the greatest threat to sustaining the outbreak.

CONTROL PROGRAM

During the 2009 ILT outbreak, a series of meetings occurred with industry representatives, private veterinarians, and British Columbia Ministry of Agriculture and Lands veterinarians. The goal of the meetings was to plan a collaborative strategy aimed at bringing the outbreak under control. The measures that were implemented as a result included enhanced communication (especially between meat birds and processors), enhanced on-farm biosecurity, a designated route for transportation of diseased birds to avoid other poultry farms, altered manure handling practices on infected farms (including an in-barn heat treatment of 40°C for three days prior to clean-out), enhanced cleaning and disinfection of barns and equipment (including live haul trucks), the discontinuation of the use of CEO vaccines, and an alternate vaccination strategy.

Because broiler chickens are marketed at an age that precludes vaccination with conventional CEO or TCO vaccines, due to withdrawal times, alternatives were sought. A recombinant vaccine composed of a turkey herpesvirus with genes from laryngotracheitis virus (Innovax®-ILT, Intervet Schering-Plough Animal Health), available in the United States, was viewed as a viable solution. This vaccine was not licensed in Canada at the time of the outbreak and had to be imported using a permit for emergency use of a veterinary biologic.

The alternate vaccination strategy was initiated on August 24th. The recombinant vaccine was only used in the "hot zone", the geographic area in which most of the cases occurred. Broiler birds only received the recombinant vaccine (given at the hatchery). Specialty meat birds and pullets, both layer and broiler breeder, received both the recombinant vaccine (at the hatchery) and TCO vaccine by eye drop (at seven weeks of age for specialty meat birds and 17-21 weeks of age for pullets).

ILT cases continued to occur for a short time after the vaccination program began, with a cluster of 12 cases occurring in the month immediately after the control strategy was initiated. No cases were reported in vaccinated birds. The last case in a broiler flock was reported on October 15th and this flock had not been vaccinated due to a miscommunication.

CONCLUSION

The implementation of the overall control program appears to have been successful. The control strategies were developed and implemented as a result of the consultations between the industry and veterinarians from private practice and government. While it can be suggested that the outbreak would have

subsided anyway with the approach of autumn, during which time previous outbreaks had diminished, it was felt that the discontinuation of the use of CEO vaccines, along with the use of the recombinant vaccines played a significant role in gaining control of the outbreak.

Table 1. Number of cases by poultry type.

Flock type	Number of cases	Number of farms
Broiler	28	25
Broiler breeder	7	7
Specialty (Taiwanese chicken, silkies, etc.)	7	6
Layer (including pullet, layer, and layer breeder)	14	12
Total reports	**54**	
Repeats *(2nd diagnosis on a farm)*	*6*	

ONSET OF IMMUNE RESPONSE IN BROILERS TO CONVENTIONAL AND RECOMBINANT DERIVED INFECTIOUS LARYNGOTRACHEITIS VIRUS (ILTV) VACCINES ASSESSED SEROLOGICALLY (ELISA AND VIRUS NEUTRALIZATION) AND BY INFRAORBITAL SINUS CHALLENGE

John K. Rosenberger[A], Sandra C. Rosenberger[A], and Toria Boon[B]

[A]AviServe LLC, Delaware Technology Park, 1 Innovation Way, Suite 100, Newark, DE, USA
[B]University of Georgia (Veterinary Student)

INTRODUCTION

Infectious laryngotracheitis (ILT), a readily transmitted respiratory disease of chickens caused by an alphaherpesvirus, is economically significant in the Unites States and other parts of the world because of the potential for increased mortality and marked performance losses. The clinical expression of the disease varies from a mild "vaccine-like" response to severe respiratory disease with significant mortality. Typically ILT has a longer incubation period than other common respiratory diseases such as infectious bronchitis and lentogenic Newcastle disease. Disease caused by less virulent forms of ILTV can be difficult to differentiate and to identify in the commercial environment particularly when flocks are co-infected with vaccine or field strains of IBV or NDV.

ILT can be successfully controlled by vaccination and increased biosecurity measures. For many years ILT has been managed by vaccination with chicken embryo origin (CEO) and/or tissue culture origin (TCO) vaccines administered by eye drop, aerosol, or in the drinking water. The CEO derived vaccines tend to be the most efficacious but are of concern to some producers because they can negatively impact livability and performance.

In more recent years recombinant vaccines have been introduced that can be applied by *in ovo* or subcutaneous injection that insures uniform application and eliminates negative side effects that may be associated with conventional vaccines.

The purpose of this report is to describe antibody based methodologies that can be utilized to measure ILTV antibody responses in broilers vaccinated with both conventional and recombinant ILTV vaccines and to document how vaccinated birds respond serologically to a challenge with virulent ILTV.

PROCEDURES

Embryos were obtained from a commercial hatchery and vaccinated on the 18th day of embryogenesis with rHVT/ILTV (Innovax-ILT). Unvaccinated embryos from the same breeder flock were hatched and vaccinated at 16 days of age orally with tissue culture or CEO ILTV vaccine. At 16, 22,

29, 36, 43, and 50 days of age all birds including controls were bled for ILTV antibody assays (virus neutralization and ELISA - BioChek B.V. Burg. Bracklaan 57, 2811 BP Reeuwijk, The Netherlands). Half of the vaccinates and controls were challenged at 29 days and antibody responses compared.

RESULTS AND CONCLUSIONS

1. ILTV challenge of vaccinates could be serologically differentiated from antibody responses to vaccination alone with either the tissue culture or recombinant vaccine.

2. An ILTV specific antibody response to CEO vaccination could be readily demonstrated by ELISA (BioChek) or VN testing. However in well vaccinated birds there was no detectable change following ILTV challenge suggesting the birds were fully protected.

3. ILTV serology can be a useful diagnostic tool for monitoring response to ILTV vaccines in a commercial environment and for documenting challenge in both vaccinates and unvaccinated birds.

ACKNOWLEDGMENT

BREAK IN "STEADY STATE" EATING PATTERNS AND ITS POSSIBLE ROLE IN THE PATHOGENESIS OF DIFFERENT DISEASES IN COMMERCIAL BROILER CHICKENS

Shahbaz-ul-haq and Stewart J. Ritchie

Canadian Poultry Consultants, 30325 Canary Court, Abbotsford, British Columbia, Canada V4X 2N4

SUMMARY

Four cases of high mortality in commercial broiler chickens, aged between 18-24 days were presented to Canadian Poultry Consultants Ltd (CPC) at different times for disease investigation. Each one of them had a history of disruption in "steady state" eating patterns due to different reasons such as significant feed spill due to malfunctioning equipment, malfunctioning inlet vent, collapsed barn ceiling due to heavy snow and a malfunctioning feeder line hopper switch. After post-mortem and diagnostic workup, three cases were diagnosed with inclusion body hepatitis and one with necrotic enteritis. Based on the history of these birds and diagnostic findings, the break in "steady state" eating patterns was considered to play an important role in the pathogenesis of infectious diseases in commercial broiler chickens.

INTRODUCTION

Steady state feed consumption refers to the eating pattern of a broiler. Broilers in a comfortable environment with full lights or nearly 24 hours of lights and constant access to feed and water, consume both feed and water at a steady state rate throughout the day and night. Individually, broilers are meal eaters. If broilers have easy access to feed, they will eat about every four hours and will drink several times during a four hour feeding cycle (15).

The main objectives of this case report analysis are to highlight the importance of steady state eating patterns in commercial broiler chickens and its possible role in the prevention of infectious diseases, to understand the importance of a thorough history and its role in the diagnosis of the poultry disease and the development of control and prevention programs and to demonstrate the importance of properly maintained automated equipment in preventing diseases.

MATERIALS AND METHODS

The commercial broiler chickens in the four cases presented ranged from 18-24 days old and were raised in standard commercial all- in/all-out operations. Chicks were vaccinated against Marek's disease on day 18 *in ovo* at the commercial hatcheries. Diets were commercial starter crumbles and grower pellets, all of which included an anticoccidial and an antibacterial. The parent stocks were not vaccinated against inclusion body hepatitis.

The necropsy of three cases revealed dark congested body muscles, petechial hemorrhages on the thigh muscles, swollen livers with focal diffuse hemorrhages, and red swollen kidneys. Liver samples were submitted to different diagnostic laboratories for further investigations including polymerase chain

reaction test and histopathology to confirm the suspected cases of inclusion body hepatitis.

In the fourth case, distended intestines lined with brownish diphtheritic membrane was observed (17).

RESULTS AND DISCUSSION

In three cases presumptive diagnosis of inclusion body hepatitis was confirmed by polymerase chain reaction test and histopathology. Histopathological findings included generalized acute coagulative necrosis associated with abundant intranuclear inclusion bodies in the liver. The fourth case was diagnosed as necrotic enteritis based on the gross post-mortem findings.

In all cases, the disruption of steady state was considered to result in litter eating and excessive stress. Stress could cause high levels of corticosterone and many changes take place in the bird with a high level of corticosterone. Birds under stress from almost any cause have a lymphatic involution with atrophy of bursa of Fabricius, thymus, and spleen. In general, due to increased circulating corticosterone, resistance to viral disease is decreased (13). Previous studies have shown that adenovirus strains can rapidly exploit opportunities presented, such as when the health of the birds is compromised by co-infection with chicken infectious anemia virus (CIAV) or infectious bursal disease virus (IBDV) (9,10,11,12). These co-infections primarily cause immunosuppression as does stress.

The present commercial broiler chickens grow at a very fast rate i.e., a typical broiler chicken from 18-24 days consumes 89-125 g of feed and gains 60-76 g of weight per day (1). Disruption in feed could cause a response that would be sufficient to create immunosuppression. Disruption in a steady state eating patterns for extended periods is similar to problems reported to be associated with feed withdrawal. Studies on controlled feed withdrawal have demonstrated decreased tensile strength of the intestines (2), heavy sloughing of intestinal mucosal cells due to cell necrosis (14), loss of the protective mucus layer (18), and increased propensity for food borne pathogens, such as *Salmonella*, to attach to the intestinal epithelia (3). Some studies have found *Clostridium perfringens* to be the principal obligate anaerobic bacterium in the intestinal tract of chicken (6,16). Other studies have shown that *C. perfringens* can be found in feces, soil, dust, contaminated feed and litter (7,8). In various outbreaks of necrotic enteritis, contaminated feed (4,5,20) and contaminated litter (19) have been incriminated as sources of infection.

One factor common to all these cases was restricted access to adequate feed intake caused by significant feed spill due to malfunctioning equipment, malfunctioning inlet vent, collapsed barn ceiling due to heavy snow, and a malfunctioning feeder line hopper switch. Any of these factors may have contributed to increased litter eating, stress, and subsequent infection.

It is possible that the disruption of steady state eating patterns and the subsequent diagnosis of an infectious disease were entirely coincidental. A thorough history taking is considered extremely important in providing the information required to develop disease control and prevention strategies. In subsequent flocks at all four farms there was no reoccurrence of disease.

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FEED-RELATED CATASTROPHIC MORTALITY IN GAME BIRDS

Benjamín Lucio-Martínez

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

ABSTRACT

A study of catastrophic mortality in pheasants and chukar partridge associated with feed demonstrated high levels of botulinum toxin, rarely found in the feed (1). The feed involved in this case caused very high mortality in pheasants and chukars. Specific-pathogen-free (SPF) white leghorns did not develop clinical signs when reared using the suspect feed.

CASE HISTORY

In the middle of July of 2009, a producer with many years of experience raising game birds in Western New York State presented 10 six-week-old chukars from a flock with a history of high mortality. About 150 of a 300 bird flock were found dead on the morning of the third day after receiving and placing them in a large wire-floor cage. Another 100 died during the day. On the next day, during the visit to the farm only two chukars were found alive, lying on their breast with the legs stretched out.

In 2007 the producer had lost several birds from botulism, and had been diligent in picking up dead birds. Before raising the chukar flock, during the 2009 season, he raised two pheasant flocks 3500 birds each from day one. The first flock had no problem at all. The second flock suffered 65% mortality in the first four weeks of age. Mortality ceased as soon as the birds were moved outdoors, and, and the producer assumed that the mortality was associated with the brooder building.

Freshly dead chukars and feed were collected during the farm visit, and brought to the laboratory.

Other than white striation of the breast muscle at post-mortem examination no other lesions were observed. Histopathological examination did not reveal lesions of diagnostic value.

FEED TRIALS

Three feed trials were conducted. Because of availability, the first animal test was conducted using four groups of 10 four-week-old SPF chickens each.

The first group received undiluted (100%) suspect feed, the second and third groups received suspect feed diluted with commercial feed to a concentration of 66% and 33%, respectively. The last group received only commercial feed.

No clinical signs were seen in these chickens. The only possible indication of an abnormality was a slight (not-statistically significant) reduction of body weight gain in the chickens receiving 100% suspect feed.

For the second experiment 12 six-week-old chukars were divided into two groups of six chukars each. One group received undiluted suspect feed and the other undiluted commercial feed. Six of the chukars receiving suspect feed were unable to move and died within 48 hours, while the six on commercial feed survived and were clinically healthy.

The third experiment involved the six surviving chukars and six four-week-old chickens. The set up was similar to the first experiment, where one chukar and six chickens received undiluted suspect feed; two chukars received suspect feed diluted with commercial feed to a 66% concentration, two chukars received suspect feed at 33% concentration, and the last chukar received 100% commercial feed. In this trial the chickens on suspect feed and the chukar on commercial feed survived until the end of the experimental period (one week). The chukar that received 100% suspect feed died within 24 hours, while the chukars that were fed 66% and 33% suspect feed died within 48 and 96 hours, respectively. Clinical signs were not observed in the chickens, and the only sign seen in chukars was paralysis, just before death.

Histological observation on chukar and chicken tissues revealed no abnormalities of diagnostic significance.

Heavy metals, aflatoxins (B1, B2, G1, G2), ochratoxin A, zearalenone, T-2 toxin, diacetoxy-scirpenol (DAS), anddeoxynivalenol (vomitoxin), and sterigmatocystin were not detected in the feed.

The Pennsylvania Diagnostic Laboratory found both Type C preformed botulinum toxin and spores in the feed and intestinal contents from dead chukars.

DISCUSSION

Botulism is commonly associated with consumption of toxin-laden invertebrates such as fly maggots from decomposed carcasses, it has rarely been associated with feed contamination. It was not determined if the origin of botulinum toxin in the feed was the feed mill or the silo in the farm itself. The use of chickens for botulinum toxin in the feed proved to be unreliable due to a lower susceptibility than pheasants and chukars.

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IMPACT OF MASH GRIND ON SHELL QUALITY

A.E. (Ted) Sefton

Alltech Inc., Guelph, ON, Canada

INTRODUCTION

"Chickens digest kafir and corn more completely when the grain was fed whole than when it was ground to meal (1)." It was later reported that digestibility improves when a position of the ration is offered as whole cereal (2). The study reported, is a continuation of this line of research from the early1900s.

MATERIALS AND METHODS

The study took place on the W.B. Coburn & Sons farm in Keswick Ridge, N.B. Canada. This Bicentennial Farm has an apple orchard and a 25,000 layer operation. Feed is made, on farm, with a Sudenga computerize, mix mill. The grain is put through a hammer mill prior to mixing, then, delivered by auger to the lay barn. Ingredients are a mixture of on farm grown and bought.

During mill repair and servicing, whole kernel wheat was sent to the mixer, then this grain, not hammer-milled, was used in the feed. As this feed was being fed less membrane eggs were noticed, as compared to normal. Out of curiosity this was repeated and compared to normal milled feed. Membrane egg numbers tended to decrease following the use of whole wheat.

As market conditions changed, wheat was removed from the diets, and the diets were formulated using corn as the cereal source. The hammer mill originally had a 3/8 in. screen. Various sized screens were used in the hammer mill, to determine the optimum screen size, based on bird performance, including number of membrane eggs.

RESULTS AND DISCUSSION

It was found that a ½ in. screen in the hammer mill produced a feed that minimized the incidence of membrane eggs. This size screen allowed occasional whole kernels of corn to pass through the hammer mill and into the feed. The ½ in. screen produced an average particle size of 1160 dgw, while the original 3/8 in. screen a finer feed of 1014 dgw.

Prior to use of the coarser screen there were approximately 17 to 18 membrane eggs per day, this reduced to 10 to 12 membrane eggs per day with the coarser screen.

Broiler (2,3), turkeys (4), and layers (5,6) have shown improvement if digestibility when all or a portion of the cereal is whole grain. In many regions it is a common practice (7) to feed a portion of the cereal as whole grain for maximum economic performance. This can, in part be explained be improved gut health (8).

The Coburn Farm has implemented the use of the ½ in. coarser screen in their hammer mill as part of their standard operating procedure resulting in:
1. Fewer membrane eggs;
2. Less electrical energy to grind the corn to the coarser particle size;
3. Greater hammer mill mixer trough-put.

ACKNOWLEDGMENTS

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INTERACTION BETWEEN MAJOR PATHOGENS IN A FINISHING TURKEY FLOCK: RESULTS OF A LONGITUDINAL STUDY

Davide Giovanardi[A], Patrizia Pesente[A], Caterina Lupini[B],
Giulia Rossi[A], Ruffoni Luigi Sperati [A], Giovanni Ortali[C], and Elena Catelli[B]

[A]Laboratorio TreValli, Corte Pellegrina, 3; 37132 San Michele Extra (VR), Italy
[B]Dipartimento di Sanità Pubblica Veterinaria e Patologia Animale, Faculty of Veterinary Medicine, Alma Mater Studiorum - University of Bologna, Via Tolara di Sopra, 50, 40064 Ozzano Emilia (BO), Italy
[C]Agricola Tre Valli, Via Valpantena 18/G, 37138, Quinto di Valpantena (VR), Italy

SUMMARY

This study investigated the spectrum of mortality-associated pathogens in a Italian finishing male turkey commercial flock. The survey mainly focuses on the interaction between avian pathogenic *Escherichia coli* (APEC), avian metapneumovirus (AMPV) and turkey hemorrhagic enteritis virus (THEV).

Cloacal swabs, rhino-pharyngeal swabs and blood samples were collected from 10 birds weekly until turkeys were 14 weeks old for virus isolation and/or PCR detection or microbiological examinations. Mortality was recorded and a sample of birds showing clinical signs, or deceased birds, were examined post mortem. When colisepticemic lesions were observed, microbiological examination was performed from

selected organs. All *E. coli* detected were assessed for the presence of virulence-associated genes and were serotyped. Random amplified polymorphic DNA (RAPD) was used to analyze the relationships between *E. coli* clones. Serum samples were examined for antibodies against AMPV, THEV, avian influenza, paramyxovirus-1, *Mycoplasma gallisepticum*, and *Mycoplasma synoviae*.

During a severe episode outbreak of mortality (0.5%) occurred in the fourth week of age, four strains of APEC were isolated and AMPV subtype B was detected. Later on, during the whole trial, eight more APEC strains were isolated from lesions of colisepticemia. THEV was detected from eighth to ninth weeks of age. The final results show that the higher mortality rate, at week four, was due to more than APEC strains and it occurred in young birds in association with AMPV infection. No role was played by THEV in increasing mortality. An APEC serotype O111 was detected; to our knowledge this is the first report of its detection in turkeys.

INTRODUCTION

Colibacillosis is considered one of the leading causes of economic loss in the poultry industry worldwide (1). Serotypes O1, O2 and O78 are present in 15% to 61% of turkey colibacillosis cases in Italy, *E. coli* where O78 is the most prevalent serotype isolated according to Circella *et al*. (4). Although *E. coli* is likely present in normal gastrointestinal flora of poultry, it is thought that only specific strains are endowed with virulence factors enabling them to cause disease. These strains are known as APEC. There are numerous field and experimental evidences that many factors can predispose or exacerbated colisepticemia, including viral and bacteria infections. AMPV and THEV infections are considered to play a relevant role in turkey colibacillosis (1).

AMPV is an RNA virus which is the type species of the genus Metapneumovirus in the *Paramyxoviridae* family. It is the casual agent of turkey rhinotracheitis (TRT). At present, four AMPV subtypes have been characterized: A, B, C, and D subtypes (5).

Hemorrhagic enteritis is an acute viral disease of turkeys caused by a DNA virus of the family *Adenoviridae*, genus *Siadenovirus*, i.e. turkey hemorrhagic enteritis virus (THEV). THEV – induced disease is characterized by depression, splenomegaly, intestinal hemorrhage, and immunosuppression followed by secondary bacterial infection such as *E. coli* (9).

The purpose of this study was to investigate the spectrum of mortality-associated pathogens in finishing turkeys focusing on the interaction between APEC, AMPV and THEV in different phases of the birds' commercial life.

A longitudinal study was performed on a flock of male turkeys. Birds were sampled weekly for selected pathogens detection or serology. Mortality was recorded and post mortem and microbiological analysis were applied on a sample of deceased birds. Serology was performed for AMPV, THEV, avian influenza, paramyxovirus-1, *Mycoplasma gallisepticum*, and *Mycoplasma synoviae* antibody detection.

Moreover the diversity and the distribution of *E. coli* strains during the production cycle were examined using microbiological and molecular techniques. *E. coli* detections were serotyped, assessed for the presence of virulence-associated genes, and resistance to antibiotics. Random amplified polymorphic DNA was applied to analyze the genetic relationship between them.

MATERIAL AND METHODS

Sampling. Cloacal swabs, rhino-pharyngeal swabs and blood samples were weekly collected from 10 birds for virus isolation and/or PCR detection, or microbiological examinations, until turkeys were 14 week old. Mortality was recorded and all birds about to die during the trial carcasses were examined post mortem, and microbiological examination were performed from animals with clear lesions of colisepticemia.

***E. coli* bacteriology.** Selected viscera (brain, pericardial sac, liver, spleen and joints) from carcasses were cultured into 3% sheep blood agar and Eosin – Methylene Blue agar (EMB, OXOID) and incubated aerobically at 37°C for 18 to 24 hr. The identification of *E. coli* was based on biochemical features.

***E. coli* serotyping.** Serogrouping of isolates was performed at IZSLER (Brescia, Italy).

***E. coli* antibiotic sensitivity test.** A sensitivity test (Kirby-Bauer) for nine antimicrobial agents was performed on all the *E. coli* isolated strains by the standard disk procedure. The antibiotic used were ampicillin, amoxicillin, oxytetracycline, gentamycin, trimethoprim-sulfamethoxazole, apramycin, amino-sidin, colistin (OXOID), and enrofloxacin (Bayer).

***E. coli* virulence genes.** PCR for the presence of virulence-associated genes was performed for all *E. coli* isolates. Strains were inoculated into EC broth (Difco), incubated aerobically at 37°C for 12 hr and then submitted for PCR. DNA was extracted with Prepman Ultra (Applied Biosystems). Amplification of each virulence associated genes *iucD, tsh, papC, fimC* (7), *fyuA* and *irp-2* (6), was done with Qiagen Multiplex PCR kit (Qiagen). The PCR products were separated by electrophoresis on 2% of agarose gel stained with SYBR safe DNA gel stain (Invitrogen).

E. coli **random amplified polymorphic DNA (RAPD).** RAPD technique was used to analyze the genetic relationships between the *E.coli* clones. Bacterial DNA was extracted with Prepman Ultra and quantified by spectrophotometer. Twenty ng of DNA was used as template in the RAPD kit Ready-to-Go beads (GE Healthcare). The kit was used as described by the supplier with primer 1290 (8). Amplification as follow was done: Five min denaturation at 95°C, 45 cycles of one min at 95°C, one min at 36°C, and two min at 72°C. Amplification products were resolved by electro-phoresis on 2% agarose gel SYBR safe DNA gel stained. The image was captured using Gel Doc 2000 (BIO-RAD). The fingerprinting was analyzed with Gel Compare II (version 2.0, Applied Maths) and the measure of the similarity was based on densiometric curves using Pearson correlation. A dendrogram was generated by unweighed pair group method with arithmetic average (UPGMA).

AMPV RT-nested PCR. A subtype specific RT-nested PCR, based on G gene sequence, able to differentiate A and B subtypes, was used to detect and type AMPV from dry swabs and isolates. RNA was extracted from pools of ten dry swabs and cDNA prepared followed by nested PCR using the method described by Cavanagh *et al.* (3).

AMPV isolation on TOC. Virus isolation was performed in chicken embryo tracheal organ cultures (TOC) (2). Swabs were pooled and the supernatants were used to inoculate TOCs. Ciliostasis was taken as the initial indicator of the presence of the virus. AMPV identification and subtyping was determined by RT nested PCR previously described, the RNA was extracted from the inoculated medium.

THEV PCR. DNA from cloacal swabs and spleen samples collected from the examined carcasses, was extracted with PureLink Genomic DNA kit (Invitrogen) as described by the supplier. Amplification was done with Taq PCR Master Mix (Qiagen) in 20 μL reaction mix containing 2 μL DNA (20 ng/μL) and 0.2 μM primers specifically for the detection of THEV developed by Pesente on Hexon gene (forward "GGC ATG GGC AAC TAT CCT AA" and reverse "TAG GAA CAC TGC CAA AAC CC"). PCR reaction conditions were: Two min denaturation at 95°C, 35 cycles of 30 sec at 94°C, 30 sec at 55°C, 30 sec at 72°C, and finally a cycle at 72°C for 10 min. The PCR products were separated by electrophoresis as described before.

Serology. Antibodies to aMPV were detected with an ELISA kit "Home Made" and with commercial kits for THEV (Synbiotics, Kansas City, MI, USA), *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS), avian influenza *Orthomyxoviru*s (AIV), and avian *Paramyxovirus* type 1 (APMV-1) (IDEXX, Westbrook, MA, USA).

RESULTS

Mortality and necroscopy findings. The higher weekly mortality was 0.5% and was registered during the fourth week of age. During this week the birds showed respiratory signs like snicking, swollen infraorbital sinuses, and nasal exudate. The overall mortality at the end of the survey was of 3.76%.

In most carcasses examined, postmortem lesions, including pericarditis and airsacculitis, were observed; these were highly suspicious of colisepticemia due to avian pathogenic *E. coli*.

E. coli **bacteriology, serotyping, and antibiotic sensitivity.** Thirteen *E. coli* strains were isolated. They were oxidase negative, non-hemolytic, catalase positive and all but one of them were lactose positive, with characteristic greenish-black metallic sheen on EMB agar. The API commercial differentiation system identified all the isolates as *E. coli*. Eight *E. coli* serotype O78 were isolated in different times in all the cycle from two weeks up to the end of the survey, one *E. coli* O111 (lactose negative) isolated at day 79 and four not typeable strains at 27, 58, and 66 days. The antibiotic sensitivity test on eight strains *E. coli* O78 isolates showed the same pattern with resistance to ampicillin, amoxicillin, oxytetracycline, and sensitivity to trimethoprim-sulfamethoxazole, gentamycin, apramycin, colistin, aminosidin, and enrofloxacin.

E. coli **virulence genes.** Virulence associated genes *iucD*, *tsh*, *fimC*, *irp-*, *2* and *fyuA* were present in all *E. coli* O78 strains while all of lacked *papC* gene thus all *E. coli* O78 belonged to the same pathotype. *E. coli* O111 was positive for all virulence associated genes searched. All these isolates were considered as APEC.

E. coli **RAPD.** Phylogenetic analysis was used to determine if there were any genetic similarities between the eight *E. coli* O78, of the same pathotype and pattern of antibiotic resistance, the *E. coli* O111 and the four untypeable strains. The RAPD analysis performed using primer 1290 revealed four distinct patterns. *E. coli* correlated with similarities in their RAPD DNA patterns were clustered. The most interesting clusters were: cluster I which consisted of one *E. coli* O78 isolate and *E. coli* O111; cluster II which consisted of all the remaining seven *E. coli* O78. To test the reproducibility of the RAPD technique, the samples were analyzed in two independent reactions. Results obtained showed no loss or shift in the position of banding patterns.

Detection of AMPV and isolation on TOC. AMPV subtype B was detected by RT-PCR and isolated on TOC from 23 day old turkeys

Detection of THEV. The virus was detected by PCR form cloacal swabs from 59 day old birds and

spleens from carcasses examined at days 66, 72, 79, and 94.

Serology. An early remarkable peak in AMPV antibodies titers has been observed during the four to six weeks of age. This was followed by a rise in THEV antibodies titers from the 11th week of age that lasted up to the end of the trial. No antibodies to MG, MS APMV-1 and AIV were detected during all the study.

DISCUSSION

During the cycle, eight strains of APEC O78 were isolated at different ages from week two to 14. Seven of them possess the same virulence genes and have high genomic similarity belonging to the same cluster. This means that this APEC O78 was the most prevalent in the production cycle. The higher weekly mortality in the fourth week was caused by infection of APEC O78 in combination with AMPV subtype B, while other APEC strains, one O111 and four non typeable strains, were not responsible for important clinical outbreaks later in the cycle. Moreover, in absence of AMPV later in the cycle, APEC O78 was not able to create severe episodes of mortality but only sporadic cases of colibacillosis and arthritis.

In terms of mortality, and based on clinical data, THEV did not lead to clinical hemorrhagic enteritis nor induced episodes of severe colisepticemia.

The detection of the APEC O111, to our knowledge, represents the first report of this strain in turkeys. APEC 0111 has been reported at least two times in chickens (Zanella *et al*. (12) and Trampel *et al*. (10)) during severe episodes of infection in laying hens. Zanella strain's virulence genes (*E.coli* kindly provided by Prof. Zanella) are the same as of the turkey one. The RAPD analysis performed on both strains revealed similar profiles, probably due to the high genomic similarity. It is thought that these strains have the same clonal origin. Further studies are needed to better understand its intrinsic pathogenicity.

It is widely believed and experimentally reported that co-infection with AMPV and *E. coli* can lead to an increased morbidity and a higher incidence of gross *E. coli* lesions (11). Our findings support the notion that AMPV may act as a primary agent predisposing to *E. coli* colonization and invasion.

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ENTERIC DISEASE INTERACTIONS IN COMMERCIAL POULTRY

D. Karunakaran

Danisco Animal Nutrition, Waukesha, WI

Intensive and sustainable animal production is very crucial for agricultural based economies. Today's poultry industry to be sustainable needs new technologies and science based interventions. New tools are absolutely essential to control and reduce enteric disease challenges. It is also important to be able to quantify and measure the challenges before and after the implementation of new technologies.

Animals have become more vulnerable to potentially harmful microorganisms such as *Escherichia coli* and *Clostridium* spp. In an attempt to control some of these challenges, the use of both therapeutic and sub-therapeutic antibiotics has been widespread. However, public health concerns and consumer pressure to reduce the use of antibiotics in poultry production is increasing. This has logically led to an increased interest in other methods of enhancing poultry performance and helping the bird to withstand subclinical disease challenge. Hence, biotechnology companies are increasing the investment of time and money to look at alternatives to maintain growth and performance in poultry.

Clostridium spp. is typically one of the most ubiquitous microorganisms in poultry production systems. As anaerobic bacteria, *Clostridium perfringens* and *Clostridium septicum* continue to become more prevalent in commercial poultry production. To control clostridial diseases, it is increasingly evident that one needs to better understand the gut microorganisms involved in the GIT that maintain the balance between beneficial flora and pathogens. Clostridial spores are typically found in high numbers in both the litter and the GIT of poultry. These organisms are capable of producing an array of extra-cellular enzymes and toxins that degrade host tissues

Over the last several years Danisco Animal Nutrition has worked extensively with many producers to better understand the microorganisms involved in clostridial diseases of poultry. This work has revealed an incredible amount of genetic diversity within the species of both *C. perfringens* and *C. septicum*. Both organisms are often present in a bird experiencing cellulitis or gangrenous dermatitis. Interestingly, this work also suggests that multiple strains of each species are often involved. Another important observation is that clostridial bacteremia is almost always associated with these diseases. It is not unusual to observe bacteremia in seemingly healthy broilers or turkeys.

Although *C. perfringens* and *C. septicum* have been the most commonly observed toxigenic clostridia, other species such as *C. cadaveris*, *C. sordellii*, and *C. tertium* have been identified as well. A better understanding of these organisms and the diseases they cause is a prerequisite for the development of new intervention strategies to control these diseases.

Avian pathogenic *E. coli* (APEC) in the GI tract is a specific subset of pathogenic *E. coli* that cause extraintestinal diseases of poultry. APEC is naturally present in the intestinal microflora of healthy birds and infections are enhanced or initiated by secondary environmental stress and host of predisposing factors. Colibacillosis refers to any localized or systemic infection caused entirely or partly by avian pathogenic *Escherichia coli* (APEC) and is the most frequently reported disease in surveys of poultry diseases or condemnations at processing.

Danisco Animal Nutrition has also researched the prevalence, distribution, and diversity of APEC within large integrated turkey, layer, and broiler companies. We find a large amount of diversity within the APEC communities both between sites as well as within sites at many of these companies. The level of APEC can range from 10^3 to 10^6 CFU/g or higher in younger birds. Research also indicates that similar APEC isolates can have different combinations and number of virulence factors associated with them. In general, APEC has been found to be more prevalent and more virulent among younger birds.

There may be multiple means to impact the gut microbial environment. One such method is by the continuous addition of a heat stable bacterial culture to the feed. This is done as a way to prevent colonization by potentially pathogenic microorganisms while also enhancing the host's immune system.

SALMONELLA ENTERITIDIS IN POULTRY AND PEOPLE: AN OUTBREAK REPORT

W. Cox and M. Leslie

B.C. Ministry of Agriculture and Lands, Animal Health Branch
1767 Angus Campbell Rd., Abbotsford, BC, V3G 2M3

In 2007, the BC Centre for Disease Control (CDC) observed a rise in the number of cases of illness in humans due to SE Phage Type (PT) 13. While normally seen only sporadically in people, this PT was showing a rise in incidence and prevalence, similar to the situation observed in other provinces in Canada. The outbreak spanned the summer of 2007 between June and September. An investigation was commenced and, in spite of quick follow-up interviews with cases, a specific food source could not be identified.

As part of the *Salmonella* Pullorum testing program in Canada, fluff samples from registered hatcheries in BC are submitted to the Animal Health Centre (AHC) for culture. A side benefit of this program is the isolation of SE when it is present in a flock. In early 2008, the number of SE isolates from fluff samples began to rise and these isolates were typed to PT 13. The rise in isolates of this particular PT in light of the human outbreak caused us to further investigate the apparent rise in hatchery samples.

In early 2008, traceback showed that SE/PT13 isolates from hatchery fluff samples came only from hatching eggs imported from the U.S. The history of SE/PT13 in other provinces, however, showed that the organism first appeared only in imported eggs but soon started to emerge from local flocks. Public health concerns together with the details on the source of the organism was compiled and presented to hatchery management and their veterinarians and to the Hatching Egg Commission. Collectively, the hatchery managers put pressure on the offending supplier of US eggs to manage the problem. Soon after these actions were taken, the frequency of isolations of SE/PT13 from hatchery fluff samples quickly dropped and soon disappeared.

Subsequent to the SE/PT 13 outbreak in people, a new outbreak was identified, this time associated with SE/PT 8. This outbreak was more profound than that observed for PT 13, having more cases and being more prolonged. The normal incidence of SE/PT8 in humans is one to eight cases per month; beginning in mid 2008, however, health authorities reported between five and 20 cases per week through November of that year, then picking up again in January 2009 and extending into fall of 2009. The total number of reported cases exceeded 250.

Initially, public health was unable to identify a common food source for the reported cases; however, small clusters of illness were associated with various restaurants and bakeries. Coincidentally, health inspectors discovered many of these premises using ungraded eggs for food preparation. The eggs, which were confiscated, included both hatching and table eggs and were of very poor quality, often cracked and contaminated with fecal material. This finding revealed a large, illegal distribution network of ungraded eggs.

Under the premise that eggs were the most likely source of illness, a case control study was done by BCCDC. The results showed that there was a two times higher risk of infection associated with the consumption of eggs, a significant increase.

Paralleling the rise of cases in humans was a substantial rise of isolates of SE/PT 8 from hatchery fluff samples. In 2007, there were seven fluff sample SE isolates, 32 in 2008, and 66 in 2009. Additionally, there were four, eight, and 19 diagnostic isolates of SE for 2007, 2008, and 2009, respectively. At least 50% of the SE isolates were PT 8, but many of the remaining ones were untyped.

The substantial rise in the frequency of SE isolates from diagnostic samples was concerning. Normally not associated with illness or mortality in chickens, these isolates were considered to be the cause of mortality in mostly young broiler chickens, predominantly in the first week or two of life, but also seen in older birds. It was evident that this strain of SE/PT 8 was becoming a threat not only to human health but now also to poultry health.

In addition to the findings implicating ungraded eggs in the human outbreak, a genetic connection between human and poultry isolates was established. Multi Locus Variable Number Tandem Repeat Analysis (VNTR or MLVA) was applied to all of the Animal Health Centre SE isolates, 21 human isolates, and 15 retail chicken isolates collected from BC grocery stores during 2008. A large cluster of samples with a specific VNTR pattern emerged that included human cases, fluff samples, diagnostic chicken samples, and isolates from retail chicken. Other SE isolates from table egg layer environmental samples appeared to rule out table eggs from supply managed farms as a source of this particular isolate.

In addition to laboratory support and excellent communication, public health worked on two fronts to impact the ongoing outbreak. The first approach was to promote good food handling practices to the public. At the same time, inspection officers continued to identify and deal with food preparation premises such as restaurants illegally using ungraded eggs. On the agricultural side, attention turned to the illegal sale of large lots of ungraded eggs to distributors and the rising prevalence of SE in hatching egg flocks.

An educational campaign was launched to address the issues with producers. They were advised of the problem in humans and the potential role that they might be playing in that outbreak. Of particular importance was the sale of hatching eggs to distributors. This action resulted in a significant drop in the number of eggs moving outside of the legal distribution channels, as measured by the number of unsettable eggs being transported to the pasteurization plant.

However, there was also much concern over the transfer of SE to broiler chicks and not only the potential human health risk but also the poultry health issue evident there. Consequently, the BC Broiler Hatching Egg Commission launched a program of farm monitoring for SE combined with aggressive mitigation strategies developed between farms identified with SE and the farm's veterinarian. The measure of success over the coming months will be a reduction in SE isolations from routine fluff samples plus a reduction in the number of isolates from farm environmental samples.

The concerted effort between public health and veterinarians both in industry and the BC Ministry of Agriculture and Lands has been an important collaboration in protecting human health. Collectively, the hatching egg producers in BC have taken an important role in the solution to a problem after being made aware of and recognizing the part they may have played in a human health situation.

MONITORING ON *SALMONELLA* INFECTIONS IN TURKEY FLOCKS IN GERMANY AND EUROPEAN UNION CONTROL MEASURES

Hafez Mohamed Hafez

Institute of Poultry Diseases, Free University Berlin, Königsweg 63, 14163 Berlin, Germany
hafez@vetmed.fu-berlin.de

In spite of significant improvements in technology and hygienic practice at all stages of turkey production accompanied with advanced improvement in public sanitation salmonellosis remains a persistent threat to human and animal health. The significant increase in the number of reported food borne outbreaks world-wide has altered our view for food safety. In many countries high incidence of salmonellosis in man appears to be caused by infection derived from contaminated poultry meat. The poultry industry has realized that prevention or reduction of food borne hazards can be achieved only through good management practices on the farm and during transport as well as logistic slaughtering and hygienic processing and distribution. In general the main strategy for control of *Salmonella* in poultry should include: Cleaning the production pyramid from the top by culling infected flocks, hatching egg sanitation and limiting introduction and spread at the farm level through Good Animal Husbandry Practices (GAHPs). To achieve GAHPs, effective hygiene measures should be applied to poultry houses, their environment and the feed. In addition, reducing *Salmonella* colonization by using feed additives, competitive exclusion or vaccines are further possibilities. All this measures should be accompanied with governmental supervisions and legislations (5,6,7).

Current legislations in the EU. In November 2003, the European Parliament Council Regulation 2160/2003/EC (1) on the control of *Salmonella* and other specified food-borne zoonotic agents was passed. This regulation covers the adoption of targets for the reduction of the prevalence of specified zoonoses in animal populations at the level of primary production, including meat turkey. After the relevant control program has been approved, food business operators must have samples taken and analyzed for the zoonoses and zoonotic agents. The flocks should be sampled also by the competent authority.

Specific requirements concerning flocks of turkeys. In June 2008 commission regulation (EC) No 584/2008 of implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards a Community target for the

reduction of the prevalence of *S.* Enteritidis and *S.* Typhimurium in turkeys was put into force. The Community target is the reduction of both *Salmonella* serovars to the maximum percentage of fattening and breeder flocks remaining positive to 1% or less by 31 December 2012 (4). The testing scheme necessary to verify progress in the achievement of the Community target is set out in the Annex of this regulation and shall apply from 1 January 2010.

All flocks of fattening and breeding turkeys should be investigated on the initiative of the food business operator within three weeks before the birds are moved to the slaughterhouse. In addition, flocks of breeding turkeys should be investigated during the rearing at day-old, at four weeks of age and two weeks before moving to the laying phase: During the laying period they are to be investigated at least every third week at the holding or at the hatchery.

Sampling by the competent authority in breeding turkey flock with at least 250 birds should be carried as follows:

- Once a year, all flocks on 10% of holdings with at least 250 adult breeding turkeys between 30 and 45 weeks of age but including in any case all holdings where *Salmonella* Enteritidis or *Salmonella* Typhimurium was detected during the previous 12 months and all holdings with elite, great grandparents and grandparent breeding turkeys; this sampling may also take place at the hatchery.
- All flocks on holdings in case of detection of *Salmonella* Enteritidis or *Salmonella* Typhimurium from samples taken at the hatchery by food business operators or within the frame of official controls, to investigate the origin of infection.
- Each time the competent authority considers it necessary.

Sampling by the competent authority in holdings with at least 500 fattening turkeys should be carried as mentioned by breeding flocks

Specific requirement concerning fresh poultry meat. Starting from 12/12/2010 fresh poultry meat may not be placed on the market for human consumption when *Salmonella* was detected. The criterion laid down does not apply to fresh poultry meat destined for industrial heat treatment or another treatment to eliminate *Salmonella* in accordance with Community legislation on food hygiene (1).

Further regulation concerning use of antimicrobials. Generally, according to Commission regulation (EC) No 1177/2006 (3) antimicrobials shall not be used as a specific method to control *Salmonella* in poultry. In some cases however the use of antimicrobial can be permitted.

Monitoring on *Salmonella* Infections in Turkey Flocks in Germany. In present study 10243 commercial turkey flocks were monitored for *Salmonella* between 2001 - 2009. Two boot swabs samples were collected from each monitored flock three weeks prior to slaughtering and examined bacteriologically as follow: Samples were pre-enriched in Buffered Peptone Water (1:10) and incubated at 37°C for 24 hours. Then selectively enriched in Rappaport Vassiliadis (RV) broth (1:100) and incubated at 41.5°C for 48 hours. Since 2009 the enrichment was carried out using Semi-solid Rappaport Vassiliadis (MSRV) (2). Then after the samples were streaked on Brilliant Green Phenol Red Agar (BGA) and Rambach plates and incubated at 37°C for 24 hours. *Salmonella* suspected colonies were identified serologically using slide agglutination tests.

The obtained results showed that a continuous reduction in the prevalence of all *Salmonella* serovars could be detected (Table 2). In 2002 until 2004 strong reduction was observed from 18.1% to 5%. Between 2007 and 2009 the number of positive test flock varied between 3.2% to 5.0%. The prevalence of *S.* Typhimurium showed similar reduction. *S.* Enteritidis could not be detected in all examined samples since 2006 (Table 1).

CONCLUSION

With respect to the Council Regulation 2160/2003/EC (1) on the control of *Salmonella* and other specified food-borne zoonotic agents, starting from 12/12/2010 fresh poultry meat may not be placed on the market for human consumption when *Salmonella* was detected. The criterion laid down does not apply to fresh poultry meat destined for industrial heat treatment or another treatment to eliminate *Salmonella*. Until now it is not clear what is meant by *Salmonella* – all serovars or only ST and SE?

The obtained results still reinforce the fact that it is essential and important to continue the efforts on reducing *Salmonella* infections.

In general the major strategy to control *Salmonella* should include cleaning the production chain from the top in aim to prevent the vertical transmission and hygienic measures throughout the production chain to prevent the lateral transmission. In all cases agent surveillance and monitoring programs must be adapted and followed strictly in aim to allow early intervention. In addition, since the success of any disease control program depends on farm and personal sanitation, it is essential to educate people involved in poultry production about microorganisms, modes of transmission and raise general awareness for the reasons behind such control programs.

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Table 1. Results of bacteriological examinations.

Year	No. of tested flocks	No. of positive flocks	No. of ST positive flocks	No. of SE positive flocks
2001	1460	199 (13.6%)	26 (1.78%)	9 (0.6%)
2002	1506	272 (18.1%)	15 (0.99%)	1 (0.07%)
2003	1416	174 (12.3%)	6 (0.42%)	1(0.07%)
2004	1333	67 (5.0%)	19 (1.43%)	0 (0.0%)
2005	1330	69 (5.2%)	20 (1.50%)	2 (0.15%)
2006	1088	66 (6.1%)	3 (0.28%)	0 (0.0%)
2007	835	29 (3.5%)	6 (0.72%)	0 (0.0%)
2008	641	21 (3.2%)	1 (0.16%)	0 (0.0%)
2009	634	32 (5.0%)	2 (0.32%)	0 (0.0%)

ACTIVE SURVEILLANCE OF *SALMONELLA* TYPHIMURIUM DT104 AT BROILER BREEDER FARM, HATCHERY, AND PROCESSING PLANT LEVELS

C. Philippe[A], D. Slavic[B], M. Guerin[C], and B. Sanei[D]

[A]Ontario Hatcheries Association, Guelph, Ontario, Canada
[B]Animal Health Laboratory, Laboratory Services Division, University Of Guelph, Guelph, Ontario, Canada
[C]Department of Population Medicine, University Of Guelph, Guelph, Ontario, Canadaa
[D]Ontario Ministry of Agriculture Food and Rural Affairs, Guelph, Ontario, Canada

The Canadian broiler and breeder industry is very concerned about the possible transmission of potential pathogens through the food chain to consumers. *Salmonella* Typhimurium DT104 has been a concern due to its multiple antibiotic resistance profile. Currently in Ontario, breeder flocks are monitored for *Salmonella* via a provincial program (the Ontario Hatchery Supply Flock Policy - OHSFP). Flocks

confirmed positive for *Salmonella* Enteritidis or *Salmonella* Typhimurium DT104 are destroyed according to the industry established insurance program. Producers are compensated via a Poultry Insurance Exchange program (PIE). A small scale case-study in 1998 in Ontario showed that *S.* Typhimurium DT104 is associated with low risk of transmission through the broiler production chain (1).

In March 2009, a breeder flock tested positive for *S.* Typhimurium DT104 at the pullet barn and at the breeder barn. The hatchery accepted hatching eggs from the infected flock, provided that no *S.* Typhimurium DT104 be isolated from fluff samples (taken every hatch). Active surveillance was conducted for 15 weeks worth of egg production (27 weeks through 41 weeks old). This project accumulated additional scientific data of *S.* Typhimurium DT104 at the breeder barn, hatchery and at the processing plant (live haul) for transmission and dissemination of this pathogen through the broiler production chain.

Environmental samples at the breeder barn were taken six times during the 15 week study period (40 samples each time). Eggs laid at the time of breeder barn sampling were followed at the hatchery. Fluff samples and day old chick papers were sampled on the day of hatch. Finally, 500 birds were sampled via cloacal swabs when they arrived at the processing plant (live haul area).

Dissemination of *S.* Typhimurium DT104 from the breeder barn all the way through the processing plant was identified and was associated with disease stressor in the breeder flock. Positive samples at each sampling points were identified twice, when the breeder flock was 32 and 36 weeks old. In addition to the sampling protocol mentioned, *S.* Typhimurium DT104 was also identified several times from early chick mortality cases during that period of time. Positive samples were also identified between 37 and 41 weeks old but not at all sampling points. Due to higher than anticipated dissemination of the pathogen, the breeder flock was destroyed at 41 weeks old.

(The full length article will be submitted for publication in *Avian Diseases*.)

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EFFECTIVE *SALMONELLA* CONTROL – COMMERCIAL POULTRY OPERATIONS

Robert O'Connor

Foster Farms

SUMMARY

This talk reviews the success of the commercial broiler industry in reducing *Salmonella* presence on raw poultry since 2005.

ANTIBIOTIC FREE BROILER PRODUCTION COMPARISON TO CONVENTIONAL

Neil Ambrose

Sunrise Farms, 13542 73A Avenue, Surrey, BC, V3W 1C9, Canada

ABSTRACT

The production of broiler chicken meat without the use of in feed antibiotics has been in evolution for many years now. The concept started in the European Union where prophylactic antibiotic use seemed to be of major issue to consumer and health authority alike.

Concerns over antibiotic resistance stemming from continual use of antibiotics in broiler production for many years spurned the drive to grow chicken meat for human consumption without reliance of pharmaceutical additives in the life of a broiler chicken. Success of such non medicated programs has been mixed at best and largely due to the realization that commercial broiler production is not an easy process if the modern day growth objectives of broilers are to be realized. Regardless, large numbers of broiler chickens are now produced within the global commercial industry without pharmaceutical use in order to serve the needs of the marketplace.

In this study, average daily weight gain growth curves together with end of production cycle performance parameters from large scale commercial broiler farms in south western British Columbia, Canada will be compared between antibiotic free and conventional production programs.

BROILER STOCKING DENSITY AND THE EFFECTS ON CARCASS QUALITY AND BIRD WELFARE IN WESTERN CANADA

Neil Ambrose

Sunrise Farms, 13542 73A Avenue, Surrey, BC, V3W 1C9, Canada

ABSTRACT

The broiler industry in Canada is governed by a supply management system that attempts to match production with marketplace demands. In addition to managing the system of chickens that are grown, there have been developed audited national food safety, biosecurity and animal care programs that are incorporated into the production standards for each Province. However, there is little held within these well meaning programs that take into consideration the real customers of the chicken industry, that being, the retail chains, restaurant groups and food service suppliers who are the organizations that poultry processors deal with on a daily basis. Processors now more commonly require compliance with third party audit requirements that analyze broiler bird care and welfare. The standards of a retail chain or fast food restaurant may not match those of a Provincial Canadian chicken marketing board, thus placing industry at its own peril as they continue follow and argue for productivity based broiler stocking density allowances rather than consideration of the end customer. In this study, current stocking densities utilized in the Provinces of British Columbia and Alberta will be discussed with particular importance focussed on carcass quality and bird welfare.

INTERACTIVE PROBLEM-SOLVING OF FIELD CASES INVOLVING COMMERCIAL POULTRY – AN AUDIENCE PARTICIPATION PRESENTATION (THE SEQUEL)

Mark C. Bland

Cutler Associates, Napa California

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

Case 1. Commercial white leghorn pullets seven weeks of age experiencing mortality in one of six brooder barns.

History. A commercial layer brooder farm located in Southern California experienced an increase in mortality in a seven-week-old commercial flock of white leghorn pullets (Lohmann). There are six brooder buildings on site. Barns 1, 2, 3, and 6 raise about 36,000 chicks, which were brooded on the second floor. Barns 4 and 5 brood approximately 100,000 chicks each in a cage brooding system on the second floor. At eight weeks of age, chicks on the second floor of Barns 1, 2, 3, and 6 move into grow-out cages on the first floor. Approximately 33,000 chicks from the second floor of Barns 4 and 5 move into grow-out cages downstairs on the first floor. The rest of the pullets remain upstairs. The vaccination program uses a live program with three live infectious bursal disease, two *Salmonella* and Newcastle/bronchitis vaccines and one live MG and pox/AE vaccines. An additional live Newcastle/bronchitis and *Salmonella* vaccination is administered at 12 and 14 weeks respectively.

At seven weeks of age, pullets in Barn 5 had a sudden increase in daily mortality over a five-day period:

- Week 6: 18, 19, 17, 19, 18, 16, 19 (0.13% weekly).
- Week 7: 21, 31, 279, 221, 180, 130, 75 (0.98% weekly).

Ranch manager noted that the pullets looked dehydrated, lethargic, and the droppings were green.

Ten fresh dead pullets were submitted to the CAHFS Lab in San Bernardino. Main pathological findings were as follows:

- Proventricular mucosal hemorrhages (moderate to severe) in the proventricular/ventricular junction.
- Muscular hemorrhages of the legs.
- Enlarged, edematous bursas with marked hemorrhage.
- Cloacal petechiation (moderate).

QUESTIONS.

a) List or identify your differential diagnoses to the high mortality?

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

c) What further test or tests would you request from the lab?

d) What if any, additional samples would you like to take from the farm?

e) What recommendations would you make to treat/control/prevent the problem from reoccurring?

Case 2. Mortality/gait issues with 17-day-old commercial Loong Kong specialty meat birds.

History. You are out in the field with the live production supervisor on your way to Farm A. Farm A has two barns growing two flocks of commercial Loong Kong (brown feathers) birds for the specialty live bird markets. Each flock consists of 8,000 cockerels and 8,000 pullets separated by a fence. Barn 1 is 17 days of age and Barn 2 is 27 days of age. On your visit, the farm manager states that there is increased mortality in Barn 1, primarily in the pullets. In addition, there are many chicks, which cannot walk very well or cannot walk at all and lay on their sides. According to the manager, the cockerels in Barn 1 look fine. You enter the pullet pen in Barn 1 and observe that approximately 2% to 4% of the pullets have difficulty in walking. Of these, you observe wing walking, walking on their hocks and numerous chicks

on their side unable to walk. In addition, you observe about 20 to 25 (0.25%/day) chicks that have recently died.

a) List or identify your possible problems/ differential diagnoses.

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

c) Besides visiting the pullets in Barn 1 is there anything else you would like to do or see?

d) Do you have any additional questions for the live production supervisor?

e) What samples would you like to submit for further diagnostics? What procedures or tests would you request?

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

g) What is your recommendation to treat the flock and/or affected birds?

h) What steps would you suggest to prevent the problem from occurring in future flocks?

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

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

a) What are your possible diagnoses?

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

c) What samples, procedures, tests would you submit?

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

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

DISEASE TRENDS AND INTERESTING CASES-DELMARVA

Daniel A. Bautista[A], Jose Miguel Ruano[B], and Jack Gelb Jr.[B]

[A]University of Delaware-Lasher Laboratory, Carvel Research and Education Center,
Georgetown, DE 19947
bautista@udel.edu
[B]University of Delaware, Department of Animal and Food Sciences, College of Agriculture and Natural Resources,
Newark, DE

CASE SUMMARIES

Respiratory complex. Respiratory cases submitted to the Lasher laboratory have remained constant notwithstanding the increase of necropsy cases. This is due in part to the shift of the proportion of cases to non-respiratory cases. The overall increase in necropsy submissions since 2004 is due to an active poultry industry program of submitting birds from flocks experiencing more than 3/1000 daily mortality (regardless of cause) for avian influenza testing. Nevertheless, the respiratory cases still follow the traditional pattern of increase during winter months. In addition, the NDV/IBV respiratory disease incidence closely correlated the incidence of CEO-derived LT vaccine reaction. It is well documented that CEO LT vaccine reaction exacerbates the incidence and severity of NDV and IBV respiratory disease. Virus isolation points to IBV as the major isolate from respiratory cases. These IBV isolates are further characterized as Arkansas strain (>90%), followed by Massachusetts, Connecticut and Del 072 in order of prevalence.

Vaccinal laryngotracheitis (VLT). There recent LT outbreak started in early 2006. The molecular epidemiology points to close homology to CEO vaccine strains. The LT break was followed by

widespread CEO and recombinant LT vaccination program that controlled the outbreak. CEO vaccines were generally administered by the drinking water route at approximately 14 to 21 days of age. Fowl pox-LT and HVT-LT vaccine were administered *in ovo*. Some flocks receiving HVT-LT vaccine developed LT but mortality and morbidity in these flocks was considerably lower than in non-vaccinated susceptible flocks. After vaccination was discontinued, a few mild cases with low mortality were reported. These cases were thought to be vaccine-related since previous flocks had been vaccinated without cleanout or heating of the poultry houses before placement of unvaccinated flocks. Primary isolation of field strains in the laboratory using dropped chorioallantoic membrane or cell culture remains difficult.

Severity of LT clinical signs and lesions are mild to moderate, very similar to that seen in adverse CEO vaccine reactions. A consistent flock observation is a reported sudden drop in water consumption, followed by onset of LT-compatible clinical signs-eye lesions, respiratory distress. All suspect LT cases evaluated by RT PCR and histopathology (eyelid and trachea), the latter for confirmation. The incidence of LT CEO adverse vaccine reactions is closely related to the incidence of IBV or NDV associated respiratory complex. It is well documented that LT CEO vaccine reactions exacerbate respiratory complex incidence and severity (2). This is also seen as an increase in airsacculitis and septicemia-toxemia condemnations in the processing plants.

Gangrenous dermatitis. There was a significant gangrenous dermatitis (GD) epizootic starting in 2005. There is variable incidence among companies. Traditional viral immunodeficiency parameters (i.e. IBD progeny challenge data) cannot fully explain the increased incidence of GD. In time, patterns start to emerge that suggests ionophore use as a predisposing factor in the incidence of GD. Ritter, *et.al.* (4) reported that the highest incidence of GD occurred during the use of ionophores during the grower phase (19 to 36 days), followed by a lower incidence of GD when chemical anticoccidials are used in the grower feed, and the lowest incidence was correlated the use of cocci vaccination (4). A similar pattern is observed with ionophore use vs. cocci vaccine in another company. Further work indicated that ionophores shift the gut microflora to from a predominant *Lactobacillus* spp. to predominantly *Clostridium* spp. (5). Mitigating measures used to control GD include shifting to cocci vaccine programs, in-house litter composting (3), scheduled litter cleanouts, and prompt treatment with penicillin or copper sulfate.

Coccidiosis and necrotic enteritis. The incidence of coccidiosis and necrotic enteritis (NE) correlated with the shift from conventional ionophore and chemical-based anticoccidial drugs to cocci vaccine programs. It is thought that the relatively high environmental infective dose due in the used litter posed a significant challenge to the cocci vaccine program. NE incidence did not always follow clinical coccidiosis. There is reason to believe that changes in the feed formulation, high environmental loads of *Clostridium perfringens* in built-up litter, the absence of the gut antimicrobial effect of ionophores, as among the main factors in increased incidence of NE in the face of normal cocci vaccine cycling. In-house litter composting was reported to have a positive effect on the incidence of necrotic enteritis in field (1).

Runting and stunting syndrome (RSS). The incidence of RSS is generally tied to seasonal changes primarily winter to spring. It has been shown that environmental stress can trigger the clinical manifestation of this infectious disease. Rota, astro and reoviruses have been reported to cause RSS. Autogenous vaccination, clean-out, and in-house composting were used to control RSS.

Chick quality issues, uneven flocks, lameness, and colibacillosis. There has been a significant rise in the cases presenting as lameness (down on hocks, deformed tibiometatarsus, slipped tendon, synovitis) leading to scratches, cellulitis, and polyserositis. These lameness cases are tied to early incidence of dehydration, deformed legs, and small uneven chicks. These small chicks from younger breeder flocks have a tough challenge competing with their bigger counterparts. They are more predisposed to scratching, colibacillosis, and late viral respiratory complex. We investigated the incidence of spinal abscesses and lameness due to *Enterococcus cecorum* in lame broilers. There were no spinal abscesses observed and bacterial isolation yielded no *Enterococcus* spp. The predominant isolate in the pericardial sac and hock joints was *E. coli* and *Staphylococcus* spp.

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FIELD EVALUATION OF A BACTERIN/TOXOID FOR THE CONTROL OF GANGRENOUS DERMATITIS IN TURKEYS

Marion J. Morgan, N. Pumford, G. Tellez, A. Wolfenden, and B. Hargis

Department of Poultry Science, University of Arkansas, 1270 W. Maple Street, Fayetteville, Arkansas 72701

ABSTRACT

Dermatitis or cellulitis is an emerging disease in the post-antibiotic era. The causative agent of turkey gangrenous dermatitis has been identified as *Clostridium septicum*. *C. septicum*, a member of the black-leg consortium of pathogens, is well controlled in cattle and sheep by the use of bacterins and/or toxoids. The purpose of this project was to evaluate the efficacy of an autogenous bacterin/toxoid prepared against a virulent isolate of *C. septicum*. The bacterin/toxoid was prepared by formalin in-activation of an overnight culture of *C. septicum*. The killed vaccine was adjuvanated with 15% aluminum hydroxide gel. The bacterin was administered sub-cutaneously at day-of-hatch followed by subcutaneous boost during the brood stage of commercial turkeys. Data collected from the field indicated a) a significant reduction in mortality associated with cellulitis in vaccinated vs. unvaccinated flocks and b) a delay of several weeks in the appearance of first mortality in vaccinated vs. unvaccinated flocks.

SUMMARY

Clostridium septicum, a member of the black-leg consortium of anaerobic pathogens, has been implicated in the disease process variously known as gangrenous dermatitis, cellulitis, or clostridial myositis (1). Prior to the removal of prophylactic antibiotics from common usage, infection from *C. septicum* was rarely seen (2). However in the post-antibiotic era *C. septicum* has emerged as a significant pathogen in both turkeys and broiler-breeder chickens. *C. septicum* isolates show a great deal of cultural homogeneity and produce relatively few toxins, the primary toxin being the lethal Alpha toxin, which is similar in structure and action to the lethal toxin of *Aeromonas hydrophila* (3,4). *C. septicum* and the other members of the black-leg group of species are well controlled by vaccination in cattle and sheep. With this in mind, a killed bacterin/toxoid was prepared from field isolates of *C. septicum* demonstrating significant levels of toxin production. The bacterin was shown in the laboratory to induce an antibody response in test animals and was provided to a turkey integrator in the Arkansas/Missouri region for field trials. This presentation is a brief synopsis of the results from the first year.

MATERIALS AND METHODS

Bacterin/toxoid preparation. *C. septicum* was grown in Tryptic Soy Broth (Difco) to which 0.5 grams of sodium thioglycollate (Sigma) per liter was added prior to autoclaving. The cultures were incubated over night at 37°C under aerobic conditions. After 24 h the cultures were tested for the presence of Alpha toxin by a hemolysis assay using chicken erythrocytes. The culture was inactivated by the addition of formaldehyde. Aluminum hydroxide gel (Rugby) was added to a final concentration of 15% with 2-phenoxyethanol (Sigma) added as a preservative. The vaccine was packaged and provided to a regional integrator for field evaluation.

Field trials. In the first study involving four houses on one farm (A), poults in two houses were unvaccinated. Poults in the remaining two houses received 0.5 mL of the bacterin/toxoid subcutaneously at seven weeks. Weekly mortality associated with *C. septicum* was observed and recorded.

In the second trial poults in two houses on three separate farms (B, C, and D) were not vaccinated at day-of-age, but were either given the bacterin/toxoid 0.5 mL subcutaneously in the neck at seven weeks or raised without vaccination. Weekly mortality due to gangrenous dermatitis was observed and recorded.

In a third trial, poults on six farms (E, F, G, H, I, and J) received vaccination on day one and were boosted in the field at seven weeks. Weekly mortality due to *C. septicum* was observed and recorded as in the first study.

RESULTS

Trial 1: On farm A, two houses of turkeys that received no vaccination in the hatchery at day-of-hatch nor later in the field had an average mortality from *C. septicum* of 2.25%. Mortality was first observed in

DISCUSSION

The results from the trials described above demonstrate that mortality associated with *C. septicum* infection may be ameliorated or even eliminated by timely administration of a bacterin/ toxoid developed against *C. septicum*. It also appears from the data that a priming vaccination followed later by a boost in the field provides better protection than a single inoculation given at day-of-age or in the field.

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week 10 of grow out. Birds that were not primed in the hatchery but vaccinated in the field had an average *C. septicum* associated mortality of 1.18%. Mortality was first observed in week 11.

Trial 2: The average mortality due to *C. septicum* on these three farms was 1.63%. Mortality was first observed as early as week nine.

Trial 3: The average *C. septicum* induced mortality on the six farms in this trial was 0.42%. First observed mortality appeared at week 12 on one farm but not until week 14 on four of the others.

Table 1. Summary of results.

Farm ID	Date Placed	Vaccinated in Hatchery	Vaccinated in field	% Mortality from Clost.	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Wk 13	Wk 14	Wk 15	Wk 16	Wk 17	Wk 18
A	2/11/09	no	no	3.17%	0	0	0	2	1	8	20	16	27	46	41	17
A	2/11/09	no	no	1.34%	0	0	0	0	0	1	5	4	2	13	42	30
A	2/11/09	no	yes	1.20%	0	0	0	0	0	1	3	0	0	9	12	22
A	2/11/09	no	yes	1.17%	0	0	0	0	0	1	3	4	3	14	13	7
B	2/21/09	no	no	2.47%	0	0	0	0	3	6	3	4	15	38	8	
B	2/21/09	no	yes	1.57%	0	0	0	0	0	4	2	4	5	13	30	
B	2/21/09	no	yes	2.08%	0	0	0	0	2	2	2	4	23	50	11	
C	3/4/09	no	no	1.80%	0	0	5	8	2	3	1	8	12	5	2	2
C	3/4/09	no	yes	1.55%	0	0	1	4	1	4	4	21	9	11	23	6
C	3/4/09	no	yes	2.22%	0	0	5	11	3	7	31	21	14	13	14	7
D	3/9/09	no	no	0.10%	0	0	0	0	0	0	0	0	0	0	0	2
D	3/9/09	no	yes	0.12%	0	0	0	0	0	0	0	1	0	0	0	2
E	3/16/09	yes	yes	0.08%	0	0	0	0	0	0	0	3	0	0		
F	3/16/09	yes	yes	0.11%	0	0	0	0	0	0	0	1	0	0		
F	3/16/09	yes	yes	0.11%	0	0	0	0	0	0	0	1	0	0		
G	3/21/09	yes	yes	1.75%	0	0	0	0	0	0	7	8	21	9		
H	3/26/09	yes	yes	0.05%	0	0	0	0	0	2	0	0	0	0		
I	3/28/09	yes	yes	1.00%	0	0	0	0	0	0	0	0	0	4		
J	4/8/09	yes	yes	0.00%	0	0	0	0	0	0	0	0	0	0		

CHARACTERIZATION OF *CLOSTRIDIUM PERFRINGENS* ISOLATED FROM CASES OF CELLULITIS IN TURKEYS

Anil J. Thachil, Arpita Ghosh, and Kakambi V. Nagaraja

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

Cellulitis in turkeys has been causing a significant economic loss for turkey producers. Its impact has caused significant concern for it to be currently considered as the high priority disease of turkeys in Minnesota and elsewhere. Cellulitis is associated with acute mortality. The mortality is reported to be as high as 2 to 3% per week in the affected flocks.

Cellulitis usually appears at the age of 13 to 16 weeks and persists until the birds are marketed. But more recently, even eight week old birds were found to be affected with cellulitis in the field (1). The lesions have been seen in various areas of the body, including the breast, abdomen, legs, thighs, groin, and the back of the bird. Interestingly, in most cases of cellulitis there appears to be no trauma to the skin. Palpation of the affected areas often reveals crepitation due to gas bubbles in the subcutis and musculature. At necropsy, there is accumulation of large quantities of bubbly, serosanguinous fluid in the subcutis (1). The cellulitis condition in turkeys appears different from the gangrenous dermatitis (GD) reported in broiler chickens (2). The underlying musculature in cellulitis may have a cooked appearance in severe cases. The liver and spleen are often enlarged and may contain large necrotic infarcts.

Diagnostic laboratories have consistently isolated *Clostridium perfringens* and *Clostridium septicum* from lesions of cellulitis in turkeys. Experimental induction of cellulitis lesions and mortality in turkeys was successful with subcutaneous injection of either *Clostridium perfringens* or *C. septicum* cultures indicating the significance of these *Clostridia* in causing cellulitis (1,3). Recently, *Clostridium septicum* is reported to be more potent in causing cellulitis lesions and mortality than *Clostridium perfringens* in turkeys. However, infection with either *C. septicum* or *C. perfringens* can cause cellulitis lesions and mortality in turkeys (1). The affected tissues in both cases contained large amounts of gelatinous exudates and gas bubbles, most of which are seen in the subcutaneous and inter-muscular connective tissues (1).

Clostridium perfringens, which produces a huge array of invasins and exotoxins, is an important pathogen of animals and humans. *Clostridium perfringens* type A *is* a major pathogen responsible for causing cellulitis in turkeys. Our earlier studies have shown that *Clostridium perfringens* type A toxoid is protective against cellulitis in turkeys (4). The immune reactive components of *Clostridium perfringens* that play a role in immunity are not well understood. The objective of this study was to examine the secretory components of *Clostridium perfringens* isolates that causes cellulitis in turkeys. In brief, *C. perfringens* isolates that appear to be potent in causing cellulitis and those which did not produce cellulitis in turkeys were examined. They were allowed to express toxins in suitable media. The culture supernatant from these isolates was subjected to SDS-PAGE analysis and two-dimensional gel electrophoresis to separate the proteins. A western blot was performed using convalescent sera from the birds exposed and non-exposed to *C. perfringens* toxins. The reactive toxin components were identified by MALDI-TOF mass spectrometry.

Toxin-PCR was conducted as previously described (5). All the *Clostridium perfringens* isolates were found to be type A by toxin-PCR. The amount of toxin production varied with the isolates. The isolates were found to be varied in their production of secretory toxins like phospholipase, hyaluronidase, collagenase, and Dnase. Our results suggest differences in the toxin expression between *C. perfringens* isolates that causes cellulitis in turkeys. Similar studies with *Clostridium septicum* isolates are in progress.

Little is known about the distribution and sources of *C. septicum* in poultry production facilities. Presently, based in the laboratory isolation results, *Clostridium septicum* appears to be a major contributor in the etiology of cellulitis in turkeys. However, not much information is available regarding the pathogenicity or the disease causing potential of *C. septicum* in turkeys. Administration of a toxoid vaccine made from clinical isolates of virulent *C. septicum* also offered protection in turkeys against clostridial cellulitis in the field (6). Despite the importance of cellulitis in turkeys, there is very little known about the basis of immunity to this infection, although immunization is an obvious approach to control.

More studies are warranted at this point regarding the role of clostridial organisms in causing cellulitis in turkeys as well as genomic and proteomic characterization of these organisms.

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QUAIL ULCERATIVE ENTERITIS VACCINATION WITH A PARTIALLY ATTENUATED LOWER INTESTINAL COCCIDIAN

D. A. Anderson[A,B]

[A]Georgia Poultry Laboratory Network, Oakwood, GA
[B]Prairie Poultry Services, Loveland, CO

ABSTRACT

Quail ulcerative enteritis is a common disease of pen-raised bobwhite quail that results in high morbidity, excessive mortality, and chronic poor performance. A strain of lower intestinal coccidia was selected for shortened prepatent period and tested for reduced pathogenicity and as potential vaccine candidate against ulcerative enteritis. The strain was administered orally to four repetitions of 100 day old bobwhite quail at 100, 200, and 300 sporulated oocysts per bird and exposed to litter contaminated with the non-attenuated strain of coccidia. Birds were monitored daily for ten weeks for morbidity, livability, and weight gain. Additionally, ten birds were sacrificed at two, four, six, eight, and 10 weeks post-inoculation and examined for coccidial development and the presence of ulcerative enteritis. Livability and weight gain was not significantly different for any of the dosages, but was significantly improved over the sham inoculated controls. Protection against ulcerative enteritis ranged from 16-32% as compared to sham inoculated controls. This partially attenuated strain appears to have the potential for reducing the effects of ulcerative enteritis in pen-raised quail.

BACKGROUND

Quail ulcerative enteritis is a common disease of pen-raised bobwhite quail that results in high morbidity, excessive mortality, and chronic poor performance. In some cases, it appears lower intestinal or cecal coccidiosis may predispose the quail to onset of ulcerative enteritis.

In previous experiments by the author, use of non-attenuated strains of lower intestinal or cecal coccidia produced a decrease in coccidial development and decreased losses to ulcerative enteritis (12-21%) when exposed to contaminated litter. To further investigate the possible relationship between coccidia and ulcerative enteritis, a strain of lower intestinal coccidia was selected for shortened prepatent period (142 hrs vs. 168 hrs).

METHODOLOGY

The strain was administered orally to four repetitions of 100 day old bobwhite quail at 100, 200, and 300 sporulated oocysts per bird and exposed to litter contaminated with the non-attenuated strain of the coccidia. Two additional groups of 100 bobwhite chicks were sham inoculated with half of them exposed to litter contaminated with the non-attenuated strain of

coccidian and the other half held as non-exposed controls.

The birds were monitored daily for ten weeks for morbidity, livability, and weight gain. Additionally, 10 birds were sacrificed at two, four, six, eight, and 10 weeks post-inoculation and examined for coccidial development and the presence of ulcerative enteritis.

RESULTS AND DISCUSSION

Coccidial development was not statistically different between treatment groups, but was significantly improved compared to sham inoculated exposed controls. Livability and weight gain was not significantly different for any of the dosages, but was also significantly improved over the sham inoculated exposed controls. All three dosage regimens protected

against ulcerative enteritis ranging from 16-32% as compared to sham inoculated controls.

The inoculation of the partially attenuated strain of coccidia was able to reduce the development of coccidiosis as well as reduce the incidence of ulcerative enteritis in the presence of contaminated litter. It allowed the birds to maintain weight gains and livability consistent with non-exposed controls.

CONCLUSIONS

It appears that in some cases, coccidiosis may predispose birds to ulcerative enteritis. Reduction in coccidiosis may result in decreased incidence of ulcerative enteritis. This partially attenuated strain appears to have the potential for reducing the effects of ulcerative enteritis in pen-raised quail.

CONTROL OF CLOSTRIDIAL DERMATITIS IN A COMMERCIAL TURKEY PRODUCTION FACILITY WITH PHYTONUTRIENTS: A TWO YEAR FIELD STUDY

Arun K. Bahl

Bahl Farms/Consulting Inc., 17519 Jetton Road, Cornelius, North Carolina, 28031
abahl@mi-connection.com

At the "Gold Medal Panel on Turkey Cellulites" more than 100 representatives from the turkey and chicken industry, academia, government, and allied community gathered in the Twin Cities in December 2009 to learn from each other and develop a plan for minimizing the impact of this disease.

Based on the presentation and discussions it was obvious that controlling clostridial dermatitis will require a multi-factorial approach in an attempt to reduce the suspect organism(s) in the bird, the environment plus attempts to increase the bird's immunity, to these microorganisms.

A Michigan heavy turkey tom producer experienced clinical clostridial dermatitis with mortality in all seven flocks marketed during production year 2008. Each flock was water medicated with Penicillin G Potassium at recommended levels for therapeutic purposes to reduce and control economic losses.

A novel approach was taken it an attempt to control clostridial dermatitis at this farm during 2009 production year. This farm has three different ages when in full production, a double brood growout operation.

Five flocks have been marketed so far in 2009. No clinical losses or dermatitis associated with clinical dermatitis has occurred. No therapeutic antibiotic water or feed medication has been necessary either. Two more flocks are to be marketed before December 31, 2009 production period.

Continuous feed intervention starting at placement time with phytonutrients and periodically with water soluble phytonutrients and other natural nutritional products has resulted in complete absence of clostridial dermatitis. Actual farm data will be presented.

EXPERIENCES WITH USING A LIVE ATTENUATED *MYCOPLASMA SYNOVIAE* VACCINE (MSH) TO ELIMINATE *MYCOPLASMA SYNOVIAE* FROM A BROILER-GROWING ENTERPRISE

R. J. Jenner

SUMMARY

Mycoplasma synoviae (MS) infection is often under-diagnosed as a significant cause of respiratory disease, lameness, impaired welfare and immuno-suppression-induced poor performance in broiler flocks.

The process of moving from MS-positive status to MS-negative status in a broiler-growing operation takes a coordinated and systematic approach. In combination with other mitigation steps, MSH vaccine can be the cornerstone of a successful MS elimination program by eliminating vertical transmission.

Personal experience with MSH vaccine in Australia has delivered major and economically significant improvements in egg production and health in breeders, productivity and health in broilers, plus considerable reductions in antibiotic usage and tangible improvements in bird welfare. Supporting data will be presented which demonstrates the gains that can be made with the use of a live attenuated MS vaccine in a broiler breeder vaccination program.

INTRODUCTION

Mycoplasma synoviae is an important pathogen of broiler breeder flocks. Its significance is often masked by other more apparent pathogens, like *Mycoplasma gallisepticum* (MG), infectious bronchitis, fowl cholera, and infectious coryza. However, as control of these other pathogens improves it can become apparent that MS contributes significantly to unsatisfactory production performance in broiler breeder flocks. Additionally, vertical transmission of MS is a major contributor to chronic respiratory disease (CRD) and suppurative tenosynovitis, along with the risk of condemnation due to airsacculitis, in infected broiler breeder progeny.

Management of MS in broiler breeder flocks has historically been based around strict quarantine and extensive use of antibiotics. The development of an attenuated live vaccine has given an opportunity to eradicate MS from broiler breeder and growout operations far more successfully and simply than ever before. MSH vaccine persists in the respiratory tract, stimulating a continuous local immunity, preventing vertical transmission and eliminating the need for antibiotics.

In the early 1990s a broiler growing operation in Queensland, Australia was experiencing significant production and health problems due primarily to MG and MS. The introduction of a live, attenuated vaccine against MG (Vaxsafe® MG Strain TS-11) into the breeder vaccination program led to the eradication of MG over an 18 month period. Subsequent to this, Vaxsafe MS (Strain MS-H) was introduced into the Australian industry in 1995, and was used to eliminate MS from the same enterprise.

METHODS

In order to become MS-free it was important to ensure our day-old parent progeny was MS-free. This was achieved through an extensive antibiotic regime using in-feed and in-water tylosin. Chicks were placed into an all-in-all-out rearing facility and strict quarantine was observed until after vaccination. It was critical that wild-type MS was not introduced prior to vaccination. Vaccination was by eye drop at three to four weeks of age.

Once vaccinal immunity had been achieved, the process of achieving MS-freedom was relatively simple. It was important that no mycoplasmacidal antibiotics are used during the life of a vaccinated flock, as life-long immunity is dependent on continuous tracheal colonisation.

Prior to the advent of vaccination, strategic antibiotic medication was used in broiler flocks to reduce the severity of MS infection. However, as MS-vaccinated breeder flocks became the major, then eventually, the only source of fertile eggs, antibiotic therapy was reduced and eventually stopped altogether. Normal biosecurity practices were maintained throughout the interim phase.

As MS is very short-lived outside the host, elimination of MS from the broiler growout was centered around preventing between-flock horizontal transmission. It was important to maintain an all-in-all-out system of depopulation and repopulation in order to break the cycle of reinfection. Thus a two-pronged approach of eliminating vertical, then horizontal transmission was the principle behind MS elimination.

RESULTS

Both breeder and broiler flocks were monitored extensively using a rapid serum agglutination (RSA) test during the elimination program. Unfortunately, the RSA response to MSH vaccine is very weak, due to the non-invasive nature of the vaccine. However the author has successfully used RSA to monitor the success of vaccination for many years. A simple, crude comparison can be made between vaccination challenge (with low, intermittent RSA response) and wild challenge, where the RSA response is strong and persistent.

Broiler flocks were also monitored using RSA throughout the elimination phase. Even though no focussed active reduction strategies were introduced to the broiler growout, there was a gradual reduction in prevalence of MS over a twelve month period, to the point where all flocks were MS-negative on both RSA and ELISA. Broiler flocks were regularly monitored for the next five years in order to identify any sources of horizontal transmission. During this period, only one flock broke with MS which was subsequently found to be due to a nearby population of finches which had entered a shed during the batch.

CONCLUSION

MS is a debilitating disease of both broiler breeders and broilers which can be very successfully managed using a combined approach of vaccination and good basic biosecurity practices.

COMPARATIVE TESTING OF TURKEYS FOR *MYCOPLASMA IOWAE*

David H. Ley

Department of Population Health and Pathobiology
College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606
david_ley@ncsu.edu

Evidence from experimental infections and clinical cases suggests that *M. iowae* may be associated with chondrodystrophy in turkeys (1,5,7). However, the origins (primary and/or multiplier breeder flocks) and the precise role (primary, secondary, predisposing, synergistic, other) of *M. iowae* in the pathophysiology of the disease; and its prevalence, extent and economic importance, are all presently undetermined. This information is necessary if *M. iowae* is to be considered for inclusion in monitoring and control programs such as the United States National Poultry Improvement Plan. Key epidemiologic and economic data, especially disease prevalence correlated with presence or absence of the organism, need to be developed using the most sensitive and specific tests available.

Samples from several flocks of young turkeys suspected of having '*M. iowae* disease' were tested by mycoplasma culture (2), and one real-time (6), and two end-point (3,4) PCR methods for definitive diagnosis and to evaluate the relative effectiveness of these methods. Mycoplasma cultures were problematic due to high rates of contaminant overgrowth, but *M. iowae* isolates were made confirming the clinical impression. High *M. iowae* positive rates were obtained with real-time PCR (modified and tested by S. Callison, GTCAllison, LLC, Mocksville, NC) and one of two end-point PCR methods, with real-time PCR showing the highest sensitivity. Application of real-time PCR to clinical cases and epidemiologic studies should be useful in determining the role of *M. iowae* in chondrodystrophy of turkeys.

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

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IMMUNOHISTOCHEMICAL DEMONSTRATION OF MYCOPLASMA ANTIGENS IN BRAIN LESIONS IN THE ENCEPHALITIC FORM OF *M. GALLISEPTICUM* INFECTION IN TURKEYS

H. L. Ainsworth[A], D. Welchman[B], R. Irvine[C], and A. M. Wood[D]

[A]Veterinary Laboratories Agency (VLA) Bury, Rougham Hill, Bury St Edmunds, Suffolk IP33 2RX UK
[B]VLA Winchester, Itchen Abbas, Winchester, Hampshire SO21 1BX UK
[C]VLA Luddington, Luddington, Stratford-upon-Avon, Warwickshire CV37 9SJ UK
[D]VLA Lasswade, Pentlands Science Park. Bush Loan, Penicuik, Midlothian EH26 OPZ UK

ABSTRACT

Brain lesions associated with an encephalitic form of *Mycoplasma gallisepticum* (MG) infection in turkeys were first described in the 1940s (4) but have been recorded infrequently since. Case reports from California in 1991 (1,2) described brain lesions associated with both MG and *Mycoplasma synoviae* (MS) in fattening turkeys and pointed out that much of the literature current at that time failed to mention the neurological changes that can be associated with MG infection. Between 2003 and 2007 marked neurological signs including torticollis, opisthotonus, and ataxia were reported in small numbers of fattening turkeys in a few mostly smaller flocks being raised for the Christmas market in various parts of England and Wales and were referred to Regional Laboratories of the UK Veterinary Laboratories Agency (VLA) for investigation. The neurological signs in some cases had initially caused notifiable disease to be suspected triggering movement restrictions and an investigation to rule this out. Some but not all flocks had a history of previous or concurrent respiratory disease with airsacculitis and sinusitis, raising the possibility of MG involvement. Laboratory investigations included serology, bacteriology, mycoplasma culture (or PCR), virus isolation, and histopathology. Some flocks showed serological evidence of MG involvement but attempts to isolate MG from necropsy material were unsuccessful. Histopathology of brains revealed a recurring theme of meningeal vasculitis, focal encephalomalacia, and non-suppurative encephalitis similar to lesions described in the encephalitic form of MG infection (2,3,4). Immunohistochemical (IHC) labelling allowed retrospective confirmation of MG involvement in the meningoencephalitis. Specific MG labelling was obtained within the lesions in blood vessels in meninges and brain in one or more birds from five of eight cases examined by IHC. Brain lesions in turkeys similar to those caused by MG (2) have been described in association with MS (1). In the present cases IHC revealed no evidence of possible MS involvement.

(The full-length article will be submitted to *Avian Pathology*.)

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INDUCTION OF *ESCHERICHIA COLI* PERITONITIS IN LAYERS

W. J. M. Landman[A,B], A. Heuvelink[C], and J. H. H. van Eck[B]

[A]Animal Health Service (GD), Arnsbergstraat 7, 7418 EZ Deventer, the Netherlands
[B]Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584 CL Utrecht, the Netherlands
[C]Food and Consumer Product Safety Authority, Zutphen, the Netherlands

SUMMARY

Four experiments were conducted to study the pathogenesis of *Escherichia coli* peritonitis in layers and assess the feasibility of various natural infection routes compared to intravenous and intraperitoneal inoculation. Intratracheal inoculation proved the most successful route, 64 to 93% of birds developing peritonitis and/or bacteraemia. Second best was aerosol exposure, 43 to 71% of birds developing *E. coli* disease. After intravaginal inoculation it ranged from seven to 57%, while after oral inoculation it varied between 0 and 28%. All groups infected through natural infection routes differed significantly regarding peritonitis and/or *E. coli* septicemia from the negative control group except the orally inoculated birds and the intravaginally infected hens of Experiment 2. There was no significant influence of intraperitoneal egg yolk inoculation and the age at inoculation (23 or 33 weeks) on the occurrence of *E. coli* peritonitis and/or *E. coli* septicemia. PFGE showed that all reisolates belonged to the same genotype as the inoculated strain indicating that the pathology found was induced by the parent strain. Induction of *E. coli* peritonitis was successful by all natural infection routes, but most prominently through the respiratory tract suggesting that air transmission may play an important role in the pathogenesis of *E. coli* peritonitis in the field. Ascending contamination of oviduct from the cloaca may also be of significance.

INTRODUCTION

Salpingitis and/or peritonitis due to *E. coli* are considered the major causes of mortality in laying hens (1). They are also regarded as the most important cause of mortality of female birds in other species, especially ducks and geese (1,2). Besides increased mortality, egg production can be impaired in case the oviduct is affected.

Hens with salpingitis show varying degrees of dilatation of the oviduct, which appears thin-walled and filled with caseous material. This material may be laminated and contain egg remnants. In case the inflammatory process remains confined to the oviduct, clinical signs will, except for lack of egg production, largely be absent.

Peritonitis is characterized at macroscopy by the loss of the healthy aspect (smooth, shiny, humid, and transparent) of the peritoneum and omentum. Hyperemia, purulent exudate and fibrin along with yolk material can be observed in the abdominal cavity of hens with peritonitis, which typically become acutely ill and die quickly. The disease frequently affects highly productive hens.

The pathogenesis of *E. coli* salpingitis/peritonitis remains obscure. Although a number of infection routes have been proposed in literature, such as translocation from the respiratory tract, translocation from the intestine, and ascending contamination of the oviduct from the cloaca, scientific evidence for their existence is lacking.

To date, there is only one report describing a single experiment on the induction of *E. coli* peritonitis through intravaginal application of *E. coli*, suggesting the feasibility of an ascending infection from the cloaca (3). More recently, a second paper describing the induction of *E. coli* peritonitis after intratracheal inoculation in a very low number of experimental layers also infected with *Mycoplasma synoviae* has been published (4).

In the present study, the induction of salpingitis/ peritonitis was assessed experimentally in layers using parenteral and natural infection routes, and an *E. coli* isolate originating from a spontaneous case of *E. coli* peritonitis in commercial brown layers.

MATERIALS AND METHODS

Birds. Brown layer hens were obtained from a commercial flock. The *Mycoplasma gallisepticum*, *M. synoviae*, and *Salmonella* Enteritidis free status of the flock was assessed by means of serology.

The birds were fed *ad libitum* with a standard commercial layer diet and had free access to drinking water. Light was supplied for 16 hours each day.

Experimental design. Four experiments were made. Experiment 1 consisted of six groups of 14 hens each. The half of each group (n = 7) was given 25 mL egg yolk intraperitoneally immediately before *E. coli* inoculation at the age of 23 weeks. Groups 1 to 5 were

inoculated with *E. coli* either intravenously, intraperitoneally, intratracheally, intravaginally or orally. Group 6 acted as a negative control group. Birds of the negative control group that were not inoculated with egg yolk were sham inoculated with peptone saline intraperitoneally. Experiment 2 was *grosso modo* a replicate of 1 with the difference that none of the birds were given egg yolk intraperitoneally and that the intravenous, intraperitoneal, and control group were smaller (n = 7). Birds were inoculated at the age of 26 weeks. Experiment 3 consisted of groups of 14 birds except the control group (n = 7). Group 1 was exposed to *E. coli* intratracheally, Group 2 to an aerosol of *E. coli* once and Group 3 to an *E. coli* aerosol four times. Group 4 was sham inoculated intratracheally. Experiment 4 was performed as replicate where hens were infected with *E. coli* at 23 or 33 weeks of age. Birds were infected either intravenously, intratracheally, via aerosol, intravaginally, or orally. Each group harbored 14 layers. A negative control group (n = 7) sham inoculated was also included.

Inocula. *E. coli* (chicken/NL/Dev/SP01404Cou/05) originating from a commercial brown layer flock with increased mortality due to peritonitis and isolated from bone marrow of perished birds using the appropriate media and biochemical identification, was used to prepare the inocula (5,6). Preparation of bacterial suspensions in peptone saline and assessment of their concentrations were performed following international standards (7,8). Birds received 10^{8-9} cfu individually, except the intravenously inoculated birds of Experiment 1 and 2, which received 10^7 cfu each and aerosol exposed birds. The latter group was estimated to have uptaken approximately 10^{6-7} cfu.

Aerosol fluids and assessment of aerosol concentration in isolator air were essentially performed as described elsewhere (9). In order to calculate the uptake of *E. coli*, a ventilation volume of 24 l/hour/kg chicken was used, which was calculated using the average minute ventilation of White Leghorns hens (261 mL/min/kg) and White Leghorn cockerels (535 mL/min/kg) (10,11). The average minute ventilation of White Leghorn hens and cockerels was used as that of brown layers was not available and these hens are generally heavier.

Postmortem and further analysis. Postmortem was performed on all birds that died during the experiment and of all surviving experimental hens at the end of each study (4, 3, 2, and 2 weeks after inoculation for Experiment 1, 2, 3, and 4, respectively). Surviving birds were stunned using a mixture of CO_2 and O_2, and bled by incision of the *vena jugularis*. Macroscopic lesions including the occurrence of peritonitis were recorded.

All birds were subjected to bacteriological analysis of bone marrow of the femur as described

under inocula. All reisolates and the inoculum strain were stored at -80°C for Pulsed-Field Gel Electrophoresis (PFGE) analysis.

PFGE analysis of reisolates. Clonality of reisolates was assessed by means of PFGE, which was essentially performed as described (12).

Statistics. Fisher's exact test was used to perform pairwise comparisons of treatment groups within and between experiments.

RESULTS

Experiment 1. Peritonitis could be induced through all tested infection routes, although there were marked differences. The rates of *E. coli* peritonitis for the intraveneous, intraperitoneal, intratracheal, intravaginal, and oral route in birds not inoculated with egg yolk intraperitoneally were 86 (6/7), 57 (4/7), 86 (6/7), 57 (4/7) and 28% (27), respectively. The rates of *E. coli* peritonitis for the intravenous, intraperitoneal, intratracheal, intravaginal, and oral route in birds inoculated with egg yolk intraperitoneally were 86 (6/7), 86 (6/7), 100 (7/7), 57 (4/7) and 28% (2/7), respectively. The rate at which *E. coli* was isolated from bone marrow paralleled the occurrence of peritonitis.

Experiment 2. *E. coli* peritonitis was induced after intravenous, intraperitoneal, intratracheal, intravaginal, or oral inoculation in 57 (4/7), 71 (5/7), 71 (10/14), 7 (1/14), and 0% (0/14) of birds, respectively. *E. coli* was reisolated from bone marrow of four, three, eight, and one bird of these groups except the orally treated birds, respectively.

Experiment 3. Peritonitis was induced in 64 (9/14), 50 (7/14) and 43% (6/14) of intratracheally inoculated birds, birds exposed once to *E. coli* aerosol, and birds exposed four times to *E. coli* aerosol, respectively. *E. coli* was reisolated from bone marrow of 7, 6, and 4 birds of these groups, respectively.

Experiment 4. The percentage of birds with peritonitis infected at 23 or 33 weeks of age was 14 and 21% (2/14-3/14), 86 and 69% (12/14-9/14), 64 and 71% (9/14-10/14), 57 and 43% (8/14-6/14), and 0% for the intravenously, intratracheally, aerosol, intravaginally, and orally infected birds, respectively. *E. coli* was reisolated from bone marrow of 14 and 12, 12 and 8, 7 and 10, 7 and 8, and 0 birds of these groups, respectively.

In all experiments, almost all birds with peritonitis died within 48-72 hours after *E. coli* inoculation. Peritonitis was never found in control chickens, while bacteriology of bone marrow always was negative. The results of all experiments have been summarized in Table 1.

DISCUSSION

The intraperitoneal inoculation of egg yolk did not influence significantly the induction of peritonitis, which is in contrast with the work of Gross and Siegel (3). Therefore, the results of birds with and without egg yolk inoculation of Experiment 1 were presented together in the Table. Peritonitis was induced by all inoculation routes and most prominently after intravenous, intraperitoneal, and intratracheal inoculation. It was induced in fewer birds inoculated intravaginally or orally.

Experiment 2 was performed to confirm the previously obtained data. Indeed, largely similar results were obtained except for the oral inoculation route, which did not yield a single case of *E. coli* peritonitis or septicemia. Moreover, for the intravaginally inoculated chickens significantly fewer cases of peritonitis were induced compared to Experiment 1.

In view of the high success rate of intratracheal inoculation, Experiment 3 focused more closely on this transmission route. Similar rates of *E. coli* peritonitis were obtained after exposing birds to an aerosol of this microorganism compared to intratracheal inoculation. There was no significant difference between layers exposed to an *E. coli* aerosol once or four times consecutively.

Indications for age-related susceptibility to *E. coli* peritonitis were not found as there was no significant difference in the rate of peritonitis and/or *E. coli* septicemia in hens infected at 23 or 33 weeks of age (Experiment 4). In this experiment, the intravenously inoculated birds received a higher dose (10^{8-9} cfu) of *E. coli* compared to Experiment 1 and 2 (10^7 cfu). This resulted in acute mortality without peritonitis of most birds (approx. 10-12/14) within 24 hours after inoculation; however, bacteriology of bone marrow was always positive for *E. coli*.

Although peritonitis was the most prominent lesion at post-mortem, in a number of cases airsacculitis, pneumonia, and pericarditis were also encountered, especially in birds infected via the respiratory tract. Salpingitis was seen in a very few cases only.

Most birds suffering from peritonitis succumbed within 48-72 hours after *E. coli* inoculation, and in some cases as in the intravenously inoculated layers of Experiment 4, birds even died within 24 hours, stressing the peracute/acute nature of this form of *E. coli* disease, which is in agreement with field observations. It typically affects highly productive hens at the start of egg production, which die quickly: birds are often found dead on their nests still being in good condition, often with fully productive ovaries and an egg in the oviduct.

PFGE showed that all reisolates belonged to the same genotype as the inoculated parent strain, indicating that the clinical and pathological lesions found were induced by it.

Induction of *E. coli* peritonitis was highly successful by all natural infection routes, but most evidently through the respiratory tract suggesting that air transmission may play an important role in the pathogenesis of this condition in the field. The disease was also induced in a significant number of birds by intravaginal inoculation, suggesting that an ascending contamination of the oviduct from the cloaca may also contribute to spontaneous cases of *E. coli* peritonitis. The latter is in agreement with studies performed by Gross and Siegel (3), who induced peritonitis after intravaginal inoculation in 43% (6/14) of layers, while after intraperitoneal inoculation it was 71% (10/14).

Although Raviv and co-authors (4) were also able to induce *E. coli* peritonitis in layers after intratracheal inoculation, their success rate was very low (4.6-7.8%) compared to our results (64-93%). This may be explained by the choice of challenge strain: they used a strain originating from colibacillosis in broilers, while here a strain originating from peritonitis in commercial layers was used. Another difference was the breed of chicken used. In the present work brown layers were used instead of White Leghorns. Genetic lines of poultry vary in their resistance to *E. coli* infections and this may influence the outcome of animal experiments (13).

Remarkable was the fact that disease could be induced without any trigger at high rates via the respiratory tract and to a lesser degree after intravaginal inoculation. This underlines the high virulence of this particular strain and the fact that primary pathogenic *E. coli* strains occur in commercial layers.

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Table 1. Percentage of layers with *E. coli* peritonitis and/or *E. coli* septicemia per experiment and per inoculation/exposure route.

Route	*E. coli* dose (cfu)	Experiment number and age (weeks) at inoculation/exposure									
		1 (23)		2 (26)		3 (28)		4a (23)		4b (33)	
		n	%	n	%	n	%	n	%	n	%
Intravenous	10^7 or 10^{8-9}*	14	86^A	7	$57^{A,B,D}$**	-		14	100^{A}***	14	86^{A}***
Intraperitoneal	10^{8-9}	14	$71^{A,B}$	7	71^A	-		-		-	
Intratracheal	10^{8-9}	14	93^A	14	71^A	14	64^A	14	$86^{A,B}$	14	69^A
Aerosol 1x	10^6	14	-		-	14	50^A	14	64^B	14	71^A
Aerosol 4x	10^7	14	-		-	14	$43^{A,B}$	-		-	
Intravaginal	10^{8-9}	14	$57^{A,C}$	14	$7^{B,C}$	-		14	57^B	14	57^{A}***
Oral	10^{8-9}	14	$28^{B,C,D}$	14	0^C	-		14	0^C	14	0^B
Sham	0	14	0^D	7	$0^{C,D}$	7	0^B	7	0^C	7	0^B

*Intravenously inoculated birds of Experiment 1 and 2 received 10^7 cfu each, while hens of Experiment 4 received 10^{8-9} cfu.

**Intravenously inoculated birds of Experiment 2 not significantly different from the negative control group due to the smaller size of groups.

***Birds with peritonitis and/or positive *E. coli* culture of bone marrow.

Figures with the same superscript within columns are not significantly different from each other ($P > 0.05$).

PFGE. PFGE showed that all reisolates, in contrast to control strains, had the same banding pattern and were therefore considered genetically identical.

UNUSUAL LESIONS OF *E. COLI* AFFECTING THE BURSA OF FABRICIUS, CECA, AND PROVENTRICULUS IN BREEDER TURKEYS

H. L. Shivaprasad[A] and S. Kariyawasam[B]

[A]California Animal Health and Food Safety Laboratory System –Tulare branch, University of California, Davis
[B]Department of Veterinary and Biomedical Sciences, Animal Diagnostic Laboratory, The Pennsylvania State University

Escherichia coli is one of the most common and important pathogens in chickens and turkeys responsible for significant economic losses to the poultry industry. *E. coli* can cause various syndromes in poultry including omphalitis, colisepticemia, peritonitis, salpingitis, cellulitis, synovitis, osteomyelitis, swollen head syndrome, panophthalmitis, enteritis, coligranulomas, etc. However, significant lesions in the bursa of Fabricius and ceca associated with *E. coli* have not been reported in poultry before.

Sixteen live and dead turkeys, eight to 11 weeks of age from a flock of 4000 multiplier breeder turkeys were examined because of anorexia and increased mortality (4% in two weeks). Postmortem examination of the turkeys revealed small bursa of Fabricius some of which contained multiple to numerous pale nodules with or with out fibrinous exudate, small pale yellow nodules in the mucosa extending in to the serosa of the ceca and in the mucosa of proximal proventriculus. Histopathology revealed severe lymphoid depletion in the bursa of Fabricius and in addition the lesions in bursa, ceca, and proventriculus were composed of fibrino-suppurative and/or granulomatous inflammation associated with large numbers of Gram negative bacteria. *E. coli* were isolated from these organs. Other lesions in these turkeys included granulomas in the lungs and air sacs associated with fungi of *Aspergillus* spp. and crop and esophageal mycosis due to *Candida* spp. Most of these turkeys also had protozoal enteritis due to coccidia, cryptosporidia and trichomonads, and *Blastocystis*.

Six isolates of *E. coli* (four from bursa of Fabricius and one each from cecum and proventriculus) were selected for further investigation and subjected to serotyping, phylogenetic typing, virulence genotyping, and pulsed-field gel electrophoresis (PFGE). Of the four bursal *E. coli* isolates, two fell into phylogenetic group D, which is typical of certain human extraintestinal pathogenic *E. coli* (ExPEC), whereas the other two belonged to B1 and A groups. *E. coli* isolates from proventriculus and cecum were classified as belonging to phylogenic groups A and B1, respectively. Virulence genotyping, which used several genes known for their association ExPEC or intestinal pathogenic *E. coli* (diarrheagenic *E. coli*) virulence, revealed that these *E. coli* from bursa of Fabricius were more related to ExPEC than they were to intestinal pathogenic *E. coli*.

The turkeys were negative for MG, MS, MM, AI, NDV, and IBDV by serology. Bursa of Fabricius was negative for virus including IBDV by virus isolation.

It is most likely that *E. coli* was the primary pathogen causing the pathology of the bursa of Fabricius, which resulted in lymphoid depletion, immunosuppression and secondary fungal and protozoal infections, and ultimately responsible for the increased mortality in the turkeys.

EFFICACY STUDY OF A LIVE *E. COLI* VACCINE IN BROILERS AGAINST THREE FIELD ISOLATES FROM THAILAND

Kalen Cookson[A], Jiroj Sasipreeyajan[B], Veerasak Letruangpunyawuti[C], and Yong Ming[C]

[A]Pfizer Poultry Health, Research Triangle Park, NC
[B]Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand
[C]Pfizer Poultry Health, Bangkok, Thailand

INTRODUCTION

E. coli induced chronic respiratory disease is a leading cause of broiler mortality and condemnations. Recent field surveys have revealed two interesting findings. First, most *E. coli* isolates coming from diseased flocks contain a preponderance of virulence genes, as measured by PCR analysis, while commensal fecal isolates tend to contain very few (3,4). These virulent isolates are referred to as APEC (avian pathogenic *E. coli*). Second, there is tremendous diversity in O serotypes within even a limited geographic region (3). Last year, we reported a study which demonstrated that a live *E. coli* vaccine (serotype O78) gave cross-protection against three heterologous APEC isolates (2). The current study was conducted in a similar fashion in Thailand using *E. coli* isolates from that country.

MATERIALS AND METHODS

Four hundred commercial straight-run broilers were divided into two treatments at day of hatch. All birds were coarse sprayed with a modified live Newcastle vaccine (Avinew-Merial) and infectious bronchitis vaccine (H120) while half also received Poulvac® E. coli. Birds were raised in isolation and fed a typical broiler ration *ad libitum* throughout the course of the study. At 28 days of age the two groups were weighed, then subdivided into four different challenge groups: No challenge, APEC O1, APEC O2 and APEC O78. Challenge inocula were administered intratracheally (IT) at a dose of approximately 10^{10} colony forming units (CFU)/bird. Dead and moribund birds were removed daily until termination of the study at 35 days, when all birds were weighed and necropsied. Lesions of colibacillosis were recorded and airsacculitis lesions were scored using the following scale: 0) none, 1) mild suds, 2) moderate suds or multifocal exudate, and 3) heavy suds or severe, profuse exudate. Feed was weighed at various intervals so feed conversion rate (FCR) could be calculated.

RESULTS

Pre-Challenge. There were no significant differences in body weights and feed conversion between the Poulvac E. coli vaccinates and the controls at 28 days of age ($P > 0.05$). However, there was a significantly ($P < 0.05$) higher mortality rate (6.06% vs. 1.98%) in the controls which resulted in a two-point difference in adjusted feed conversion (not statistically significant). Culturing of lesions from dead birds showed that two of four vaccinates and seven of 11 non-vaccinates tested had E. coli. By 35 days of age, vaccinates were significantly heavier (1,515g vs. 1,485g) with a better feed conversion (see Table 1).

Post-challenge. Based on mean air sac lesion scores in the control group, APEC O78 was the most pathogenic isolate, followed by APEC O1, then APEC O2 with scores of 1.75, 1.42, and 0.83, respectively. There were no significant differences between vaccinates and non-vaccinates, however. The very low lesion scores from APEC O2 challenge correlated with the minimal effects of this challenge isolate on performance parameters in either group. In addition, APEC O1 and O78 both increased feed conversion in controls (though not statistically significant). The greatest contrast between vaccinates and non-vaccinates was seen in comparing APEC O1 challenge results. While this isolate did not affect performance in the Poulvac E. coli vaccinates, challenge controls had 1.19 higher FCR. Finally, APEC O78 caused the highest FCR in both groups but weight gains were

twice as depressed in the non-vaccinated birds (6.06% vs. 11.67%).

DISCUSSION

Prior to challenge, there was a significant difference in total mortality between vaccinated and non-vaccinated birds. Vaccinates also had a lower mean air sac score (0.33 vs. 0.71). These differences might be attributed to vaccinates acquiring immunity against a low-level E. coli challenge that was naturally present in the isolation facilities - E. coli was isolated from 2/4 dead vaccinates compared to 7/11 non-vaccinates (one bird was not tested due to decomposition). In fact, lower mortality in "non-challenged" birds receiving Poulvac E. coli has been reported in previous chicken studies (1,2). A difference in immune status to E. coli in the face of a low-level exposure might also explain why the vaccinated controls were significantly heavier at the end of the study.

The disease involvement in this challenge study tended to be about one grade less than had been seen in a previous cross-protection study (2). This may be one reason why little to no difference was seen between vaccinated and non-vaccinated groups. However, a significant difference was seen in final weights, while weight gains – although not statistically analyzed – were numerically higher in vaccinates against all three APECs. On average, vaccinated birds had 40% better weight gain after their respective challenges. It is hard to show significant FCR differences with the relatively small sample sizes used in this study but, at least numerically, vaccinates had lower FCRs than their respective non-vaccinated birds in each instance. On average, this difference was nearly 50 points (see Table 1).

While the method of challenge in this study did not result in E. coli lesions as severe as a previous cross-protection study (2), the performance cost of E. coli still was manifested in lower body weights and increased feed conversion. Birds vaccinated with Poulvac E. coli at day of hatch were more spared from these performance losses, especially against APEC O1.

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Table 1. Summary of 35 day mortality and performance (one week after APEC challenges).

Day of hatch vaccine	APEC challenge	Body weight (g)	Weight gain % (28-35d)	Rate of feed conversion	% Mortality
None	None	1,483.3[b]	18.83	2.54	0.0
	O1	1,475.2[b]	14.01	3.40	4.2
	O2	1,540.0[a]	18.66	2.70	4.2
	O78	1,439.1[b]	6.06	3.84	8.3
	3-APEC Average	1,485.4[B]	12.91	3.31	5.6
Poulvac E. coli	None	1,584.6[a]	29.85	2.25	0.0
	O1	1,579.1[a]	21.15	2.21	4.2
	O2	1,524.9[a]	21.18	2.57	0.0
	O78	1,440.0[b]	11.67	3.66	4.2
	3-APEC Average	1,514.7[A]	18.00	2.85	2.8

[a,b,A]Groups having a different letter are not statistically different, based on Duncan's multiple range test for performance values and Chi-square analysis for mortality (*P*<0.05).

GEL DROPLETS FOR THE DELIVERY OF POULTRY VACCINES IN THE BARNS

Eng H. Lee and Majed Al-Attar

Vetech Laboratories Inc.131 Malcolm Road, Guelph, Ontario, Canada N1K 1A8

SUMMARY

Gel droplets for the delivery of coccidiosis vaccines in the hatchery have been used successfully for the past few years. However, to use the same delivery in the poultry barns, it was necessary to add a "sticky" edible gum such as xanthan gum to the existing formulation. This addition helped the suspended vaccine droplets to linger on the back of birds longer and for easier pickups. When this modified gel droplets delivery was used to deliver an IBD vaccine to one-week old SPF chicks, colored tongues were found in over 80% of the treated chicks. Fourteen days post vaccination (PV), seven of 10 vaccinated birds were positive by ELISA tests compared to nine of 10 controls vaccinated by gavage. At 18 days PV, all 10 gel droplet vaccinated birds became positive. Similar results were obtained in the repeated test. When used for the delivery of a *Salmonella* vaccine, 14 of 15 vaccinated birds were found to be positive. A scaled-up version of what described here, most likely, will not require the time consuming withdrawal of

chlorine from the water before vaccination, and take away the worry of the same disinfectant that might cause vaccine failures after the water is restored.

Generally poultry vaccines are either live or inactivated and they may require different routes of administration. The most common methods are: Through drinking water, spraying, subcutaneous, or intramuscular routes (4). Sometimes, consideration for the routes of infection is the most natural for the application of the vaccine to stimulate good immune response. Poxvirus vaccines, for example, must be given in a manner that causes the vaccine to penetrate the skin (2).

The oral route of vaccination via drinking water is a common practice in administering live viral, bacterial, and parasitic vaccines for poultry. Usually, successful drinking water vaccination requires a lengthy preparatory procedure and many precautions to be considered before, during, and after vaccination. All these are needed in addition to the importance of maintaining the quality drinking water for vaccine administration (3). Two main concerns or disadvantages with water vaccination are: The uneven vaccine distribution affecting the amount of intakes and the inactivation of the vaccine before it is ingested. Added to these, the laborious steps that must be followed to achieve successful drinking water vaccination and the large quantity of water that must be used (3).

Vaccines to protect against common poultry diseases such as infectious bursal disease (IBD) and salmonellosis are good examples of orally administered poultry vaccines. Many types of IBD vaccines are now available; the live attenuated, the immune complex vaccine, or the inactivated oil-emulsion adjuvanted vaccines (8,6). Although these IBD vaccines can be administered by subcutaneous or *in ovo* injection or by spray, the most common route of administration is still through drinking water.

The use of gel droplets spray delivery system as a method of vaccinating chicks as well as turkey poults against coccidiosis in hatcheries and barns have been used successfully for many years (5,7). The ease, the uniformity of delivering the coccidial vaccine and the efficacy of the vaccination procedure as shown by the improvement of feed efficiency (7) may reflect the protection obtained against the clinical disease after vaccination. The present work is an extension for the use of the gel droplets spray method to replace the more laborious method of the drinking water vaccination of chicken against IBD and *Salmonella*. This method may be extended to replace the water vaccination for a number of other viral and bacterial diseases in the poultry houses. Therefore the aim of this study is to explore the possibility of replacing water vaccination by the gel droplets delivery system.

MATERIALS AND METHODS

Experimental chickens. Broiler chicks were hatched in our laboratory from specific pathogen free (SPF) eggs obtained from Sunrise Farm Inc. and were used throughout the experiments. The chicks were placed in single-use cardboard boxes and housed in a disinfected isolated quarter. Feed and water were supplied *ad libitum*.

The gel diluent. The Gel-sprayed vaccines were delivered with 1.3% of the 60/40 gel diluent of Vetech Laboratories Inc. and 0.1% of xanthan gum was added (Lee, USA Patent pending).

IBD virus (IBDV) vaccine preparation. Vaccine stabilizer was prepared by suspending 0.7 g of skim milk powder (Bermudez and Stewart-Brown, 2003) in 250 mL of distilled water. The lyophilized 2500 doses IBDV vaccine (S-706, Merial, Canada) was first dissolved in 5 mL distilled water and 2 mL of this reconstituted vaccine were added to the 250 mL of stabilizer to make a total of 1,000 doses. To this mixture a suspension of *E. acervulina* was added as markers for vaccine take at 300 oocysts per bird for Experiment 1. Inoculation of control birds was performed by using two 1 mL syringes to withdraw 0.75 mL of each of this vaccine suspension with constant agitation. For the gel droplets, about 6.2 g of gel diluents and 0.2 g of food color were then added and all mixed into a suspension and transferred to a 500 mL hand sprayer.

Experiment 1. IBD gel-spray vaccination. A total of 25 SPF chicks were used in this experiment. Control blood samples of 0.5 to 0.7 mL were collected from the jugular vein of 10 randomly selected chicks a day before vaccination. At seven days of age the 25 birds were divided into three groups and vaccinated as follow. Chickens in group 1 (six birds) were vaccinated by water gavage of reconstituted IBDV vaccine. Chickens in group 2 (six birds) were vaccinated by gavage of the gel-spray containing IBDV vaccine. Chickens in group 3 (13 birds) were vaccinated by droplets spraying of gel containing the IBDV vaccine.

Vaccine Take. The presence of coccidial infection was used as an early indicator for possible IBDV vaccine take in Experiment 1. One bird each from groups 1 and 2 and two birds from group 3 were examined on Day 5 post inoculation (PI) for the present of lesions in the duodenum. The rest of the birds were examined for the present of oocysts in their fecal samples collected on Day 6 PI.

Experiment 2. IBD gel-spray vaccination. The IBDV vaccine was prepared as in Experiment 1 except Immucox[®] (Vetech Laboratories Inc. Guelph Ontario, Canada) was added to the gel of group four. This experiment was done with 25 birds, divided into five groups. Group 1 of four chickens served as non-

vaccinated controls. Chickens in group 2 and 3 of five chickens each were vaccinated by water gavage and gel gavage respectively. Groups 4 were vaccinated by gel gavage containing Immucox and group 5 of six chickens were vaccinated by gel spray.

Antibody response to IBD vaccination. All sera collected before and after vaccination in Experiments 1 and 2 were tested for presence or absence of antibody to IBDV by ELISA test. This was done by the Laboratory Services of the Animal Health Laboratory of the University of Guelph, Guelph, Ontario.

Salmonella **vaccine.** A live mutant of *Salmonella* Typhimurium vaccine (Salmune®) was used (CEVA, Lenexa, KS, USA).

Salmonella **gel spray vaccination.** This experiment was done to determine if commercially available live *Salmonella* vaccine can be uniformly delivered by the gel-spray method and if it can be used as an alternative method to water vaccination without being affected by coccidiosis vaccine (Immucox) when mixed. Before vaccination, cloacal swabs as negative controls were randomly collected from 10 birds, four days before any of the 12-day old SPF birds were vaccinated. Then, the 25 birds were vaccinated and divided into two groups of 15 birds each in the sprayed groups and 10 in the gavage group. The prepared Gel-spray that mixed with the vaccines was plated for *Salmonella* identification. Gel-sprayed birds were sprayed at a rate of one spray per three birds which is equal to about one recommended dose (0.25 mL/bird). The positive control birds were inoculated by gavage with the recommended dose of Salmune (0.25mL) through a 1-mL syringe.

RESULTS

Serum antibodies response to IBD vaccination. In Experiment 1, the mean ELISA titers of chickens vaccinated by water or gavage and droplets spraying are shown in Table (1). Chickens responded well to the different method of vaccination especially at day 14 and 18 post vaccination, also, good response was obtained at day 11 post vaccination in the group which was vaccinated by the gel gavage route. The mean titers were 2946 and 2850 at 14 and 18 days post vaccination in water gavage respectively. Mean titers of 1805 and 2517 were recorded in the gel gavage group at 14 and 18 days post vaccination respectively (Table 1). The mean titer of chickens vaccinated by the gel droplets at 11 days post vaccination was low, but at 14 days post vaccination the mean ELISA was 3610 which is the highest titer compared to all the groups and to those titers at all periods post vaccination (Table 1). At 18 days post vaccination the ELISA titers were comparable for all groups (Table 1). The percentages of positive antibody titers in all groups were gradually

increased and reached 100% at 18 days post vaccination (Table 1).

Vaccine take as shown by coccidial infection. In Experiment 1, duodenal lesions were detected in one of one chick examined and oocysts were detected in four fecal samples out of five examined chickens from the group vaccinated by water gavage. In the chickens vaccinated by the gel gavage duodenal lesions were detected in one of one chick examined and oocysts were detected in all. In the group vaccinated by gel droplets spraying, duodenal lesions were detected in two of two chickens examined and oocysts were detected in six fecal samples out of 10 (Table 1).

In Experiment 2 mean ELISA antibody titers at 11 days post vaccination were generally low except in the group vaccinated by the gel gavage route it reached 2380. The highest mean ELISA titers at 18 days post vaccination reached 2595 in the group vaccinated by the gel droplets spraying that mixed with Immucox method compared to the other groups. The mean ELISA titers of all vaccinated groups were gradually increased with time, but the mean titers of the groups vaccinated by droplets gel spray method, with and without Immucox vaccine, were more uniformly increased (Table 1).

Salmonella **gel spray vaccination.** All the 10 chickens sampled before vaccination were negative for *Salmonella*. Direct plating of the vaccine revealed pure culture of the vaccine *Salmonella*. All chickens, except one, that were vaccinated by the gel droplets vaccination method including the chickens that had received Immucox and Salmune vaccines mixture; were positive for vaccine *Salmonella* when swabbed from cloaca at two days post vaccination.

DISCUSSION

Finding an alternative to drinking water in the delivery of live poultry vaccines, may be necessary partly because of the tedious procedure needed for the avoidance of chlorine before and after vaccine is applied. In addition to that, the stress of water deprivation to the birds, as well as the increase in the use of closed watering system, all of which makes water vaccination more difficult (1,2).

An alternative with coarse-water spray for IBD vaccination had recently been shown to be successful (1). The modified gel droplets application, reported here, may be another option for this application of IBD vaccination. This is supported by comparable antibody responses of these sprayed chicks to the control birds (Table1).

Similarly, the recovery of the vaccine *Salmonella* from cloacal swabs, were almost the same from the control group vaccinated by water gavage and the group vaccinated by the droplets spraying method.

Therefore, the application of gel droplets method to vaccinate against *Salmonella* was as effective as water vaccination.

This application of the gel droplets vaccination in barn to vaccinate chickens against IBD and *Salmonella* likely can be extended to deliver other poultry vaccines such as hemorrhagic enteritis for turkey and Newcastle disease for chickens.

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Table 1. Infectious bursal disease antibodies detected by ELISA test at different intervals post vaccination using gel droplets spraying vaccination method compared to oral gavage vaccination (Experiment 1).

Groups	Treatments	Coccidia		IBD (ELISA) 1-day	IBD (ELISA) 11-days	IBD (ELISA) 14-days	IBD (ELISA) 18-days
		Lesions	Oocysts				
All groups	10*/25 No treatment	ND**	ND	0/10***	-	-	-
1	Water (gavage)	1/1	4/5	-	ND	4/5 (2946)****	4/5 (2850)
2	Gel spray (gavage)	1/1	5/5	-	4/5 (2081)	5/5 (1805)	5/5 (2517)
3	Gel spray (Sprayed)	2/2	6/10	-	2/5 (704)	7/10 (3610)	10/10 (2094)

*Number of chickens tested
** Not done
***Number positive/Number tested.
( ) **** Means ELISA titers.

FIELD EVALUATIONS OF *IN OVO* APPLIED TECHNOLOGY COMPARING EMBREX® INOVOJECT® SYSTEM TO THE INTELLIJECT® SYSTEM: PERCENT HATCH

C. J. Williams

Pfizer Poultry Health, 1040 Swabia Court Research Triangle Park, NC 27703
christopher.williams2@pfizer.com

SUMMARY

Pfizer Poultry Health, in conjunction with three separate U.S. commercial broiler operations, conducted three comparative field trials evaluating percent hatch differences between two *in ovo* injection systems. In total more than 48 million eggs were injected from over 380 different breeder flocks. All trials utilized whole hatchery production evaluation methodologies over a minimum two month period. The difference in percent hatch between same breeder flocks was greater for the Embrex® Inovoject® System by 1.25% in trial one, 1.35% in trial two and 0.79% in trial three. Overall, the percent hatch difference across all three trials was 1.20% greater in favor of the Inovoject System. The difference in percent hatch equates to a significant difference in value to the poultry producers in these evaluations.

Additional testing during the conduct of trial one utilized side by side paired hatch evaluations to enable statistically significant analysis. The necropsy of unhatched eggs revealed statistical differences ($P \leq .05$) in percent hatch due to increased number of embryos that died after injection in the Intelliject® treatment group.

INTRODUCTION

In ovo vaccination in the United States is currently the standard procedure for approximately 95% of all commercial broiler chickens. Vaccines for Marek's disease virus (MDV) and infectious bursal disease (IBD) are routinely given *in ovo* (1), and the technology has been further enhanced with the recent licensing and subsequent use of HVT-vectored *in ovo* vaccines for IBD and Newcastle disease virus (NDV). The commercially applied platform for *in ovo* technology allows for delivery of several vaccine antigens simultaneously into over 50,000 eggs per hour (2,3).

From a commercial poultry production standpoint, *in ovo* vaccination must have continued quality control and ease of application during the daily operation to consistently achieve maximum vaccine efficacy, maintain chick quality and hatchability, and support subsequent bird health. Field trials can be designed and conducted to obtain comparative production data to substantiate the relative value of the technology.

The purpose of this research was to directly compare two commercially available *in ovo* vaccination systems at three different broiler production locations utilizing large scale field trials under normal hatchery operations to delineate differences between the systems with respect to percent hatch of eggs injected.

MATERIALS AND METHODS

Two commercially available egg injection systems (Embrex Inovoject System, Pfizer Poultry Heath, Durham, NC and IntelliJect system, AviTech, Salisbury, MD, distributed by Merial Select, Gainesville, GA) were compared and evaluated in three large U.S. commercial broiler hatcheries. All three hatcheries utilized ChickMaster (Medina, OH) multi-stage incubation systems. All machine operation and support procedures for *in ovo* application were completed by trained hatchery personnel per Pfizer Poultry Health and AviTech/Merial respective guidelines. Concerted efforts were made to minimize sources of variation in each trial; however, certain application and processes differ between the two injection systems. For example, the same vaccines for Marek's disease Vaccine (HVT/SB-1, Merial Select, Gainesville, GA) were used in each respective trial; however, vaccine concentrations were equalized to dosage per egg, as the Embrex Inovoject System dispensed 50 μL and the IntelliJect System dispensed 100 μL vaccine per egg. The embryos received the same antigen amount in each trial but at different concentrations due to different delivery volumes. Individual trial protocols are summarized as follows:

Trial 1. A side by side trial that compared daily production in two adjacent hallways that hatched on the same days. Injections occurred at both day 18 and day 19 of incubation, and the trial proceeded continuously for nine week duration. The total set capacity of hatchery was ~1.8 M eggs per week. Additionally, a series of detailed egg breakouts were

conducted involving both day 18 and day 19 injections. A total of four evaluations utilizing eggs from paired breeder flocks and egg randomization at injection were completed involving 116,640 eggs per treatment (eight hatchers per treatment).

Trial 2. A week on week off trial design whereby all eggs were injected by same system for alternating weekly production for a total of 10 weeks (five weeks for each system). The total set capacity of the hatchery was ~1.8 M eggs per week and all injections were completed on day 19 of incubation.

Trial 3. A week on week off trial design whereby all eggs were injected by the same system for alternating weekly production for a total of eight weeks (four weeks for each system). The total set capacity of the hatchery was ~2.2 M eggs per week and all injections were completed on day 19 of incubation.

RESULTS

Individual trial results are summarized as follows:

Trial 1. Overall percent hatch was greater in the Inovoject System vaccinated group by 1.74% with greater than 6.5 M eggs in each treatment group. When percent hatch was compared utilizing eggs from only the same breeder flock sources, percent hatch favored the Inovoject System in 75 out of 126 flocks and by 1.25% involving more than 11.5 M eggs total. Egg breakout evaluations revealed a significantly lower percentage (1.55%) of embryos that died after injection at day 18 of incubation ($P \leq .05$) in the Inovoject system treatment as compared to the Intelliject system. Differences on day 19 (0.41%) favored the Inovoject system but were not statistically significant.

Trial 2. Overall percent hatch was greater in the Inovoject System vaccinated group by 1.09% with greater than 8.5 M eggs per treatment group. In same breeder flock comparisons, percent hatch favored the Inovoject System in 64 of 102 flocks and by 1.35% involving more than 13.8 M eggs total.

Trial 3. Overall percent hatch was greater in the Inovoject System vaccinated group by 0.80% with greater than 8.5 M eggs per treatment group. In same

breeder flock comparisons, percent hatch favored the Inovoject System treatment in 96 of 152 flocks and by 0.79% involving more than 16.3 M eggs total.

In summation, percent hatch was greater in eggs injected with the Embrex Inovoject System in all three trials when compared to the IntelliJect system. In comparisons utilizing eggs only from the same breeder sources, percent hatch favored the Inovoject System in all age categories (Table 1) and by 1.20% overall with approximately 20 M eggs represented in each treatment group.

DISCUSSION

Large scale commercial field trials are used in the poultry industry to understand value and determine differences in various treatments and programs while challenged with the real world dynamics of production. The operations of a commercial hatchery present unique opportunities to conduct field trials; side by side and week on week off field trials represent excellent testing protocol. Data presented herein from three large field trials indicates differences in the two available *in ovo* injection systems. Percent hatch was greater in all three trials when eggs were injected with the Embrex Inovoject System as compared to the IntelliJect system. The difference in percent hatch and the increase in the number of viable chicks per egg set represent a significant value to the producer.

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Table 1 - Percent Hatch by Breeder Flock Age - Sum of Trials 1, 2, & 3
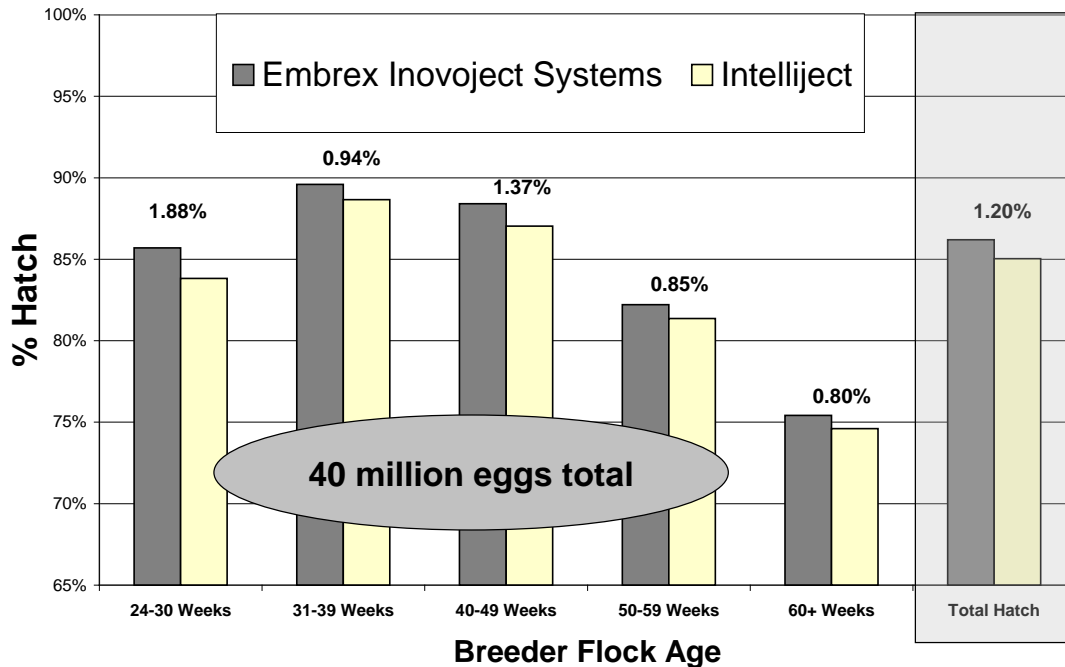
VACCINATION WITH SUBUNIT EPITOPES OF *CAMPYLOBACTER* EXPRESSED IN TWO DIFFERENT BACTERIAL VECTOR SYSTEMS REDUCES *CAMPYLOBACTER JEJUNI* IN CHICKENS

Neil R. Pumford, Sherryll L. Layton, Marion J. Morgan, and Billy M. Hargis

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

SUMMARY

Campylobacter is one of the major food-borne pathogens present in poultry products. *Campylobacter* is not only associated with gastroenteritis in humans but is also associated with chronic life-threatening diseases. Selected subunit peptide immunodominant epitopes were selected to be expressed in a *Salmonella* and *Bacillus* vectors. All vectored epitopes elicited a humoral immune response against *Campylobacter jejuni*. There was a significant decrease in *Campylobacter* levels found in the ileum after immunization with both vectored vaccines following challenge with *Campylobacter jejuni*. One of the selected vectored vaccines decreased *Campylobacter jejuni* to almost undetectable levels in both the *Salmonella* and *Bacillus* vectors. This very successful protection against *Campylobacter* challenge was repeated with similar results. Vaccine strategies may prove to be vital in decreasing world-wide food borne infections from poultry.

INTRODUCTION

Bacterial contamination of poultry products from *Campylobacter jejuni* is a major food safety issue (1,4). There is a real need for the development of an effective vaccine against *Campylobacter*. Whole cell vaccines have the potential to cause the debilitating disease Guillain-Barré Syndrome (1). Specific *Campylobacter* proteins used as a recombinant subunit vaccine have had some success (8). Expression of *Campylobacter* proteins or subunits on bacterial vectors such as *Salmonella* has been successful in vaccination against *Campylobacter* (9). There are many immunodominant proteins of *Campylobacter*. We have selected specific peptide sequences with antigenic potential from three immunodominant proteins (Cj0113, Cj0982c, and

Cj0420) in which the proteins have been shown to be good vaccine candidates (2,6,7). We expressed the peptides on the cell surface of a *Salmonella* vector system (3,5) developed in our laboratory and on the cell surface of a *Bacillus* vector (Cj0113 only).

MATERIALS AND METHODS

Recombinant *Salmonella* containing stable linear epitopes of either Cj0113, Cj0982c, or Cj0420 were constructed using our previous developed method (3). We constructed a *Bacillus* vector by integrating a stable plasmid which produces and expresses the Cj0114 epitope on the bacterial cell surface. Birds were given 10^7 to 10^8 bacteria (*Salmonella* or *Bacillus* vectors) per bird of vectored bacteria at day-of-hatch, day 11, day 21. On day 21 after the boost birds were challenged with wild-type *Campylobacter jejuni* (three strains). Colonization and invasion of the vectors were determined. Humoral immune (IgG and sIgA) response against the candidate peptides were determined using an ELISA assay. Levels of *Campylobacter* were determined by quantitative real-time PCR following challenge.

RESULTS

All three candidate *Salmonella* vectors had a good immune response with the Cj0113 candidate having the highest levels. Chickens vaccinated with Cj0420 and Cj0982 *Salmonella* vectored candidates had a two to three log reduction relative to controls following challenge with *Campylobacter*. Furthermore, with the Cj0113 vaccine candidate there was an eight log reduction of *Campylobacter* in the ileum. Similar results were found using a *Bacillus* as the vector for the Cj0113 vector candidate.

DISCUSSION

The ability of an oral live attenuated *Salmonella* or *Bacillus* vaccine vector expressing the *Campylobacter* epitope Cj0113 to reduce *Campylobacter* in market birds will help protect against food borne disease. This approach could be potentially adapted to other agents for a rapid production, safe and cost effective vaccine for the induction of mucosal immunity.

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AN INNOVATIVE PRESENTATION FOR LIVE FREEZE DRIED POULTRY VACCINES

A. Malo, P. Kühne, H. Middelbeek, and R. Biemans

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

SUMMARY

Most live poultry vaccines are currently presented as lyophilized cakes in glass vials. The new presentation consists of small, freeze dried, highly soluble spheres packed in an aluminum container instead of a single vaccine cake in a bottle. Dilution of the vaccine is fast and easy for administration via water, spray or eye drop. The vaccine presentation is optimized in terms of: Ease of use, better solubility of the vaccine and environmental friendly packaging material. Data show that live vaccines produced by the new technology are as effective as the glass vial vaccine in their efficacy under laboratory and field conditions.

INTRODUCTION

The poultry industry is working with large numbers of animals that need to be immunized against several diseases during their production life cycle. Mass application of live vaccines is therefore common practice, especially in the broilers segment. Normally this is done via spray or drinking water application.

Most of the live vaccines used currently in the poultry industry are produced as freeze dried (lyophilized) cakes in glass vials. The production of live vaccines for the poultry industry is a biological process, which means that the yield can differ from product to product and from batch to batch. For some vaccines the largest vial presentation with the current production technology ranges between 2,500 – 5,000 doses. Larger dose presentations (5,000 – 10,000 doses) are desired for mass vaccinations for practical purposes.

The preparation of a live vaccine solution for vaccination is labor intensive. The vaccines have to be dissolved in water. To open the vaccine the metal cap has to be removed and the rubber stopper that seals the vial should be opened under water to ensure that the freeze dried cake is properly dissolved. This process is time consuming, the hands of the operator will get wet, it hampers the hygiene conditions of the preparation process and it may result in injuries caused by the sharp edges of the metal caps. Besides, the empty glass vials and rubber stoppers need to be disposed accordingly.

Live freeze dried vaccine spheres. Innovative alternative technology makes it possible to lyophilize live viral vaccines into 100 μL small, highly soluble spheres instead of a single vaccine cake in a traditional glass vial (Figure 1). The lyophilized spheres have the same formulation as the currently available vaccines in glass vials. The spheres are stored in bulk at –20°C, making it possible to package larger presentations when needed (Figure 2). The lyophilized spheres are packed in light weight, easy to open, compactable and recyclable aluminum cups in different convenient dose sizes (Figure 3). These can be stored like conventional vaccines at $\pm$4°C. The sealed aluminum cups protect the vaccine from light and humidity. Color coded labels on the cups make vaccines easy to identify. For vaccine preparation, the seal of the aluminum cup is opened and the spheres are poured in the water. After stirring, the vaccine spheres will dissolve completely within seconds. For eye drop vaccination a special funnel has been developed where the spheres can be added to the diluent. No contact between the operator and the vaccine solution is necessary, improving hygiene and safety for the operator. Empty cups may be stacked up after disinfection in a chlorine solution and offered for recycling after use.

MATERIALS AND METHODS

Comparison of efficacy of lyophilized sphere vaccines versus conventional lyophilized vaccines. The efficacy of live vaccines in the classical presentation as freeze dried cake or as freeze dried spheres was compared by a challenge trial for Newcastle disease (ND) and infectious bronchitis (IB) or by the serological response after vaccination for infectious bursal disease (IBD). The chickens were vaccinated at a commercial farm under field conditions. Day old chickens were coarse sprayed with a live IB vaccine of the Massachusetts serotype (strain Ma5). Additionally a cloned ND vaccine was administered by coarse spray at two weeks of age and vaccination against IBD was administered via the drinking water at 20 days of age, because of the presence of maternal immunity in the birds. The date for IBD vaccination was based on the antibody level of 20 hatch mates (blood donors) at one day of age measured in the virus neutralization (VN) test.

Newcastle disease vaccines. Two groups each of 20 two week old conventional broilers were vaccinated with a cloned commercial Newcastle disease vaccine with at least $6.0\log^{10}EID_{50}$/dose. One group was vaccinated with the lyophilized vaccine in a glass vial while the other group received the vaccine in lyophilized sphere format. A third similar group served as control. All groups were challenged at three weeks post vaccination with the virulent Newcastle disease virus Herts strain via the intramuscular route with at least $5.0 \log^{10} EID_{50}$. The birds were observed daily for two weeks after vaccination and the mortality rate was registered.

Infectious bronchitis vaccines. Two groups each of 25 day old conventional broilers were vaccinated with the commercial Massachusetts vaccine strain Ma5 with at least $3.0\log^{10}EID_{50}$/dose. One group was vaccinated with the lyophilized vaccine in a glass vial while the other group received the vaccine in lyophilized sphere format. A third similar group served as control. All groups were challenged at three weeks post vaccination with a virulent Massachusetts strain (M41) via the ocular route with $4.5\log^{10}EID_{50}$/0.2mL. Protection was assessed by means of the ciliostasis test (1) one week after challenge. Just before challenge five birds were sacrificed from each group in order to judge the ciliostasis score correctly later on in the challenged groups. The condition of the tracheas of these five birds was later on compared with the ciliostasis score of the remaining of the challenged groups

Infectious bursal disease vaccines. Two groups each of 20 two weeks old conventional broilers were vaccinated with an intermediate cloned commercial vaccine against Infectious Bursal Disease at 20 days of age of age with at least $4.0\log^{10}TCID_{50}$/dose. The vaccination date was based on the level of maternally derived antibodies at one day of age, measured by means of the virus neutralization test by bleeding 20 hatch mates (mean VN titer: 11.65 $\log^2$). One group was vaccinated with the lyophilized vaccine in a glass vial while the other group received the vaccine in lyophilized sphere format. A third group served as unvaccinated control. Efficacy was evaluated by measuring the increase in titer in the vaccinated groups by means of the virus neutralization test at seven, 14, and 28 days after vaccination.

RESULTS

Newcastle disease vaccines. There was no difference in the level of protection of the broilers vaccinated with the lyophilized vaccine in comparison to the broilers vaccinated with the vaccine in lyophilized sphere format. Both vaccinated groups showed no evidence of clinical disease or mortality whereas the control group showed 100% of mortality within three days after challenge.

Infectious bronchitis vaccines. There was no difference in the level of protection of the broilers vaccinated with the lyophilized vaccine in comparison to the broilers vaccinated with the vaccine in lyophilized sphere format. Both vaccinated groups showed no evidence of damage in the trachea after challenge whereas the control group showed 93% of ciliostasis.

Infectious bursal disease vaccines. The results are shown in Table 1. There was no difference in the level of serological response of the broilers vaccinated with the lyophilized vaccine in comparison to the broilers vaccinated with the vaccine in lyophilized sphere format. The significant increase in antibody levels at 14 and 28 days after vaccination are a good indication for the "take" of the vaccines. The control group remained negative.

CONCLUSIONS

The results of the comparisons between live vaccines against Newcastle disease, infectious bronchitis and infectious bursal disease in lyophilized sphere format or as conventional lyophilized vaccine in a glass vial did not show any differences. The lyophilized vaccines in sphere format can therefore replace conventional vaccines in glass vials. Lyophilized live vaccines in sphere format packed in aluminum cups are user friendly, dissolve faster and are environmental friendly in comparison to conventional lyophilized live vaccines in glass vials.

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Table 1. Average log^2 virus neutralization titer for infectious bursal disease.

	Days after vaccination		
	7	14	28
Group			
Spheres Vaccinated	4.0	11.5	15.6
Glass vial Vaccinated	4.0	12.6	15.3
Controls*	4.0	4.0	4.0

Figure 1. Conventional lyophilized live vaccine showing single cake in a glass vial.

Figure 2. Bulk of lyophilized spheres ready for packaging.

Figure 3. Lyophilized spheres packed in light, easy to open, compactable and recyclable aluminum cups.

AN OUTBREAK OF PANDEMIC H1N1 INFLUENZA IN TURKEYS IN ONTARIO

D. Ojkic[A], A. Ferencz[B], H. Wojcinski[B], Y. Berhane[C], and J. Pasick[C]

[A]Animal Health Laboratory, University of Guelph, Box 3612, Guelph, Ontario, N1H 6R8, Canada
[B]Hendrix Genetics, 650 Riverbend Drive, Kitchener, Ontario, N2K 3S2, Canada
[C]National Centre for Foreign Animal Disease, Canadian Food Inspection Agency,
1015 Arlington Street, Winnipeg, Manitoba, R3E 3M4, Canada

On Friday, October 9, 2009 a turkey breeder flock experienced a 5% decrease in egg production. The affected farm (Farm A) had two laying barns and was a contract producer of grandparent breeding stock. The affected flock was 52 weeks old and had been producing hatching eggs for 21 weeks. The flock appeared healthy and had no apparent clinical signs. The same producer also had a two-barn farm which housed young birds (Farm B). By Sunday, October 11, 2009 egg production had decreased by additional 35%. There was a slight increase in mortality (less than 1%), but no clinical signs were observed. In the second laying barn egg production was normal and birds appeared healthy. Toms housed on the farm also appeared healthy. On Monday, October 12, 2009 a self-imposed quarantine was put in place until the cause of the egg production drop could be determined. On Tuesday, October 13, 2009 samples from both Farm A (laying farm) and Farm B (younger birds) were sent to the regional laboratory (Animal Health Laboratory, University of Guelph). By October 14, 2009 egg production in the affected flock had decreased by 80%. Samples from the affected barn were found influenza A-positive, but were negative for H5, H7, and H3 influenza A subtypes. Samples from other barns were influenza A-negative. On October 16, 2009 a preliminary identification of pandemic H1N1 influenza was made based on partial sequencing of hemagglutinin and neuraminidase genes. Samples were forwarded for confirmatory testing and pathotyping to Canada's National Centre for Foreign Animal Disease in Winnipeg, Manitoba. Full-length sequencing of all eight genes showed that the virus was over 99% identical to the pandemic H1N1 2009 virus from human samples deposited in GeneBank.

(The full-length article will be published.)

SEROLOGIC CROSS REACTIVITY OF SERUM SAMPLES FROM AVIAN INFLUENZA VACCINATED COMMERCIAL U.S. TURKEYS TO THE EMERGENT H1N1 INFLUENZA VIRUS

Darrell R. Kapczynski[A], Eric Gonder[B], Becky Tilley[B], David L. Suarez[A], and David E. Swayne[A]

[A]Southeast Poultry Research Laboratory, United States Department of Agriculture, Athens, Georgia, USA
[B]Goldsboro Milling Company, Goldsboro, North Carolina, USA

Because influenza A viruses of swine-origin have been shown to co-infect turkeys, when the 2009 pandemic influenza A H1N1 virus (pH1N1) emerged from swine to humans, the possibility of turkeys becoming infected with these viruses was a concern. In August 2009, pH1N1 influenza virus was identified in turkey breeders in Chile which resulted in significant drops in egg production. Later in 2009, outbreaks of the pH1N1 were detected in Canadian and U.S. (Virginia) turkeys resulting in egg production losses.

In the U.S., swine and turkeys may be reared in close proximities and vaccination of turkeys against avian influenza may include H1 and H3 influenza viruses also isolated from swine. In these studies, we tested whether sera from turkeys vaccinated against avian H1N1 viruses would cross react against the pH1N1 (A/Mexico/4108/09) isolate. Turkey breeder hens received three vaccinations with a trivalent inactivated autogenous vaccine which contained two H1N1 strains of avian origin (A/turkey/North Carolina/17026/1988 (Tk/88) and A/turkey/North Carolina/00573/2005 (Tk/05)) and a triple reassortant H3N2 avian influenza virus (A/turkey/North Carolina/16108/2003 (Tk/03).

Genetic analysis indicated greater than 92 percent nucleotide similarity in hemagglutinin (HA) gene sequence between one of the vaccine isolates (Tk/88) and the pH1N1. HI results indicate high levels of antibody were observed against the avian influenza vaccine viruses with a majority of titers ≥ 5 (log$_2$). In contrast, minimal cross reaction against the pH1N1 isolate was observed. Of 200 sera tested, only 15 were ≥ 5 (log$_2$). Taken together, these studies suggest the current turkey H1N1 vaccines may have limited cross protection against the pH1N1 virus.

CHARACTERIZATION OF LOW PATHOGENICITY NOTIFIABLE AVIAN INFLUENZA VIRUS OF H7N9 WILD BIRD LINEAGE ISOLATED FROM COMMERCIAL POULTRY

Janice Pedersen, Mary Lea Killian, Nichole Hines, Dennis Senne, and Brundaban Panigrahy

U. S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services,
National Veterinary Services Laboratories, Ames, IA, USA

SUMMARY

Circulation of low pathogenicity avian influenza (LPAI) viruses of the H7 subtype is a threat to the poultry industry for several reasons: Potential for increased pathogenicity as a result of reassortment/ mutation, production losses, carcass condemnation at slaughter and trade restriction. Since 2006, infections in poultry caused by H5 and H7 subtype viruses are reportable to the World Organization for Animal Health (OIE). In 2009, two LP notifiable AI (LPNAI) events were reported to the OIE. In April, antibodies to H7N9 and RNA to H7 avian influenza virus (AIV) were detected in broiler breeders in Kentucky (KY), and in August, a LPAI H7N9 virus was isolated from meat turkeys in Minnesota (MN). The 2009 TY/MN H7N9 virus was compared to a H7N9 LPAI virus isolated from commercial turkeys in Nebraska (NE) in 2007 and to other H7 viruses from poultry and wild birds. Nucleotide sequence and phylogenetic analysis indicated both the MN and NE H7N9 viruses are of wild bird lineages, but represent separate introductions of LP H7 AI from wild birds to poultry.

INTRODUCTION

The risk of transmission of avian influenza (AI) has increased as a result of globalization, company traffic between premises, high poultry population densities, interactions with live-bird markets, and the spread of disease from wild reservoirs. Wild waterfowl are the natural reservoir for LPAI of all subtypes. Notifiable AI is defined as an infection in poultry with H5 or H7 subtypes of AI virus, which includes both low pathogenicity and highly pathogenic viruses. Notifiable AI is reportable to the OIE. Isolation of H5/H7 virus, detection of H5/H7 specific RNA, or detection of H5/H7 specific antibodies that are not a consequence of vaccination are all reportable findings.

Low pathogenicity AI could cause a mild respiratory infection and mild to severe drop in egg production in breeders or be subclinical with no apparent signs of disease. Taking into account the risk of transmission, establishment of an infection in domestic poultry and the possibility of reassortment/ mutation of the virus to highly pathogenic AI (HPAI), control strategies are essential. Control strategies are based on a combination of depopulation, controlled marketing, movement and restocking restrictions, and emergency vaccination policies. This paper will review detections of a H7N9 LPAI in commercial poultry in 2009 and the possible epidemiological connection of the 2009 H7N9 virus to a previous isolation of H7N9 LPAI in commercial poultry in 2007.

Following the initial detection of antibodies to AI as a result of routine slaughter surveillance, an H7N9 LPAI virus was isolated from a multi-age commercial facility in NE in 2007. Subsequently, the LPAI H7N9 virus was isolated from swabs collected from a dead bird surveillance program. No H7N9 antibody or H7 viral RNA was detected with follow-up surveillance. In 2009, there were two additional detections of LPNAI H7N9 in commercial poultry: one in a broiler breeder flock in KY and an outbreak in commercial meat turkeys in MN. Swabs and serum were collected in response to a moderate decrease in egg production on a single premise in Edmonson County, KY. AI matrix and H7 viral RNA and H7N9 antibodies were detected, but no virus was recovered from the swab specimens. No evidence of H7 AI was detected following enhanced surveillance in 20 premises which had received egg placements from the hatchery.

The MN outbreak effected meat turkeys in 89 flocks (>1 million turkeys) on eight premises in four counties. H7 viral RNA (two premises) and H7N9 antibodies (six premises) were detected from multiple flocks, and a LPAI H7N9 virus was isolated from tracheal/oropharyngeal (TR/OP) swabs from meat turkeys in Meeker county, MN. The outbreak was managed with controlled marketing and depopulation over a period of five months. In addition to the LPNAI H7N9 positive flocks in KY and MN, antibodies to H7N9 were detected in two flocks where the virus or H7-specific RNA could not be detected; broiler breeder flocks in Giles and Lincoln counties, Tennessee (TN). In addition, antibodies to H7N9 and a suspect H7 real-time RT-PCR (rRT-PCR) test result were detected in meat turkeys in Illinois (IL). The IL, KY, and TN detections appeared to be isolated events as no evidence of H7 viral RNA or antibodies was found following enhanced surveillance conducted in commercial and backyard poultry. The five 2009 events have no known epidemiological connection.

MATERIALS AND METHODS

Specimens. Tracheal/oropharyngeal swabs were collected from broiler breeder flocks in KY and TN and from meat turkeys in IL and MN. Swab pools consisted of up to five swabs in 3.0 to 3.5 mL of brain heart infusion (BHI) broth. Swab pools were transported to a diagnostic laboratory within 24 h of collection for rRT-PCR and virus isolation. Serum was collected as part of routine National Poultry Improvement Plan (NPIP) surveillance from broiler breeders in TN, pre-slaughter surveillance for meat turkeys in IL, and for three premises in MN. Serum was collected from the remaining five premises in MN serum as part of enhanced surveillance, in-house company surveillance, re-population surveillance, and as part of the Minnesota Notifiable LPAI (NLPAI) plan. Serum was collected from the KY broiler breeders in response to a diagnostic investigation.

Hemagglutinin-inhibition (HI) and neuraminidase-inhibition (NI) tests. The HI test was conducted using four hemagglutinating units (HAU) of the viral isolate (for virus subtyping) or reference antigens (H1-H16) for antibody subtyping (5,12). Reference antiserums (H1-H16) were tested against four HAU of virus for isolate subtyping. The NI test was conducted as previously described with optimized dilutions of reference (N1-N9) antigens to determine neuraminidase antibody subtype and optimized dilutions of virus isolates with reference (N1-N9) antiserum for neuraminidase subtyping of isolates (8).

Virus isolation. Virus isolation was conducted in nine to 11 day-old specific pathogen-free (SPF) chicken embryos with conventional procedures as described

previously (12). Briefly, 2.0 mL of swab supernatant was diluted in antibiotic suspension, incubated for 1 h at room temperature and inoculated (0.3 mL per embryo) into the allantoic sac of nine- to 11-day-old embryonating chicken eggs. A second passage was made for all specimens that were positive by the matrix rRT-PCR and negative for virus upon completion of the 1st passage.

RNA extraction and rRT-PCR. Viral RNA was extracted from 50 µL of clarified swab supernatant with MagMAX™ AI/ND Viral RNA Isolation Kit (Ambion, Austin, TX). Extraction was conducted according to procedures described in the USDA/NVSL protocol "Real-Time RT-PCR for the Detection of Avian Influenza Virus and Identification of H5 and H7 subtypes in Clinical Samples" (AVPRO1510, available upon request), with KingFisher (Thermo, Ontario, Canada) magnetic particle processor (2,9,11). The magnetic particle processor was programmed according to USDA/NVSL standard operating procedure AVSOP1522 (available upon request). All rRT-PCR tests were conducted with AgPath-ID™ (Ambion, Austin, TX) PCR chemistry and AB7500Fast (Applied Biosystems, Foster City, CA) or LightCycler480® (Roche, Mannheim, Germany) real-time PCR instruments. Extracted RNA was screened for type A influenza by the matrix (M) rRT-PCR assay, and all specimens testing positive were tested by the H5 and H7 subtyping assays. Specimens with a cycle threshold (Ct) lower than 35 were considered positive and specimens with a Ct of 35 or higher were considered suspect positive.

Nucleotide sequence and phylogenetic analysis. Full length hemagglutinin genomic sequencing was conducted as described by Spackman *et al.* (10). The hemagglutinin gene segment was amplified by RT-PCR and directly sequenced with the BigDye terminator kit (Applied Biostystems, Foster City, CA) on an ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, CA). The remaining sequences were obtained from the GenBank. An H7 phylogenic tree was constructed using nucleotide sequences from the hemagglutinin gene and Lasergene software, version 8.0 (DNA Star, Madison, WI). Phylogenetic inference was conducted using the neighbor-joining method of inference with 1000 bootstraps.

RESULTS

HI and NI test for antibody subtyping. For the KY submission all 12 serums from House 1 and all seven serums from House 2 were positive for H7N9 antibodies. For the IL submission, seven of fifteen serums collected during routine pre-slaughter surveillance were positive for antibodies to type A

influenza by the agar gel immunodiffusion (AGID) test: All seven were confirmed positive for H7N9 antibodies by the HI and NI tests at the National Veterinary Services Laboratories (NVSL). For the Lincoln county TN submission, a total of 30 serums tested positive for type A influenza antibodies by the AGID test and were forwarded to the NVSL for HI and NI subtyping. Six of the 30 serums were positive for H7 antibodies and one serum was positive for N9 antibody. For the Giles county TN submission, 30/30 serum collected during routine surveillance tested positive for influenza A antibody by the AGID test and were subtyped as H7N9 by HI and NI tests at the NVSL. Serum collected from MN premises 1-6 were tested for AI antibodies by the AGID test in state laboratories. Antibodies to H7N9 AI were detected from premises 1-6 at the NVSL by the HI and NI tests.

rRT-PCR. For the KY flock, TR/OP swab pools collected in response to a moderate decrease in egg production were positive for matrix and H7 viral RNA. In IL, TR/OP swabs collected retrospectively to positive serology tested positive by the matrix and suspect positive by the H7 rRT-PCR tests. Swabs collected from broiler breeders in Lincoln and Giles counties, TN were positive for H7N9 antibodies but negative for H5, H7, and matrix viral RNA. Swabs collected from premises 7 and 8 as part of the MN NLPAI plan were positive for AI matrix and H7 viral RNA.

Virus isolation. No virus was isolated from TR/OP swabs collected from the KY, IL and TN investigations. H7N9 LPAI virus was isolated from TR/OP swabs from commercial tom turkeys on two separate premises in Meeker county, MN. Pathogenicity of the virus was determined by analysis of the amino acid sequence at the hemagglutinin protein cleavage site and the chicken pathogenicity test.

Nucleotide sequence and phylogenetic analysis. Nucleotide sequence and phylogenetic analysis of the hemagglutinin gene were conducted for the A/TY/NE/505577/07 (NE/07) and A/TY/MN/14135-2/09 (MN/09) H7N9 viruses to determine lineage and homology. The amino acid sequence of the hemagglutinin protein cleavage site was compatible with LPAI for A/TY/NE/505577/07/H7N9 (NVPENPKNR/GLFGAI) and TY/MN/14135-2/09/ H7N9 (NVPEKPKTR/GLFGAI) viruses. A BLAST search with GenBank H7 hemagglutinin sequence data found the A/TY/NE/505577/07 to be 97.1% similar to A/Ruddy turnstone/NJ/518/2006 H7N3 and A/Northern shoveler/NC/6412-050/2005 H7N3. The A/TY/MN/14135-2/09 H7N9 virus is 97.5% similar to A/Mallard/MN/Sg-00230/06 H7N3 and 97.1% similar to A/Blue-winged Teal/OH/566/06 H7N9. BLAST search analysis of the nucleotide sequence indicated

both H7N9 viruses are highly similar to LPAI originating from wild birds. Phylogenetic analysis of the hemagglutinin nucleotide sequence (Fig. 1) shows the MN/09 and NE/07 viruses to be closely related to H7N3 viruses isolated from wild birds and poultry in North America, but distinct from the HPAI H7N3 isolated from commercial poultry in Chile in 2002. The MN/09 virus also shares a close relationship to H7N2 viruses isolated from commercial poultry and birds in the live bird markets in the Northeast United States and Florida.

DISCUSSION

Antibody detection by the AGID or enzyme-linked immunosorbent assay (ELISA) is the basis for most NPIP commercial poultry surveillance for AI in the United States. Initial detection of AIV infection in TN, IL, and MN flocks was the result of routine pre-slaughter surveillance. Confirmatory HI and NI testing conducted at the NVSL confirmed the presence of AI antibodies of the H7 and N9 subtype. rRT-PCR has been shown to be an effective tool for the surveillance of AI in both commercial poultry and wild aquatic birds throughout the world (1,2,3,4,6,7). Due to the increased diagnostic sensitivity as compared to virus isolation in embryonated chicken eggs, viral RNA has been reported from poultry and wild bird specimens when virus has not been recovered, as was the case in KY. The rRT-PCR test is a sensitive surveillance tool and will error on the side of detecting false positive results when compared to the confirmatory test which is virus isolation. The diagnostic sensitivity (DxSN) of the matrix and H7 rRT-PCR assays are 88.2% and 97.5%, respectively (2,9). Nucleotide sequence BLAST search analysis and phylogenetic analysis indicated the MN and NE H7N9 viruses are closely related to H7 viruses isolated from wild birds. Phylogenetically, NE/07 and MN/09 are members of the same clade, but members of different clusters within a single clade. In addition, the amino acid sequence at the hemagglutinin protein cleavage site has asparagine (N) at positions -2 and -5 for TY/NE/2007 and threonine (T) and lysine (K) at positions -2 and -5, respectively. Both the Minnesota and Nebraska H7N9 viruses are of wild bird lineages, but represent separate introductions of LP H7 AI from wild birds to poultry.

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Figure 1. Phylogenetic tree for the hemagglutinin gene of H7 viruses.
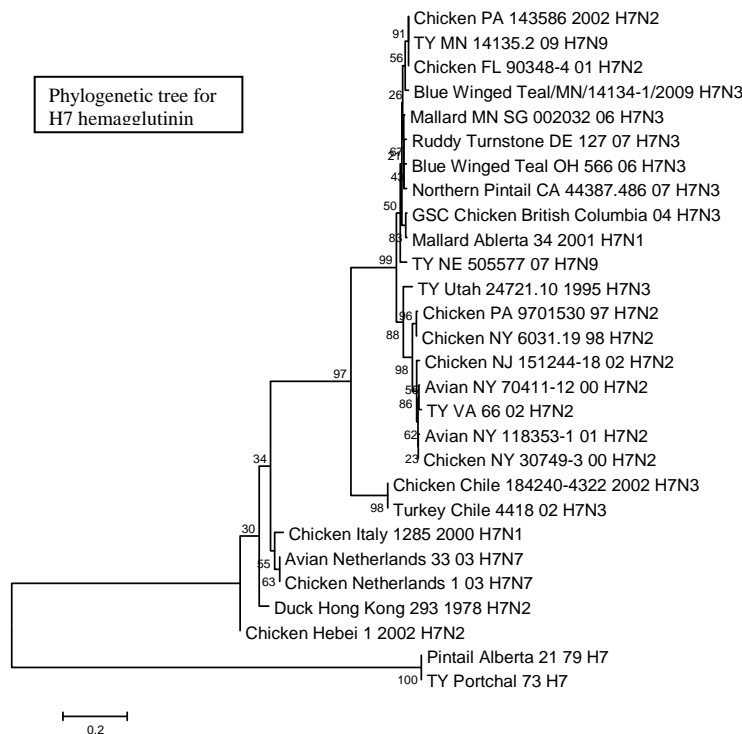
ISOLATION OF AN H2N8 AVIAN INFLUENZA VIRUS FROM A COMMERCIAL TURKEY FLOCK

Peter Woolcock[A], Carol Cardona[B], Jinling Li[B], and Bruce Charlton[C]

California Animal Health and Food Safety Laboratory System, UC Davis
[A]Davis Branch, [C]Turlock Branch
[B]Veterinary Medicine Extension, UC Davis

SUMMARY

An influenza virus was isolated from tracheal tissue collected from a commercial turkey flock in California submitted to the California Animal Health and Food Safety Laboratory. The birds were housed in a multi-house facility with some birds being outdoors and protected by netting from free flying birds.

The isolated virus was typed as H2N8 by genotyping. The nucleotide sequences of all eight genes (PB2, PB1, PA, HA, NP, NA, M, NS) of the virus were determined allowing for comparisons to be made with those of other avian influenza virus sequences in public databases.

The virus was isolated in September which coincides with the August/September onset of the north to south migration of wild waterfowl, particularly of pintail ducks. The gene sequences will also be compared with those of isolates obtained from free flying wildfowl and resident waterfowl that were obtained during this time period in California and from other parts of the Pacific flyway.

DEVELOPMENT AND PRELIMINARY EVALUATION OF A NOVEL BACTERIAL VECTORED VACCINE SYSTEM AGAINST AVIAN INFLUENZA

S.L. Layton[A], C.J. Kremer[A], M.J. Morgan[A], A.D. Wolfenden[A], L.R. Berghman[B], Y.M. Kwon[A], and B.M. Hargis[A]

[A]Department of Poultry Science, Division of Agriculture, University of Arkansas, Fayetteville, AR
[B]Department of Poultry Science, Texas A&M University, College Station, TX

ABSTRACT

Avian influenza (AI) is a significant human and animal health problem worldwide. Development of vaccines for effective control of AI virus in poultry and wild birds is in high demand. Most AI vaccines target the immunodominant antigens such as hemagglutinin (HA) and neuraminidase (NA); however, these vaccines can only provide protection for particular AI serotypes. Among the many vaccine strategies, *Bacillus*-based vaccines that carry antigenic properties of AI is a promising strategy. Our laboratory has recently created several *Bacillus*-vectored vaccine candidates which carry and produce six antigenic epitopes of AI. Experimental vaccination studies in chickens demonstrated that vaccinated chickens exhibited significantly increased M2e-specific and HA-specific IgG titers when compared to chickens receiving the wild type *Bacillus* strain. These data results provide evidence that the *Bacillus*-vectored vaccines may simultaneously induce robust acquired immune responses against multiple vectored epitopes.

Highly pathogenic avian influenza (HPAI) is a significant public health concern and a serious economic threat to the commercial poultry industry worldwide. In the last five years, there has been a substantial increase in the number of HPAI outbreaks in poultry flocks and the number of countries reporting outbreaks continues to increase (1,2,12). Increasing the resistance of the poultry population against avian influenza will not only prevent substantial economic losses to the poultry industry due to the high morbidity and mortality associated with AI in poultry flocks, but will also reduce the significant health risk for the human population by reducing shed and thereby transmission.

Vaccination is widely considered an effective means to prevent infectious diseases, but until recently, the vaccination of poultry against AI has not been widely recommended (3,6,11,12). Current influenza vaccines target antibody production against the surface

glycoproteins, hemagglutinin (HA) and neuraminidase (11,13). However, these antigenic molecules are highly susceptible to recombination and mutations (4,5). This results in the need to frequently update the vaccine to protect against currently circulating strains. Therefore, there is a critical need for new influenza vaccines which are able to provide protective immunity against current and future AI virus strains and for poultry vaccines that can be cost-effectively amplified and delivered.

Our laboratory has recently created several *Bacillus*-vectored vaccine candidates which carry and produce six antigenic epitopes of AI. Sequences for both the human and the chicken M2e were included on the vector. M2e is the highly conserved extracellular domain of the the M2 ion transport protein of AI. We recently reported antibodies against M2e conferred protection against low pathogenic AI challenge (7). In addition to the two M2e sequences, two linear, highly conserved peptide sequences from each the HA moiety (HAUA, HALB) and the nucleoprotein (NP) region of AI were included on vector. When mice were inoculated with a synthetic peptide vaccine generated against highly conserved antigenic peptides of HA they developed neutralizing antigen specific antibodies which protected them from a heterologous influenza challenge (9,10). In a separate experiment, two synthetic peptides were once again used to vaccinate mice only this time they were generated against conserved epitopes of NP known to stimulate a T-cell response. Mice in this experiment exhibited an antigen specific cell mediated immune response and were protected from a low pathogenic H3N2 and H2N2 challenge (8). These two lines of evidence facilitate the belief that both the humoral and cell mediated branches of the immune system must be stimulated to completely protect against an avian influenza infection. A potential immunostimulatory sequence, CD154 or HMGB1, was also added to each *Bacillus*-vector created.

These newly created vaccine candidates were evaluated in a preliminary experiment for their ability to induce a humoral immune response against three of the expressed AI epitopes: M2e, HALB, and HAUA. Day-of-hatch (day 0) chicks were obtained from a local commercial hatchery and randomly distributed into five treatment groups (n=15/treatment group). All chicks in each treatment group were tagged and numbered. The chicks were orally infected by gavage with 0.25 mL of saline or 10^6 cfu/chick and 10^8 cfu/chick of the *Bacillus* vector expressing the six AI epitopes linked to CD154 (BS/AI/CD154) or the *Bacillus* vector expressing the six AI epitopes linked to HMGB1 (BS/AI/HMGB1). Each treatment group was housed in an individual floor pen on fresh pine litter and provided water and feed *ad libitum*. On days 11

and 21 post-hatch, the birds were given a booster vaccination of the same treatment they received on Day 0. Also on days 21 and 31, blood samples were collected from each of the tagged birds and the serum was removed for later use in determining antigen specific IgG serum antibody response by antibody capture ELISA. Data are presented numerically in the form of S/P (sample mean − negative control mean)/(positive control mean − negative control mean) ratios ± SEM in Table 1. These data indicate that the treatment groups receiving either vaccine candidate BS/AI/CD154 or BS/AI/HMGB1 at either 10^6 or 10^8 cfu/chick showed a significant increase in the levels of M2e, HALB, and HAUA specific IgG antibodies when compared to the saline group.

These preliminary data provide evidence that when heterologous epitopes are cell surfaced expressed on this *Bacillus* vector, antigen specific seroconversion occurs. Ongoing experiments are focusing on evaluation of live vs. inactive vaccine administration, duration of response and protection from direct influenza challenge.

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Table 1. M2e, HALB, HAUA antigen specific serum IgG antibody levels at Days 21 and 31 post initial vaccination with BS/AI/CD154 or BS/AI/HMGB1 or saline. S/P ratios (sample mean – negative control mean)/ (positive control mean – negative control mean) $\pm$ SEM.

	M2e Day 21	M2e Day 31	HALB Day 21	HALB Day 31	HAUA Day 21	HAUA Day 31
Saline	0.02 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.01
BS/AI/HMGB110[6]	0.54 ± 0.08	0.84 ± 0.10	0.52 ± 0.13	1.10 ± 0.25	0.68 ± 0.10	0.90 ± 0.11
BS/AI/HMGB110[8]	0.42 ± 0.10	0.64 ± 0.09	0.43 ± 0.05	1.30 ± 0.18	0.55 ± 0.05	1.00 ± 0.12
BS/AI/CD15410[6]	0.29 ± 0.04	0.45 ± 0.04	$.048 \pm 0.10$	$1.10 \pm .017$	0.61 ± 0.09	0.86 ± 0.13
BS/AI/CD15410[8]	0.80 ± 0.8	0.82 ± 0.23	0.37 ± 0.11	1.10 ± 0.21	0.45 ± 0.04	0.83 ± 0.09

THE GENOMES OF MAREK'S DISEASE VIRUS EXIST AS QUASISPECIES AT DEFINED INTERVALS DURING SERIAL PASSAGE-INDUCED ATTENUATION

Stephen J. Spatz[A], Isabel M. Gimeno[B], Richard L.Witter[C], and Mohammad Heidari[C]

[A]Southeast Poultry Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Athens, GA 30605, USA
[B]Department of Population Health and Pathobiology, North Carolina State University, Raleigh, NC 27606, USA
[C]Avian Disease and Oncology Laboratory, Agricultural Research Service, United States Department of Agriculture, E. Lansing, MI 48823, USA

SUMMARY

Marek's disease (MD) is a highly contagious neoplastic and neuropathic disease of chickens caused by *Gallid Herpesvirus* type 2 (GaHV-2) and is controlled through mass vaccination with the bivalent vaccine containing oncogenic *Gallid Herpesvirus* type 2 and *Meleagrid Herpesvirus* type 1 or live-attenuated strains of oncogenic GaHV-2. Attenuation of involves the serial passage of virulent GaHV-2 field isolates in avian embryo fibroblasts. In order to gain a better understanding of the genes involved in attenuation, the genomic DNA sequence of a single GaHV-2 virulent strain (648A) was determined at defined passage

intervals (p11, p31, p41, p61, p81, and p101). Biological characterization of these 'interval-isolates' in chickens indicated that the ability to induce transient paralysis was lost by passages 40 and the ability to induce persistent neurological disease was lost after passage 80, coincident with the loss of neoplastic lesions (1). Deep sequencing of the interval-isolates allowed for a detailed cataloguing of the mutations that exist within a single passage and the frequency with which a given mutation occurs in subsequent passages. Gross genetic alterations were identified in both novel and well-characterized genes and cis-acting regions involved in replication and cleavage/packaging. Deletions in genes encoding the virulence factors vLipase, vIL8 and RLORF4 as well as a deletion in the promoter of ICP4 appeared between passages 61 and 101. Three mutations in the virus-encoded telomerase (vTR) which predominated in late passages were also identified. Overall fourteen SNPs were identified in the genomes for passages 11 through 101. The proportion of the SNPs within the genomes varied greatly between individual passages (Table 1). Few genetic changes were absolute, present in 100% of the sequences within a passage. This indicates that serial passage of GaHV-2 results in the generation of quasispecies of varying proportions and represents the first sequencing report of the existence of quasispecies for an alphaherpesvirus.

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Table 1. The genomic DNA sequence at defined passage intervals (p11, p31, p41, p61, p81, and p101).

Location	SNP	648 passage number						Common Name
		11	31	41	61	81	101	
MDV002/079 vTelomerase	Arg^{180}Pro G to C							RLORF1 Arg-rich protein RNA subunit of telomerase
MDV003/078	Leu^{17}Ser							Spliced gene encoding vIL8
MDV003/078	Gln^{96}Arg							Spliced gene encoding vIL8
5'MDV006.6/075.1 3' MDV007/074	G to A							B68, 14 kDA protein RLORF12
MDV008/073 MDV009 MDV009.5/073.4	Gly^{9}Arg Pro^{161}Leu Arg^{45}Lys							RLORF14 LORF1 RLORF13
MDV054	Val^{359}Ala							U$_L$41, virion host shutoff (VHS)
MDV059	Glu^{59}Lys							U$_L$46, VP11/12
3' MDV082/102	A to G							RSORF1
MDV084/100	Val^{1871}Ala							Immediate Early ICP4
MDV084/100	Ser^{1641}Pro							Immediate Early ICP4
MDV084/100	G^{189}A Silent							Immediate Early ICP4
5' MDV086.1/097.9 3' MDV086.4/97.3	T to C							
MDV088	Asp^{133}Gly							Immediate Early ICP22
MDV096	Ser^{205}Pro							US8, Glycoprotein E

VECTOR HVT-IBDV VACCINATION (VAXXITEK®) IN COMMERCIAL LAYERS

F. Perozo[A], F. Perez[A], F. Rojo[B], and R. Fernandez[B]

[A]University of Zulia Veterinary College, Maracaibo, Zulia Venezuela
[B]Merial Select, Inc. Gainesville, GA, USA

SUMMARY

The objective of this work was to assess layer infectious bursal disease virus (IBDV) vaccination using VAXXITEK®, a turkey herpesvirus (HVT) vector vaccine expressing the IBDV viral protein 2 (VP2). At one day of age 7,500 ISA-Brown layers were vaccinated with the vector vaccine by the subcutaneous route and compared along rearing and production with the same amount of birds vaccinated with a live IBDV program (days 7 and 18 intermediate and intermediate plus, respectively). The results showed significantly higher bursal indexes and lower bursal lesions at different time points in the VAXXITEK group, suggesting better bursal integrity when compared with the live program. Molecular analysis at 28 days of age detected field strains only in the traditional group, suggesting protection against field viruses at a critical point for IBDV infection. At 29 weeks of age higher egg production (83.23% vs. 80.35%) and lower mortality (2.96% vs. 3.55%) were observed in the HVT-IBDV vaccinated birds. These results indicate the suitability of single dose of the vector vaccine for IBDV control in commercial layers.

Infectious bursal disease control is currently attempted using live and/or killed vaccines for the dams and/or offspring. A different approach for IBDV vaccination is the immunization of chickens using viral vectors expressing the VP2 of the IBDV. The aim of this work was to evaluate the efficacy of a HVT-IBDV vector vaccine (VAXXITEK) for IBDV control in commercial layers and compare it with traditional live IBDV vaccination.

MATERIALS AND METHODS

At one day of age, 7,500 ISA-Brown layers were vaccinated with the HVT-IBDV vector vaccine by the subcutaneous route and compared along rearing and production with the same amount of birds vaccinated with a traditional live vaccine program (days 7 and 18 intermediate and intermediate plus IBDV, respectively). The criteria to evaluate vaccination efficacy included: The bursal index, the bursa size, and the lesion scores. Molecular IBDV detection using reverse transcriptase polymerase chain reaction (RT-PCR) and direct nucleotide sequence to evaluate field virus colonization and production parameters were also evaluated.

RESULTS AND DISCUSSION

Recently, experimental and field work has demonstrated the suitability of the vector HVT-IBDV vaccination for classical and variant IBDV control in meat type chickens (2,3). This trial describes the feasibility of using VAXXITEK in layers during the rearing period, diminishing the live vaccine load in the pullets and demonstrating a novel tool for IBDV and immunossupression control. Figure 1, shows a consistent difference in the bursal size, suggesting no IBDV effect on the VAXXITEK group and bursal atrophy in the live IBDV group. The relative size of the bursa strongly correlates with appropriate immunity in poultry (1), in this trial higher bursal indexes were observed in the VAXXITEK group. Histopathology findings demonstrated IBDV related bursa damage in the live vaccinated birds. In these birds, lymphoid depletion of the follicles and follicular atrophy consistent with the higher grading were observed. Bursas were scored from one (normal) to four (severe damage).

At four weeks of age, RT-PCR detected in the live vaccinated group an IBDV similar to S706 strain, therefore different to the one used for vaccination, demonstrating colonization of the bursa by viruses present in the farm. No IBDV was detected in the VAXXITEK vaccinated birds, suggesting control of viral colonization at a critical point for IBDV infection. At 29 weeks of age an increased percentage of egg production (83.23% vs. 80.35%) and lower mortality (2.96% vs. 3.55%) were recorded in the vector HVT-IBDV vaccinated birds. Overall, these results indicate the suitability of single dose of the vector vaccine for IBDV control in commercial layers.

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VAXXITEK® is a registered trademark of Merial in the United States of America and elsewhere.

Figure 1. Size differences observed in bursas collected at seven weeks of age. VAXXITEK® vaccinated pullets showed better bursal size when compared with the live vaccine group.
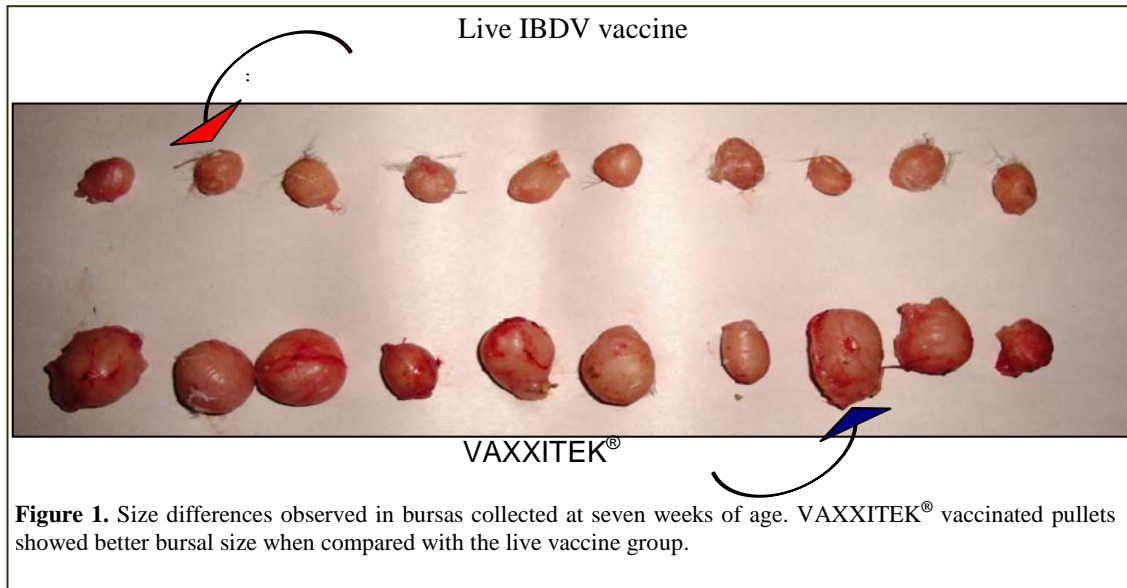
CONTROL OF VARIANT BURSAL DISEASE IN BROILERS WITH A HALF DOSE OF A VECTORED HVT+IBD VACCINE (VAXXITEK HVT+IBD) GIVEN DAY-OLD

Randall T. Bishop

Cornwallis Veterinarians Ltd, 273 Belcher Street, Kentville, Nova Scotia, Canada

Vaxxitek was given day old at one-half dose and full dose to broilers placed on a multi age broiler farm which has experienced high condemnation and performance problems over the past year. A variant infectious bursal disease (IBD) virus was isolated from this farm and consistently high ELISA xlIBD titers were found. One barn was vaccinated with a full dose and the other four barns were done with one-half dose Vaxxitek day-old. ELISA titers are done with the Idexx xlIBD and Symbiotic Proflock Plus systems to determine the efficacy of the vaccine and the presence of field virus. Performance data is compared.

EFFECTS OF *CRYPTOSPORIDIUM BAILEYI* ON THE BURSA OF FABRICIUS OF SPF CHICKS

Hayet Abbassi[A] and Muriel Naciri[B]

[A]Department of Animal Science, University of Minnesota, St. Paul, MN, USA
[B]INRA, Station de Pathologie Aviaire et de Parasitologie, 37380 Nouzilly, France

The aim of this study was to investigate in newly hatched SPF chicks the effect of *Cryptosporidium baileyi* on the bursa of Fabricius and compare it to an intermediate strain of IBDV and the concurrent infection *C. baileyi*-IBDV. Four groups of chicks were inoculated either with *C. baileyi* at day one, or intermediate IBDV at hatching, or both agents, or a placebo. Five birds per group were sacrificed each week during five weeks and the remaining birds were sacrificed at the end of experiment at 76 days of age; the BF/BW weight ratios were measured and the bursa of Fabricius were submitted for histology. Samples of feces were monitored every other day for oocyst shedding. The *C. baileyi* infection at D1 induced statistically significant decrease in the BF/BW weight ratios between D14 and D46. Similar results were obtained with the intermediate strain of IBDV. More severe atrophies of the bursa of Fabricius were observed with the concurrent infection *C. baileyi*-IBDV, especially at D35 and D46. At D76, *C. baileyi* infected group was similar to the control, while the IBDV infected groups maintained lower BF/BW weight ratios. The patent periods (for oocyst shedding) were 20 and 28 days in the *C. baileyi* and *C. baileyi*-IBDV infected groups respectively. Once the oocysts are cleared from the bursa, the histology show only signs of follicular atrophy that are not specific for a cryptosporidial infection. In conclusion, an early oral infection with *C. baileyi* alone can induce severe atrophy of the bursa of Fabricius similar to an intermediate IBDV strain. This atrophy can be quantified by the BF/BW weight ratio and confirmed by histology.

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

EXPERIENCES USING AUTOGENOUS FOWL ADENOVIRUS VACCINE TO CONTROL INCLUSION BODY HEPATITIS

T. Inglis and D. Mitevski

Poultry Health Service Ltd., 97 East Lake Ramp NE, Airdrie, Alberta, Canada.

SUMMARY

Inclusion body hepatitis (IBH) is a disease caused by fowl adenovirus (FAdV). IBH is commonly observed in chicken flocks in Canada and many strains of FAdV are thought to be endemic. Historically, IBH has been associated with primary infectious with immunosuppressive viruses such as infectious bursal disease virus (IBDV) and chicken anemia virus (CAV). In 2003 a number of outbreaks of IBH were seen in Alberta broiler chicken flocks (aged 8 to 35 days), in which mortality rates reached or exceeded 21%. Gross, histological, and serological evaluation along with virus isolation did not reveal evidence of underlying disease(s) in these cases. Based on the diagnostic data from 103 outbreaks of IBH in broiler chickens, it was concluded that IBH was acting as a primary disease. An autogenous killed FAdV vaccine comprised of three of the strains isolated from the clinical cases, was developed to control the disease by vaccination of broiler breeders. The vaccination program began in 2006. To date, the vaccines developed have controlled the incidence and severity of disease associated with IBH in broiler chickens.

INTRODUCTION

Early reports of IBH in Canada were first identified in Alberta in 1968 (3). The condition was characterized by mortality of 5 to 8% in broiler chicken flocks three to five weeks of age. While the etiology was presumed to be viral based on the presence of hepatic intranuclear inclusion bodies it was some time before a virus was isolated. The predominant finding in these reported IBH cases was pale, swollen kidneys as well as swollen, mottled livers with a reticular pattern

of hemorrhages beneath the capsule (3,6). Subsequently, associations between IBH and underlying immunosuppressive disease have were described (1,7). Case reports from the US and Canada of IBH associated with underlying immunosuppressive diseases followed this early work for some time (4). Based on the presumption that various FAdV strains were causing primary IBH the Australian poultry industry began a live vaccination program in 1990 using a live serotype 8b FAdV (ICTV taxonomy system) (2,9). Outside of Australia, a number of killed FAdV vaccines have been created for breeder programs and reported to be successful in the control of IBH in broiler chickens (8).

CLINICAL PICTURE

In Alberta, cases of IBH were diagnosed sporadically each year in routine diagnostic submissions from chicken flocks (three to seven cases/year from 1999-2002). In July of 2003 the incidence and severity of IBH cases in broiler chicken flocks increased suddenly. The lesions seen in these cases were consistent with the classical description of pale, swollen kidneys as well as swollen, mottled livers with a reticular pattern of hemorrhages beneath the capsule (3,6). Within a year of the first cases, 35 outbreaks were diagnosed and confirmed by histological examination. Affected flocks ranged in age from 10 to 35 days of age (average 22 d) with reported mortality ranging from 1 to 26%. The mortality pattern typically seen with these cases of IBH was a rapid increase over three days followed by three days of sustained mortality after which mortality would return to normal rates overt the last three days of the outbreak. There did not appear to be any positive response to various antibiotic and supportive therapy. At the time of these outbreaks there were no documented clinical cases of IBD of CAV in any commercial poultry flocks in Alberta. The poultry population in Alberta is spread over a large geographical area with relatively low bird population densities which along with full barn clean out and disinfection between flocks contributes to a low infections disease challenge. This region has traditionally had a very low viral disease challenge and, aside from a minority of multiage broiler farms, was not using any field or hatchery vaccination to control IBDV or Marek's Disease Virus in broiler flocks.

During the first year of this new presentation of IBH the industry experienced significant losses and in 2004 a project was initiated to find a control strategy. Of the 45 cases identified that year 40 were chosen for enhanced diagnostic evaluation. IBH was confirmed in all 40 cases. There was no histological evidence of bursal damage in 23 of the 40 cases or serological response to IBD in affected flocks at slaughter. Samples from 25 of the 40 cases were tested for IBD and FAdV by virus isolation (yielding only one isolate on a farm previously using live IBD vaccine). From these cases, eighteen FAdV isolates were typed as: Ten isolates of FAdV 8a T8-A, four isolates of FAdV 8a TR-59, four isolates of FAdV 11 strain 380, one isolate of FAdV 7x11a. These isolates are consistent with the strains of FAdV described in Canada (5). Three isolates were selected from these viruses for the development of an autogenous, killed vaccine (9).

DISCUSSION

The vaccine was produced and the program was implemented at the start of 2006. By the end of 2006 the number of IBH outbreaks had decreased to 23 (with only four cases in broiler flocks from vaccinated breeders) and by the following year the number of outbreaks was down to four cases in total. The incidence of IBH has remained at this level since 2007 and would appear to support the success of this program. Monitoring of breeder flocks for serological response to FAdV using ELISA testing has been ongoing since the vaccination program was initiated and shows 78 to 100% seroconversion in properly vaccinated flocks. Historical samples from breeder flocks older than 26 weeks of age, collected prior to the initiation of FAdV vaccination, showed rates of seroconversion to FAdV ranging from 0 to 92% (average 36% positive samples). The mode of protection conferred by these vaccines has not been clear and the importance of the strains selected for protectively is unknown. It would appear that affected broiler farms can show repeated IBH outbreaks if the viral load is not reduced with adequate cleaning and disinfection. A number of cases of IBH were seen in broiler chicken flocks in Saskatchewan sourced from a vaccinated breeder flock which showed partial vaccination failure. The evidence of horizontal transmission within a farm from one flock to the next (chicks from the same source of vaccinated breeders) as well as cases of suspected vertical transmission of virus from partially vaccinated breeders may suggest that vaccination with these killed FAdV vaccines primarily interferes with vertical transmission of the virus rather than a protective antibody affect at the broiler flock level.

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CATASTROPHIC POST-PLACEMENT MORTALITY IN 21,000 CHICKEN BROILER FLOCK

Lloyd J. Weber[A] and Marina L. Brash[B]

[A]Lloyd J. Weber Consulting Services, 519 Maltby Road W, Guelph, Ontario, N1L 1G3, Canada
[B]Animal Health Laboratory, University of Guelph, Guelph, Ontario, N1H 6R8, Canada

Twenty-one thousand chicken broilers were placed in a 15,000 square foot barn at 7:30 a.m. on a cool, very windy day (Day One) in late March 2009. The truck delivery crew placed the broilers in the brooding area and left within 50 minutes and reported good barn conditions with no concerns. The same delivery truck then delivered the balance of the truckload to a neighboring farm with no reported mortality problem.

The owner's son checked the barn at 8:03 a.m. and felt that chicks did not follow him to the door. At 11:30 a.m., the owner checked on the barn and became concerned about mortality of a few hundred birds. A sample of 23 dead birds were picked up at the farm for post mortem. Twenty one birds had moist feed in the crops. The post mortem findings suggested dehydration because of the dark shanks with no evidence of yolk sacculitis. The next day (Day Two) the grower reported about 80% mortality within the preceding 24 hours.

A farm visit followed later in the afternoon on Day Two to assess the severity of the problem and to check out barn conditions. A University of Guelph Engineering gas detector was used to assess carbon monoxide and carbon dioxide concentrations. The exercise turned out to be major history taking. Ventilation fan operation, heater function, water and feed were discussed. Sanitation agent usage between crops and in water lines was clarified. Recent pesticide treatment of the barn was questioned. Brooding temperature and location of mortality was also observed with virtually 100% livability in remaining 2,500 cockerels located at the far end of the barn. Nine other farms received feed from the same batch with no problems.

I suggested placing 100 chicks immediately the next morning (Day Three) in the identical environment. Since the birds survived and looked great after 24 hours, the total barn was repopulated that same day with excellent livability.

Flock history failed to identify the exact cause of high mortality, however lesions seen at necropsy, including cyanosis of shanks and beaks, marked pulmonary congestion and edema and histological lesions of acute hepatic peracinar necrosis and reduced pulmonary parenchymal aeration were suggestive of hypoxia. Carbon monoxide or carbon dioxide or exposure to an unknown gas was suspected as the cause of mortality but could not be confirmed.

MULTIPLE BROILER BREEDER FLOCKS WITH ESOPHAGEAL LESIONS

R. M. Ouckama[A] and M. Brash[B]

[A]Maple Lodge Farms Ltd., Hatchery Division, 101 Fox Road, Port Hope, ON L1A 3V5
[B]Animal Health Laboratory, University of Guelph, Box 3612, Guelph, ON N1H 6R8

SUMMARY

A cluster incidence of obvious and unusual proliferative esophageal lesions was found in several broiler breeder flocks of various ages mid February 2009. Histological findings varied in degree between birds, but hyperplasia of the surface of esophagus epithelium and squamous cell metaplasia of the esophageal mucosal glands was evident in all flocks. The flocks were widely dispersed geographically in south western Ontario, different owners, associated with three different hatchery locations but shared a common feedmill source. Since that time additional flocks have been identified associated with other feedmills in both Ontario and Quebec. The postmortem findings are compatible with those reported in the literature for vitamin A deficiency, but the etiology in this case has not been determined.

Squamous cell metaplasia of the mucosal glands of the esophagus (and occasionally oropharynx, paraocular glands, sinuses, proventriculus, and bursa of Fabricius) is a characteristic lesion of vitamin A deficiency (VAD) in chickens, along with increased susceptibility to candidiasis (1,3,4,6,8). The squamous metaplasia of the mucosal glands block the opening of the gland and result in accumulation of debris and the nodular proliferation of the esophagus seen grossly post mortem. Postmortem observations of proliferative coalescing nodules on large areas of distal esophagus and histologic finding of squamous metaplasia of mucosal glands of esophagus are rare in modern commercial broiler breeder flocks. This case reports these findings in a cluster of flocks in Ontario in February 2009 and the results of further investigation.

CASE REPORT

The initial presenting case was a 27 week old broiler breeder flock that was behind in production and weight goals despite adequate feed allocation. The distal third of the esophagus in all birds submitted showed prominent nodular proliferation, and based on the classic histological lesions that were characteristic of a vitamin A deficiency, this was listed as the most likely possibility in the case. Since the primary source of vitamin A is feed, seven other flocks, various ages (16 to 28 weeks and a 53 week) using the same feed source were submitted to Animal Health Lab in Guelph as a survey to determine if there was any evidence in additional flocks. No clinical signs suggestive of vitamin A deficiency were observed across the flocks as a group. (Listlessness, unsteady gait followed by stunted growth, emaciation, sudden drop in egg production and hatchability, lacrimation, and eventual blindness (1,3,4,6,8).) These cases were sampled between February 13 and 18, 2009.

In all eight flocks submitted, raised, white, individual or coalescing proliferative nodules were found in large areas of distal third of esophagus. Histology showed hyperplasia of surface epithelium, mild to moderate to severe squamous metaplasia of the mucosal glands of the esophagus with near complete replacement of the normal columnar mucous secreting glandular epithelium by stratified squamous epithelium in some sections. Plugs of squamous epithelium protrude from the ostia of the glands above the surrounding esophageal mucosal surface. Concurrent focal candidiasis was found in two of the flocks. Two other flocks also showed degenerative changes of infraorbital sinuses (jumbling, flattening, and deciliation of epithelial lining) with mild inflammatory rhinitis. One flock showed segments of squamous epithelium of conjunctiva of the eyelids with no inflammation. Gross examination of the esophagus, oropharynx, and trachea are routine in every submission for postmortem. The esophageal lesions were not observed in other broiler breeder flocks of various ages submitted for examination during this time.

No virus was isolated in eggs from cecal tonsil, lung, or trachea. The flocks were MS and MG negative by plate agglutination and serology titers by routine ELISA for AE, NDV, IBV, IBD, and reovirus were as expected for vaccination program. Analysis of liver tissue from four flocks resulted in 552.21 µg/g, 719.78 µg/g, 734.40 and 1265.10 µg/g dry matter vitamin A. There is no established avian reference value for the assay used in any avian species (laboratory communication). In adults of most domestic animals hepatic vitamin A concentrations associated with recommended vitamin A dietary concentrations are in the order of 300-1000 µg/g dry matter (1). Based on this all the liver samples were within normal range.

For pullet and breeder feed collected at the farm December to February, vomitoxin levels ranged from 1.1 to 1.9 ppm. Vitamin A levels for these same samples were 4960 to 6050 IU/kg for the December dated and 6190 to 8750 IU/kg plus one at 2220 IU/kg for the February dated. Primary broiler breeder recommendations are in the order of 10,000 IU/kg and 5000 IU/kg is not sufficient for modern breeds (1,7). There is degradation with time of up to 5 to 6% per month, so held samples would be expected to be lower values. Reports from the feedmill and premix company common to all these cases were that all retainer samples at the feedmill tested within normal expected ranges for vitamin A and for mycotoxins and the feed analysis for other parameters including minerals were within normal range.

Unexplained hatchability drops associated with early embryo mortality (three to four days incubation) were reported in some of the flocks. In the initial presenting flock, egg yolk from eggs laid on date of submission had a retinol level of 1796 ng/g, which was reported as equivalent activity vitamin A 5.981 IU/gm. Literature reports a minimum of 2.4 IU vitamin A/gm yolk for adequate embryo development and the concentration of vitamin A in the yolk increases directly with feed concentration (7,8,9).

Since this time six additional flocks in Ontario (7 to 23 wks) plus seven flocks in Quebec (26 to 56 weeks) with different feed mills, were found with similar lesions.

DISCUSSION

In the literature squamous metaplasia in the mucosal glands of the esophagus is characteristic of vitamin A deficiency (VAD). As a fat soluble vitamin with storage in relation to the fat stores, the length of time on a deficient diet before depletion will depend on the amount of fat storage and the age of the bird. Older hens would take a minimum of four to seven weeks on deficient diet before any clinical signs are evident. The plasma concentration of vitamin A is maintained until the liver storage is exhausted, then plasma levels reduce. Hepatic concentration is the best indication of status of vitamin A in the bird (1,3). Egg yolk levels vary directly with the dietary concentration intake and to the bird's ability to effectively metabolize and mobilize vitamin A. Low concentration in yolk results in weak embryos, early embryo mortality, reduced growth and delayed development of the embryo and incubation stressors may increase requirement for proportion of embryos (7,8,9).

Insufficiency of vitamin A can occur in several circumstances. Inadequate amount in feed source can result from formulation errors, mixing errors, vitamin quality, vitamin potency or deterioration with time at the mill or premix level. It would be rare in a commercial operation to have this occur repeatedly over the several weeks required to deplete the bird's stores. The lower values on the limited test may be from age or storage conditions of the sample. Inactivation of the vitamin can occur with high heat (as occurs in feed processing) if not adequately protected or stabilized, through oxidation, catalytic effects of trace minerals and peroxidation of polyunsaturated fats. Breeder feeds have relatively low fat content and in this case no added fat, plus less likelihood for rancidity in the winter weather. The mineral levels were reported as normal.

Interference with absorption in the gut can occur with competition at the absorption sites from high levels of the other fat soluble vitamins, especially D (7). Variation in absorption ability can occur when dietary fat levels are too low to optimize fat soluble absorption, damage to the intestinal wall from parasites (e.g. coccidia, nematodes), viral (e.g. reovirus, malabsorption syndrome), bacterial, mycotoxin, or heavy metals. However, these would be expected to show other signs of disease post mortem and would involve general nutritional status, not a specific vitamin alone. No pathogenic bacteria, viruses, or mycoplasma were isolated.

Increased demand for vitamin A above normal may occur with other disease or high stress challenges. Entering into egg production to peak is a time of very high fat metabolism and general nutritional demands. Also the modern yield breeds have a higher base demand than the previous breeds which is the reason for the high nutrient level recommendations from the primary breeding companies. In this case there was a variety of ages and production stages represented in the sample. Also normal feed formulation should have taken into account the potential losses during manufacture, storage, and the demand requirements of the commercial breed.

Interference with metabolism of vitamin A has been reported with exposure to halogenated hydrocarbons (pesticide), multichlorinated napthelene (wood preservative) (5) and in association with DON vomitoxin (2). The average vomitoxin levels in the feed were not excessive, although averaged higher than seen the previous year due primarily to the corn supply levels and this would be common to most feeds in the area. The farms involved were spread over wide geographic area with different sources of inputs, such as wood bedding or straw, and no chemical usage common to all was found.

It appears that the birds did have normal levels of vitamin A in their body at the time of the submissions with the esophagus lesions. The lesions seen were both grossly and histologically typical of vitamin A deficiency and these particular lesions have not been

reported with any of the usual viral or bacterial diseases of poultry (1,6).

Adequate vitamin A levels in the liver at the time of submission does not rule out an earlier deficiency or an interference that has now resolved itself. Once the bird is on normal intake levels the liver and egg levels return to normal very quickly, within a couple of days. Literature reports that the esophageal lesions are the first to appear in an insufficiency, experimentally after three weeks of a borderline level, and are still evident more than 10 weeks after return to supplemented diet (1,3). Repeated exposure to an irritant that produces mild chronic injury to epithelial cells commonly results in hyperplasia and although not common, metaplasia of these cells can occur. Metaplastic changes go from a more specialized epithelium to a less specialized epithelium which is most often squamous epithelium (personal communication H. John Barnes). However, no common exposure to possible irritant could be determined. Through synergy, multiple mycotoxin contamination a low levels could result in irritation or new disease manifestations (2). There is always the possibility of an unknown chemical or unknown disease affecting liver metabolism or mucous membrane metabolism, although these flocks were widely dispersed with different ownership. Two cases did show areas of candidiasis (reported as secondary to esophageal damage including vitamin A deficiencies) (3,4).

At this time, the cause of the esophageal lesions and significance of the lesion to production level, general health and nutritional status has not been definitively identified.

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A CASE OF PULMONARY HEMORRHAGE IN BROILER BREEDERS

Jenny Fricke[A], Darko Mitevski[A], Tom Inglis[A], and Victoria Bowes[B]

[A]Poultry Health Services Ltd, 97 East Lake Ramp NE, Airdrie, AB, T4A 0C3 Canada
[B]Animal Health Centre, 1767 Angus Campbell Rd, Abbotsford, BC V3G 2M3 Canada

SUMMARY

Pullets from a flock of 6,000 broiler breeders with a history of increased mortality were submitted to a diagnostic laboratory. Post mortem examination revealed hemorrhagic lungs and frank blood present in the trachea and airsacs. Petechial hemorrhages on the heart surface and mild hydropericardium were also observed. Impression smears from lung tissue revealed large numbers of Gram positive cocci. Staphylococcal infection was suspected and the flock was treated with antibiotics. Bacteriology results later indicated the isolation of significant numbers of *Staphylococcus aureus* from the lungs. The post mortem presentation,

subsequent laboratory results and initial conclusions regarding this case will be presented.

INTRODUCTION

Staphylococcus aureus infection is quite common in broiler breeder chickens and is most often associated with cases of caseous arthritis, tenosynovitis, and/or osteomyelitis (1). In the following case report the findings associated with the isolation of *Staphylococcus aureus* from the lungs of broiler breeders presenting with pulmonary hemorrhage are detailed.

CASE REPORT

History. In October of 2009, 14 dead 16-week-old broiler breeder pullets were submitted to Poultry Health Services for post mortem examination. Several subsequent submissions were also received, all with similar history and findings. The birds originated from a flock of 6,000 birds and were accompanied with a history that 70 birds had died over the last three days. This flock had previously experienced a necrotic enteritis break at seven weeks of age, restricted to the male birds. At that time the whole flock was treated with and responded to penicillin (Pot-Pen, Vetoquinol) administered for five days via the drinking water. The flock was following a typical vaccination program for broiler breeders and all vaccines administered on the farm were delivered via the drinking water. Vaccinations had proceeded as scheduled until the necrotic enteritis break at seven weeks of age, after which point vaccination was behind schedule by approximately three weeks. Until the October 2009 submission, there had been very little mortality in the female birds.

Necropsy Findings. The predominant findings on post mortem examination were that 12 of the 14 submitted birds had coagulated frank blood in the trachea, lungs and airsacs and severely hemorrhagic lungs. The presence of petechial hemorrhages on the cardiac surface and a fibrinous fluid filled pericardium were also noted. The livers and spleens were pale, but not enlarged.

Diagnostics. Tracheal viral swabs were immediately collected to test for avian influenza (AI) and Newcastle disease (ND) by PCR. Trachea and lung were collected as fresh and fixed tissues to test for infectious laryngotracheitis (ILT) by PCR and histopathology. Swabs were collected from the liver, lung and joints for bacterial culture and identification. Samples of trachea, lung, liver, heart and spleen were collected into 10% neutral buffered formalin for histopathology. Impression smears of the lungs were examined for bacteria following Gram staining.

RESULTS

Virology. The results of PCR testing for AI, ND, and ILT were all negative.

Histopathology. There was no histologic evidence of ILT which was the primary rule out based on the gross lesions. The significant histologic lesions that were observed displayed varying degrees of severity. There was multifocal epicardial hemorrhage with mild random acute multifocal myocardial degeneration and necrosis. The liver was congested and there was a background of mild periportal aggregation of mononuclear inflammatory cells, many with karyorrhexis, and moderate generalized random single cell necrosis. There was acute fibrinoid necrosis of the larger hepatic blood vessels with fibrin pooling in the sinusoids. In some sections there was massive generalized acute multifocal coagulative necrosis associated with abundant Gram positive coccoid bacteria. In the lungs there was hemorrhage present in the airways and marked generalized acute coagulative necrosis of the parenchyma. The bronchioles and air capillaries contained fibrin, acute inflammatory cell debris, and abundant Gram positive coccoid bacteria accompanied by mild interstitial edema.

Pulmonary impression smears. Numerous Gram positive coccoid bacteria were observed in the pulmonary impression smears.

Bacteriology. Swabs which were collected from the liver yielded low numbers of *Staphylococcus* spp. and *Staphylococcus aureus*. Lung swabs yielded high numbers of *Staphylococcus aureus*, with lower numbers of *E. coli* and *Enterococcus* spp. noted. The antimicrobial sensitivity pattern for the *Staphylococcus* spp. indicated sensitivity to all antibiotics tested.

DISCUSSION

Based on the results of bacteriology and histopathology, the etiologic agent responsible for the increase in mortality and the gross post mortem findings was identified as *Staphylococcus aureus*. On histopathologic examination there was a distinct lack of embolic bacteria suggesting an inhaled route of entry for the bacteria. The severe acute coagulative necrosis of the lung, liver and heart supports a presumptive diagnosis of bacterial toxemia.

Typically it is thought that staphylococcal infections occur due to a compromised immune system and/or a break in the skin or mucous membranes (1). In this case, no predisposing factors relating to the immune system or the respiratory tract could be identified. No vaccinations were administered by spray, and there had been no changes in the barn ventilation that might account for an aerosol introduction of pathogens. The trigger for this

presentation of staphylococcal infection remains undetermined.

The final conclusion regarding this case was that pathogenic *Staphylococcus aureus* infection was the primary cause of the acute mortality. Initial treatment, prior to receiving bacteriology results, consisted of penicillin (Pot-pen, Vetoquinol) administered in the drinking water. Due to concerns regarding the possibility of *Pasteurella multocida* infection and a continued increase in mortality, the flock was then treated with and responded to enrofloxacin (Baytril, Bayer), administered for three days in the drinking water. Subsequent diagnostic submissions from this flock have revealed caseous arthritis, and *Staphylococcus aureus* has been isolated from the affected joints. Monitoring of this and subsequent flocks is ongoing.

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AN INVESTIGATION INTO CASES OF SEVERE FLUSHING IN MISSISSIPPI COMMERCIAL BROILERS

K. Jones, D. Magee, S.A. Hubbard, A. Banda, F. Wilson, and T. Cummings[A]

[A]Poultry Research and Diagnostic Laboratory, Department of Pathobiology and Population Medicine
College of Veterinary Medicine, Mississippi State University, P.O. Box 97813, Pearl, MS 39288

SUMMARY

An investigation was performed into the cause of unusual flushing in commercial broilers (over two weeks of age) from at least three commercial poultry companies in Mississippi beginning fall 2009. Flushing appeared to be renal in nature and lead to extremely wet litter, dingy feathers, bird huddling and piling, and mortality. Necropsy exam revealed primarily dehydration, swollen kidneys, and small bursae. Histological examination indicated interstitial nephritis glomerulonephritis, nephrosis, lymphoid depletion of the bursa and thymus, and general inflammation of various organs. Bacteriology repetitively isolated *Enterococcus faecalis* from multiple locations in the body as well as from the environment. Virus isolation and molecular analysis were uneventful. The condition in the field seemed to respond to complete removal of affected litter; penicillin therapy; and in some cases, the administration of aspirin.

Case history and clinical signs. A total of twenty cases were submitted to the Poultry Research and Diagnostic Laboratory (PRDL) in Pearl, Mississippi from August through November 2009. The first case submitted to the lab occurred on August 11, 2009 with complaints of an increase in mortality (an increase of five times normal), depression, reluctance to move when urged, and birds huddling and flushing. Affected birds ranged from 17 to 47 days of age with the majority of cases being between 21 to 28 days of age. Both top-end and bottom-end growers were affected. A typical scenario was that only birds in one section of one house on a farm were affected. This location was usually the cool cell end or behind the first migration fence, but not always. The majority of the house did not appear to be affected.

Necropsy findings. Birds and samples were submitted to PRDL for processing. All live submissions were humanely euthanized using carbon dioxide gas or cervical dislocation. All affected birds submitted had dirty feathers and hock and foot pad burns. Additional predominant necropsy lesions included enlarged swollen kidneys, small bursae and thymuses, and dark muscles typical of dehydration. The occasional bird also had pericardial fluid.

Histopathology. Sections of kidney, bursa, thymus, spleen, bone marrow, liver, heart, lung, trachea, proventriculus, brain and intestine were collected, fixed in 10% neutral buffered formalin, and sectioned at 4μm. Tissues were stained using hematoxylin and eosin (H&E) and were then examined by light microscopy. The most consistent lesions were interstitial nephritis, glomerulonephritis, and tubule dilation in the kidneys. Kidney lesions were characterized by multifocal interstitial lymphocytic inflammatory cell infiltration, enlarged hypercellular glomeruli, and focal areas with dilated tubules. These findings were variable and some of the kidneys were unremarkable. The changes generally were of minimal to mild severity. There did not appear to be a good correlation between gross and microscopic renal pathology, possibly suggesting some form or metabolic abnormality causing diuresis. Atrophy and lymphoid depletion were appreciated in bursae and thymuses

more so than what is typically thought of as age related changes. Follicular lymphoid depletion was also evident in spleens. Bone marrow appeared hyperplastic, and multiple tissues had evidence of congestion and inflammation (heart, lung, trachea, proventriculus, brain, and intestine).

Bacteriology. Brain/skull, liver, spleen, bone marrow, kidney, airsac, blood, water lines, and feed were cultured aerobically on 5% sheep blood agar plates (Remel, Lenexa, KS) at 37°C with 5% CO_2. Cultures were considered negative if no growth occurred after 48 h of incubation. *Enterococcus faecalis* was isolated from blood, brain/skull, water line, and feed samples. *Staphylococcus aureus* was found in airsac, kidney, spleen, brain/skull, and water line samples. *Escherichia coli* was identified from air sac, heart, brain/skull, and blood samples. In addition, *Enterococcus faecium* was found in feed sampled, and *Pseudomonas aruginosa* was isolated from water line samples. Liver samples did not yield organism growth of any clinical importance. Fungal cultures were performed on feed and an airsacculitis sample. Both were negative.

Feed analysis. Feed samples obtained from affected flocks were processed for mycotoxin (MSU CVM) and sodium (Barrow Agee, Memphis, TN) content. Samples had <0.1 ppb AFLB1, AFLB2, AFLG1 and AFLG2 and had <1 ppm of vomitoxin, T2, DAS, zearalenone, citrinin, ochratoxin, and sterigmatocys. Sodium levels were 0.2 to 0.23%. All results were considered within normal range.

Virology. Kidney, liver, spleen, proventriculus, lung, trachea, intestine, and cecal tonsils were obtained for virus isolation. Virus isolations in nine to 11 day old SPF embyronated chicken eggs (inoculation of the allantoic sac and membrane) as well as in cell cultures using previously described methods (5) were performed. Infectious bronchitis virus (IBV) and infectious bursal disease (IBD) were isolated from kidney samples via use of nine to 11 day old SPF embyronated chicken eggs. IBV was also isolated from cecal tonsils using SPF embyronated chicken eggs. Cell cultures revealed a reovirus from kidney samples and avian adenovirus from kidney and liver samples. Isolates were confirmed using real-time reverse transcription-polymerase chain reaction (RT-PCR) and molecular sequencing was also utilized using previously described methods (1,2,4). One IBV isolate from the cecal tonsils was identified as having 99% similarity with Arkansas DPI serotype, and another isolate had 99% similarity with Connecticut serotype. All IBD isolates from kidney had 96% similarity with Delaware E strain IBD.

Sections of kidney from necropsied birds were also placed in viral transport media and submitted for examination by negative-stain electron microscopy (EM) (3). No viral particles were detected in the kidney by negative stain EM.

Challenge studies and treatment. In addition, challenge studies were conducted at Mississippi State University College of Veterinary Medicine and in the field. Studies consisted of pen trials on affected litter, transfer of non affected birds to affected areas of a house, and oral gavage of affected litter/feces. No unaffected birds in these studies became clinically affected.

Interventions attempted in the field included optimizing ventilation, removal of feed, litter removal (complete cake-out of affected areas of the house) with the addition of new dry litter in some houses, the administration of antibiotics (bacitracin and penicillin), and anti-inflammatory drugs (aspirin). Successful practices included administration of penicillin, aspirin, and removal of affected litter.

DISCUSSION

Necropsy examination was unremarkable except for swollen kidneys, small bursae, and small thymuses. Histopathology indicated a low to moderate incidence of glomerulonephritis/nephritis of only generally mild severity, and pathology in bursa/thymus. Virology yielded IBDV and IBV, reovirus, and avian adenovirus. Bacteriology identified primarily *Enterococcus fecalis*. Successful intervention strategies included removal of affected litter and the addition of fresh litter, administration of penicillin, and/or aspirin.

This condition appears to be infectious but not highly contagious. It appears to affect the kidneys and not the intestines. The condition may turn out to have a seasonal occurrence, as it did not seem to cause problems after the onset of colder temperatures. If seasonal and related to heat, then metabolic and feed related influences need to be further investigated.

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CLINICAL ASPECTS OF THE IN-FIELD USE OF A PORTABLE BICHEMESTRY INSTRUMENT (ISTAT-1) IN REDUCING FEATHER PICKING AND CANNIBALISM IN BROILER BREEDERS

Daniel Venne, Couvoir Scott ltée, and Younès Chorfi

Faculté de médecine vétérinaire, Université de Montréal

SUMMARY

Feather picking, feather licking, and cannibalism all describe a syndrome in which a normal behavior pattern of the birds is altered into a pathological behavior. This type of behavior has been reported for many years in backyard flocks and commercial growouts.

This presentation describes an increase in this type of behavior in broiler breeder replacement flocks and broiler breeder layers associated with nutritional factors whose effects can be measured by in field blood gas biochemistry. Biochemistry has been used in scientific studies but far less in field clinical evaluations. This paper shows the practicality of using in field biochemistry with the iSTAT system and the effect of rapid treatment of metabolic disorders on the behavior of chickens.

Since in the case of diagnosing the possible causes of syndrome type disease is difficult at the best of times the use of new technological tools can help in the differential diagnosis and more rapid interventions for the well being of the birds and better profitability of the flocks. Rapid intervention in the case of feather picking is important since this behavior can be acquired and may become chronic if a rapid intervention and a rapid cure is not instigated.

This report describes two clinical cases of feather picking reported by one of our managers in March and in September 2009. The pullets were 56 days old and started showing signs of feather picking and aggression the previous day and the hens were 168 days old.

Differential diagnosis of feather picking and cannibalism is presented in Table 1. The factors that needed further investigation are bolded.

In the past the use of salt in the water has reduced the incidence of this type of behavior. The general recommendation is for 20 g of salt in 4 L of water. It is however risky if the feather picking in not nutritional in origin and can cause wet litters or reduced growth if given in excess. Our company had been developing normal values for blood gas and electrolytes in poultry and we decided to see if blood biochemistry could help in a better diagnosis in this case.

In both cases the birds showed blood parameters compatible with metabolic acidosis as shown in Table 2.

A treatment consisting of 125 g sodium bicarbonate in 576 L of water was instigated and the behavior was evaluated. Another blood test was done five to seven days later. The results after water supplementation are shown in Table 3.

Feed was sampled and analyzed during each visit. The results are shown in Table 4.

CONCLUSION

Treatment of the metabolic acidosis with the use of sodium bicarbonate in the water returned the birds to homeostasis and significantly reduced the feather picking. The feed analysis received two weeks after the treatment confirmed low sodium and low electrolyte balance in the diet. In field blood biochemistry can be used to elaborate rapid treatments in the case of metabolic acidosis associated with low electrolyte balance in the feed.

Table 1. Differential diagnosis of feather picking and cannibalism.

Possible cause	Observation	Probability in this case
Strain or genetic effect	Ross 308 in all flocks, with day old beak trimming	Low
External parasites	No mites or other parasites visible on the birds or in the feces	Low
Nutritional or mineral deficiencies or unbalanced diets	**New feed delivery two days before. Feed analysis takes 5 to 10 days**	**High**
Texture of feed	**Pelleted feed since the start of the grow-out**	**Possible**
Behavioral stressors	Birds were flighty	Possible
Social behavior	No transfer of birds between pens	Low
Social behavior	Low bird density	Low
Social behavior	Floor fed birds in grow out barn	Low
Environment	Light intensity grow-out barn is completely dark-out, laying barn	Low

Table 2. Blood parameters.

Blood parameter	Pullet flock	Breeder flock
Average blood pH	7.266	7.265
Average PCO2	44.60	59.47
Average HCO3	19.95	27.00

Table 3. Results after water supplementation.

Blood parameter	Pullet flock	Breeder flock
Average blood pH	7.382	7.436
Average PCO2	45.97	45.25
Average HCO3	27.15	30.20

Table 4. Results of feed analysis.

Feed parameter	Pullet flock	Breeder flock
Calcium	0.98%	0.96%
Phosphorous	0.60%	0.63%
Sodium	0.14%	0.13%
Potassium	0.58%	0.54%
Chloride	0.24%	0.20%
Electrolyte balance	141.6 mEq	138.2 mEq

INFLUENCE OF LITTER COMPOSTING ON DARKLING BEETLE (*ALPHITOBUS DIAPERINUS*) POPULATIONS, LITTER MICROBIOLOGY, AND THE ROLE OF BEETLES AS VECTORS FOR BROILER PATHOGENS

John K. Rosenberger[A], Nannette Olmeda-Geniec[B], Sandra C. Rosenberger[A], Jake Rouse[B], and Toria Boon[C]

[A]AviServe LLC, Delaware Technology Park, 1 Innovation Way, Suite 100, Newark, DE, USA
[B]Elanco Animal Health, A Division of Eli Lilly and Co., 2001 W. Main GL18, Greenfield, IN
[C]University of Georgia (Veterinary Student)

INTRODUCTION

Numerous publications and reports covering more than three decades have documented that darkling beetles (larvae and adults) in poultry houses may serve as vectors for microbes that can be important poultry and/or human pathogens. However little is known about the impact of poultry litter management or treatment strategies including but not limited to disinfection, litter replacement, litter composting, use of ammonia control amendments, or combinations of the aforementioned on beetle populations and accordingly correlations with poultry disease incidence/severity and overall flock performance.

The proposed trial was designed to assess the effects of two broiler litter management programs (composted vs. non-composted) on litter bacteriology, darkling beetle populations (adult and larvae), and the role of beetles as vectors for economically important bacterial and viral pathogens.

Information from the proposed trial could be utilized to establish specific requirements for beetle control programs with different litter management strategies while providing correlations that may be important in disease reduction and improved flock performance.

PROCEDURES

Selection of test broiler farms. Ten Delmarva broiler farms from two broiler companies (five farms per company) were utilized for the trial. Farms chosen for the evaluation represented two different litter management strategies (composting and non-composting). Information requested from the companies for each farm included breed cross and contract type (small bird, etc.), vaccines utilized, amount of time between current and previous flock, most recent removal of litter and total cleaning and disinfection, insecticide and ammonia control usage, previous five flock performance history, performance of test flock to include livability profile, condemnations, feed conversions and medication costs and antibody evaluations at processing to document exposure to specific pathogens.

Collection and enumeration of darkling beetles. Darkling beetle adult and larval forms were collected from ten Delmarva broiler houses two days post placement, at three to four weeks of age and prior to processing. Beetle traps consisting of a 12 inch section of two inch diameter PVC pipe with a roll of corrugated cardboard (brooder guard) inside were utilized. A total of eighteen traps were distributed in each of the 10 test houses during the designated collection times for a five day period. Each test broiler house was separated into three equal sized sections (brood chamber, non brood chamber 1 and non brood chamber 2) to accommodate consistent samplings. Three traps were placed on the side walls of each of the three house sections and three additional traps in each section were placed adjacent to and between feeder pans. The corrugated cardboard from traps in each section were removed and individually placed in a "zip-lock" plastic bag labeled with Farm I.D., location of trap, bird age and date of collection. The samples were placed in coolers and transported to the laboratory for evaluation.

Microbial evaluation of darkling beetles (adults and larvae) and litter samples. The number of darkling beetles (adults and larvae) were estimated and pooled separately in plastic screw cap tubes by house section and time collected. An adult pool and larval pool were prepared for each of the three house sections for a total of three adult pools and three larval pools per house for each collection period. Insect pools utilized for virus isolation attempts were frozen at -70°C until assayed. At the second collection time (three to four weeks), each insect pool was separated into two aliquots: One pool utilized for bacteriological evaluation and one for virology. The aliquot retained for bacteriology was held at refrigeration temperature (~4°C).

Virus isolation attempts were performed using a 20% weight/volume suspension of each insect pool prepared in phosphate buffered saline with antibiotics (1000 units penicillin G, 1000 µg streptomycin and 250

μg amphotericin G per mL). If insufficient number of beetles (<100) were collected a 10% weight/volume suspension was prepared. The suspension was homogenized using Stomacher apparatus/bags and then frozen and thawed three times. The homogenates were then centrifuged (1000xG) and supernatant filtered to remove tissue debris and potential contaminants. The filtered supernatant was used as inocula for SPF embryonated eggs via the chorioallantoic membrane route. Evidence of virus isolation was based upon the development of pathognomonic embryo lesions, mortality or inhibition of isolated virus with specific antisera. Established cell lines were also utilized for the identification of selected enteric virus isolates (astrovirus and rota-like virus).

Samples of unprocessed refrigerated (not frozen) beetles (adults and larvae) were retained from the second collection for isolation and identification of selected bacteria. Bacterial isolations and identification were done by the Lasher Laboratory at the University of Delaware, Georgetown, DE using well accepted and documented procedures. Assessments included the total aerobic and anaerobic counts and the number of gram-negative lactose and non-lactose fermenting organisms per unit volume of darkling beetles (adults and larvae).

Litter samples adjacent to the beetle traps were collected during the three to four week beetle collection. Approximately 20 grams of litter per individual trap site was collected from the top three inches of litter by hand with sterile gloves and placed in one bag per chamber (three samples from side walls and three samples from between the feed pans in each bag). There were a total of three bags per house. The bags were marked with farm identification, collection date and sample site and placed in a cooler with cold packs. Adult beetles and larvae were removed from the litter samples and discarded. The litter samples were thoroughly mixed and then submitted for bacterial evaluation the day of collection. Total aerobic and anaerobic counts and gram negative lactose and non-lactose fermenting organisms were enumerated per gram of litter.

SUMMARY OF RESULTS

1. Litter composting did not, by itself, appear to reduce beetle numbers, virus load or bacterial counts at three to four weeks post chick placement.

2. Viruses and bacteria isolated from beetles are those frequently found in feces.

3. Virus isolation procedures "favored" isolation of enteric viruses and infectious bursal disease virus.

4. Viruses could be isolated from beetle adults and larvae.

5. Isolation of virus from beetles at chick placement suggests "carry over" from the previous flock.

6. On a per gram basis beetles appeared to concentrate *Clostridia* spp. and some of the gram negative aerobes.

ACKNOWLEDGMENT

COMPARING *IN VITRO* LYMPHOCYTE REACTIVITY TO *SALMONELLA ENTERICA* SEROVAR ENTERITIDIS (SE) ANTIGENS IN FIVE COMMERCIAL WHITE EGG AND THREE COMMERCIAL BROWN EGG STRAINS OF LAYING HENS FOLLOWING SE INFECTION

Peter S. Holt[A], Lara E. Vaughn[A], Richard K. Gast[A], Kenneth E. Anderson[B]

[A]USDA/ARS Egg Safety and Quality Research Unit, Athens, GA
[B]Department of Poultry Science, North Carolina State University, Raleigh, NC

Infections by *Salmonella enterica* serovar Enteritidis (SE) continue to pose a serious food safety problem for the consuming public and therefore a major concern for the layer industry. As a result, investigations into factors that impact the susceptibility to or course of infection by SE can provide important clues into possible methods for reducing flock SE problems. Different chicken breeds have been shown to exhibit varying susceptibility to infection with paratyphoid salmonellae. These differences may be a reflection of enhanced immunocompetence against these organisms.

The current study was undertaken to compare the course of infection with SE in crops and intestines of five commercial white egg strains (W1-W5) and three commercial brown egg strains (B1-B3) of laying hens and attempt to correlate these infections with the elicitation of an antibody response in the crop as well as the ability of lymphocytes from the infected hens to respond *in vitro* to SE lipopolysaccharide (LPS) and SE flagella. Hens were orally challenged with 10^8 SE and crop lavage and fecal samples were collected weekly. Portions of the fecal and crop samples were incubated overnight at 37°C in Rappaport-Vassiliadis (RV) broth and 0.1 mL of this culture was plated onto Brilliant Green novobiocin (10 µg/ml) and Xylose-Lysine-Deoxycholate –Tergitol 4 (XLT4) media. After a further overnight incubation at 37°C, the plates were examined for the presence of SE. A portion of each crop sample was also centrifuged and the supernatant used for determining specific antibody responses to SE LPS using an indirect ELISA. Prior to infection and weeks 2 and 4 post infection (PI), five hens from each strain were bled, lymphocytes purified, and 100 µL of 2×10^6/mL lymphocytes were incubated with 5 µg/mL SE flagella or 20 µg/mL SE LPS. The cells were incubated for 72 h at 37°C, 5% CO_2 at which time proliferation was tested colorimetrically.

All of the white egg hens were 80 to 100% crop SE positive at one and two weeks PI and then levels decreased thereafter (Fig. 1A). This is in contrast to hen strain B3 and B1 which were 20% and 40% crop positive, respectively. However, examination of fecal shedding showed that all strains of hens were similar, with 80-100% of all the hens exhibiting fecal SE at week 1 PI. Specific anti-SE LPS antibody levels were essentially equivalent between the different hen strains weeks 1 to 4 post challenge. Lymphocyte *in vitro* response to LPS and flagella were generally low at week 2 PI and strongly increased at week 4 (Fig. 1B). Hen strain W4, however, exhibited high lymphocyte activity to both antigens prior to infection and this increased two weeks PI before decreasing at week 4. This high activity did not appear to impact crop or intestinal SE colonization as both sites were 100% SE+ at one week PI. The hen strain that exhibited low crop SE colonization, B3, responded strongly *in vitro* to both antigens and exhibited the highest response of all the strains at week 4 PI.

These results indicate that different hen strains vary in their crop colonization by SE but this cannot be extended to gut colonization. The highest specific *in vitro* response to SE antigen was observed in lymphocytes taken from the hen strain that exhibited the lowest crop SE colonization. However, similar observations were not observed with specific antibody responses. These results indicate that different strains of commercial laying hens do exhibit some resistance to infection but more studies need to be conducted to determine the mechanism.

Figure 1. Crop SE colonization (A) and lymphocyte activation by 20 µg/mL SE LPS (B) in five white egg strains (W1-W5) and three brown strains (B1-B3) of laying hens.
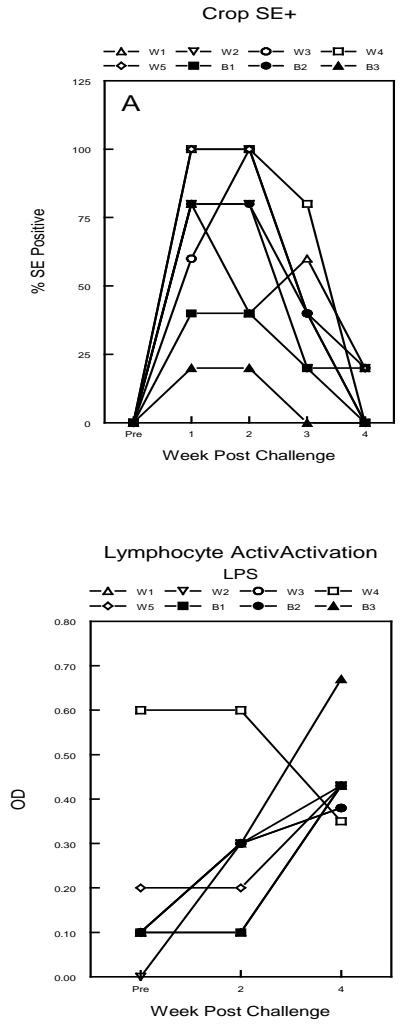
EVALUATION OF RECOMBINANT *SALMONELLA* EXPRESSING THE FLAGELLAR PROTEIN FLIC FOR ENHANCED IMMUNE RESPONSES IN POULTRY

C. J. Kremer[A], R. E. Wolfenden[A], K. M. O'Diam[B], S. L. Layton[A], N. R. Pumford[A], M. J. Morgan[A], A. D. Wolfenden[A], S. Shivaramaiah[A], B. M. Hargis[A], and K. Cole[B]

[A]Department of Poultry Science, University of Arkansas, Fayetteville, AR
[B]Department of Animal Science, The Ohio State University, Columbus, Ohio

ABSTRACT

Salmonella Enteritidis is one of the most common causes of human food-borne illness (8) and the Centers for Disease Control (2) estimates that 1.4 million cases of food borne illness due to *Salmonella* occur yearly in the United States. This organism is often transmitted to humans from poultry products that may have been contaminated pre- or post-harvest. Once poultry are infected with *Salmonella*, the colonization of the tissues will often remain until the birds are harvested and contaminated carcasses can easily spread the organism during processing (7). Vaccinating poultry flocks against *Salmonella* aims to reduce the amount of the organism shed through the feces and its ability to colonize the digestive tract, to decrease the instances of organ invasion, and to prevent contamination of eggs (5). Previous research indicates that oral vaccination of poultry with live attenuated *Salmonella* can confer a high degree of protection; protecting chicks from the challenge strain from multiplying and reduced gut colonization, even with a 10^9 challenge dose (3).

The flagellar protein fliC has shown promise in enhancing an immune response and the ability to protect against challenge of wild-type *S.* Enteritidis in poultry. The protein ends of the flagellin are highly conserved among *Salmonella* serovars and other enteric species, such as *E. coli* (6) and this antigen is recognized by CD4+ T-Cells in both humans and murine models (1). Because fliC is so highly conserved, it may confer cross-protection against multiple serovars of *Salmonella* in poultry. The objective of this study is to evaluate orally administered live attenuated *S.* Enteritidis vaccine candidates for their ability to colonize the cecal tonsils and invade the liver and spleen of commercial poultry as well as create an increased antibody response. The ultimate goal is to create a vectored vaccine that will persist in tissues long enough for an effective immune response to be mounted that will provide lasting protection to the birds without causing illness to birds or humans as well as determine if fliC as an epitope that will aid in this goal.

Several novel attenuated strains of *S.* Enteritidis were developed that express a fliC epitope on the outer membrane using a previously published method which utilizes the Red recombinase system in combination with overlapping extension PCR (4). Experiments were performed in both commercial chickens and turkeys.

In Ohio, turkeys were chosen for the experimental model to evaluate colonization, invasion, clearance, and antibody responses for vaccine candidates with and without fliC and wild type *Salmonella*. Poults were orally challenged with 10^6-10^8 cfu/poult of a wild-type *S.* Enteritidis phage-type 13A (Positive Control), $\Delta aroA/\Delta htrA$ SE-M2e (ΔSE-M2e), or $\Delta aroA/\Delta htrA$ SE-M2e-fliC (ΔSE-M2e-fliC) on day-of-hatch and d 21 post-hatch. Liver, spleen, and cecal tonsil were aseptically removed on d 7, 14, 21, 28, 35, and 42 post-hatch to determine colonization and organ invasion. Blood samples were collected on d 7, 14, 21, 28, 35, and 42 post-hatch to determine M2e-specific antibody responses.

The ΔSE-M2e-fliC candidate vaccine strain demonstrated less organ invasion compared to the ΔSE-M2e strain on d 7 ($P<0.0001$). However, no marked difference in colonization of the cecal tonsils was noted at d 7. From d 21 through d 42, no significant differences were observed in organ invasion between the vaccine candidate strains when compared to SE13A. The ΔSE-M2e-fliC candidate vaccine strain demonstrated a significant decrease in colonization of the cecal tonsils when compared to the ΔSE-M2e strain on d 14, 21, and 28 post-immunization ($P<0.05$). By d 21, the ΔSE-M2e-fliC candidate strain exhibited a higher M2e-specific antibody response when compared to the negative control and SE13A groups ($P<0.05$). Furthermore, by d 28 both ΔSE candidate vaccine strains exhibited a significantly higher M2e-specific antibody response when compared to the negative control and SE13A groups ($P<0.05$). However, no differences in M2e-specific antibody responses were observed between the ΔSE candidate vaccine strains throughout the study. Overall, the ΔSE vaccine candidate strains were able to reduce organ invasion and colonization while eliciting a humoral immune response in commercial poults. Additionally, accel-

erated clearance by the ΔSE-M2e-fliC candidate was observed.

In Arkansas, broiler chickens were chosen as the experimental model. At day of hatch, chicks were randomized, assigned to a treatment group, tagged, and orally gavaged with 0.9% sterile saline or approximately 1x10^8 cfu of ΔaroA/ΔhtrA SE-fliC (ΔSE-fliC), ΔaroA/ΔhtrA SE-fliC-chicken CD154 (ΔSE-fliC-CD154), or ΔaroA/ΔhtrA SE empty vector (ΔSE-EV). The cecal tonsils, liver, and spleen were aseptically removed from a sample of five chicks per group for individual recovery of ΔSEEV or the appropriate vector d 3 post hatch. After the d 3 samples were obtained, all groups were challenged with 1.25x10^7 cfu Salmonella Typhimurium (ST) per chick. Additionally, 15 chicks per group were individually sampled for recovery of ST and ΔSEEV or vector strain from their cecal tonsils, liver and spleen at d 10, 19, and 30. From the d 10 and d 19 samples, ceca were also removed for enumeration of ST and ΔSEEV or vector strain. At d 30, a delayed type hypersensitivity reaction test was performed to evaluate response to ST.

On d 19 (16 days post infection), neither candidate protected against ST challenge better than the ΔSE empty vector, although there was significantly less Salmonella recovered from vaccinated chickens compared to non-vaccinated controls. These studies indicate that these experimental recombinant vaccines did not protect against heterologous challenge or enhance clearance after ST challenge. The increased clearance of the candidate vaccines, particularly the vector expressing fliC alone, may have value in that the fliC epitope may decrease the clearance time of other recombinant vectored Salmonella vaccines.

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USING A LIVE *SALMONELLA* ENTERITIDIS VACCINE TO CONTROL BREAKS OF *SALMONELLA* GALLINARUM AND *SALMONELLA* ENTERITIDIS IN POULTRY IN LATIN AMERICA

Mariano Salem

Lohmann Animal Health International, Msalem@lahinternational.com

INTRODUCTION

Salmonella Gallinarum (SG), also named *Salmonella enteric* subspecies *enteric* serovar *Pullorum-Gallinarum,* is also the etiological agent of fowl typhoid (FT) (12). The disease is characterized by septicemia, high mortality, horizontal, but essentially, vertical transmission and severe internal organ damage. It is essentially a host adapted disease. Chickens and turkeys are the most commonly affected birds but quail, pheasants, ducks, peacocks, and guinea fowl are also susceptible (12).

Salmonella Enteritidis (SE), also called *Salmonella enteric* subspecies *enteric* serovar

enteritidis, is a paratyphoid *Salmonella* that also causes septicemia, transient drop in egg production, mortality in baby chicks and in birds in egg production. As a paratyphoid it has a broad range of host among birds and mammals that includes human beings.

Both bacteria belong to the "O" antigen Group "D" and contain somatic antigens 1, 9, and 12.

PUBLIC HEALTH SIGNIFICANCE

Both diseases, FT and SE, have similar though not identical clinical signs and lesions. Both diseases can be acquired by transovarian transmission, and birds become carriers of the bacteria after recovering from infection. However, besides the mortality and loss of egg production that both diseases cause in commercial poultry, SE is a common cause of zoonosis throughout the world.

Essentially, FT has been eradicated from the USA and other developed countries through serum testing, culture and sacrifice of infected breeders. On the other hand, SE continues to be present even in developed countries. Reduction of the incidence in all countries is very difficult and reduction of contamination to "accepted" levels has proven hard to achieve. Control programs against SE in poultry, thus, in humans, have been implemented in countries with the will to reduce the SE problem. In others, that include some countries in Latin America, these programs are starting to develop and others do not have a specific control program for SE. Their salmonellosis control program is aimed against *S*. Gallinarum and *S*. Pullorum only. Others officially are free of SE or free of "all salmonellas."

INCREASE IN FT IN LATIN AMERICA

Salem *et al*, 2009 (11) reported an increase in cases of FT in Latin America. However, this is not a new problem (13). In his paper, Salem (11) listed the different reasons why this is occurring. Among them:

1. Lack of sufficient FT free breeders to provide chicks for growers,

2. Keeping infected breeders in egg production,

3. Continuous exposure in infected farms,

4. Deficient biosecurity,

5. Lack of updated diagnostic reagents and procedures, and

6. Lack of report of existing cases for fear of losing export markets.

Since eradication of flocks infected with FT and SE has been eliminated as an option in some countries, treatment with antibiotics and vaccination are the options taken by poultry growers.

THE VACCINE

A live *Salmonella* Enteritidis vaccine (Avipro® Salmonella Vac E) that uses an attenuated metabolic mutant, strain Sm24/Rif12/Ssq, has been used extensively in countries in Latin American to prevent mortality, loss of egg production and reduction of bacterial shedding in the environment. This vaccine bacteria has been selected to be invasive enough to induce local immunity but harmless to chickens and other possible host including humans. The $Sm^{res} Rif^{res} Ery^{sens}$ mutations allow for easy differentiation from field strains of SE. The **ssq** is considered by WHO as an attenuation marker (anti-epidemic marker) that makes the vaccine bacteria super sensitive to quinolone antibiotics (8,9).

OTHER VACCINE CHARACTERISTICS

1. Vaccine bacteria does not seed the environment. It is shed for a short period and it doesn't survive in the litter (14).

2. Does not colonize the ovary and is not transmitted to the progeny (14).

3. Does not leave a gap in protection during the growout since it can be applied at day of age.

4. Birds vaccinated via water are fast plate agglutination negative, thus vaccination does not interfere with control programs that are based on serology.

5. Vaccine bacteria can be differentiated from field strains by sensitivity and molecular tests. Thus, do not interfere with control programs based on bacterial culture (8).

6. Does not dot reverse to pathogenicity. The probability of reversing to pathogenicity is 10^{-8} per attenuation marker x 3 markers= $10^{-24.}$ .That is a 10 with 24 zeros (8).

EFFICACY

The efficacy of live *Salmonella* vaccines has been well documented. Babu *et al*., 2004 (1) reported that live *Salmonella* vaccines considerably reduced SE shedding when compared with groups vaccinated with inactivated vaccines or with non-vaccinated groups. These results are in agreement with results obtained by Hassan & Curtis, 1990 (6); Mastroeni *et al*., 2001 (10); Gast *et al*., 1993 (4) and Harrison *et al*., 1997 (5). In their studies they confirm the better efficacy of live vaccines when compared with killed vaccines, as well as the importance of cell mediated immunity (CMI) in *Salmonella* clearance from birds. Also, Mastroeni *et al*. 2001 (10) and, Beal *et al*. 2006 (3) confirmed that clearing of *Salmonella* Typhimurium is independent of B cell function. However, Horzinek *et al*., 1997 (7)

reported that live *Salmonella* vaccines can also elicit both cell-mediated and humoral immunity at the desired (e.g. mucosal) site in the animal. There is also scientific evidence that prove that the use of only one serovar in live attenuated vaccines can protect birds against salmonellosis produced by groups B and D. This is due to the significant level of cross protection among them (3).

This live SE vaccine (Avipro Salmonella VacE) induces early protection against SE and also against *S*. Gallinarum (same somatic antigens, 1, 9, and 12) when applied at day of age via drinking water. It does this by colonizing intestinal cell, blocking receptors from field *Salmonellas* and eliciting strong CMI. Revaccination is recommended at seven and 17 weeks for continued protection during growout and during the laying period.

SITUATIONS WHEN THE LIVE SE VACCINE IS USED

1. After a break of FT or SE has been diagnosed, or suspected in a farm, the following flocks are vaccinated as a prevention, applying the first vaccine at day of age, seven, and 17 weeks. Chicks that are already infected vertically or at the hatchery will die for several weeks. Chicks that were not infected originally or during the first days of age will develop protection.

2. When a breeder flock has been diagnosed or suspected of suffering from SE or FT and the breeders are kept in production. All progeny flocks are vaccinated.

3. When the source of day old chicks is unreliable.

4. **Although, off label**, some growers have used the vaccine successfully at the beginning of an outbreak.

5. Where in existence, to strengthen SE control programs.

VACCINATION SCHEDULE

Replacement, commercial layer and breeder pullets: day of age, seven weeks, (via drinking water) 17 weeks (this last one can be applied via water or injected); however, if applied by injection, there will be circulatory antibody formation and the birds will be FPA positive.

Birds in production: In high challenge areas: Every 12 weeks, starting at 28 weeks.

Broilers: day of age.

CAUSES OF VACCINE FAILURE

1. Disinfectants and high PH in drinking water used for vaccination.
2. Antibiotic therapy, especially in the feed or water.
3. Birds that have been previously infected.
4. Misdiagnosis.
5. Bad water vaccination techniques.

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SALMONELLA AS A PREDISPOSING FACTOR TO *EIMERIA*-INDUCED NECROTIC ENTERITIS: EVALUATION OF SELECTED PROBIOTICS INTERVENTIONS

S. Shivaramaiah[A], R. E. Wolfenden[A], J. R. Barta[B], S. L. Layton[A], B. M. Hargis[A], and G. Téllez[A]

[A]Department of Poultry Science, University of Arkansas, Fayetteville, AR, USA
[B]Department of Pathobiology, University of Guelph, Guelph, Ontario, Canada

SUMMARY

Necrotic enteritis (NE) caused by *Clostridium perfringens* (*C. perf*) has increasing importance in commercial poultry. An *Eimeria* infection is regarded as an obligatory precursor to development of NE, likely due to disruption of intestinal integrity. Preliminary evidence from our laboratory suggests that a significant *Salmonella* infection, followed by a relatively high *Eimeria* challenge, accentuates the predisposition of broilers to NE as compared to an *Eimeria* infection alone. Recently, we have used this model to reproduce NE in chicks challenged with both *Salmonella* and *Eimeria*. Additionally, we have been able to suppress clinical NE by probiotic treatment in chicks challenged with *Salmonella* and *Eimeria* with selected probiotics, probiotic candidates, and antibiotics. Relative impact of specific probiotics and probiotic candidates will be discussed.

Necrotic enteritis (NE) is an intestinal disease caused by *C. perf*, a ubiquitous Gram positive bacteria and is characterized by poor performance and acute mortality. The disease has major implications because of the severe economic losses it can cause in poultry production (3,8). It is now well known that coccidiosis is an absolute prerequisite for a NE outbreak because loss of intestinal integrity as a result of coccidiosis facilitates the colonization of *C. perf* and the apparent manifestation of NE. Several studies have successfully developed reproducible models of NE by dietary modifications and immunosuppression, with coccidia being the common predisposing factor (1,3,6,7,8). Current methods of treatment include the extensive use of antimicrobials due to the absence of a suitable vaccine. Preliminary data from our laboratory indicate that a significant *Salmonella* Typhimurium (ST) infection followed by an *Eimeria maxima* (EM) challenge leads to a quicker onset of NE. The following set of experiments evaluates a reproducible model for NE in controlled challenge studies and additionally evaluate selected commercial *Lactobacillus* and potential *Bacillus* based probiotics for their ability to ameliorate NE caused by our challenge model.

MATERIALS AND METHODS

Day-old broiler chicks were randomly divided into the following treatments: Neg Control, EM + *C. perf*, ST + EM + *C. perf*, and one of three different probiotic treatments. ST challenge was performed at day-of-hatch (10^6-10^8 cfu/chick) followed by an *Eimeria maxima* infection at three weeks of age. *C. perf* challenge with a combination of two different field isolates (10^8cfu/mL in drinking water for two days) was performed 4d post *Eimeria* infection, at a time when *Eimeria* lesions are maximal. For probiotic treatment, a *Lactobacillus* based commercial probiotic (FM-B11[TM]) was administered in drinking water (10^6 cfu/mL) for 3d (D14, 15, and 16) and the potential *Bacillus* spore probiotic candidates isolated from poultry sources were mixed in the feed at a

concentration of 10^6 spores/g. At 28d of age, the experiment was terminated and the following parameters were evaluated: mortality, lesion scores, quantification of *C. perf* in the ileum, and body weight.

RESULTS AND DISCUSSION

Salmonella infected birds had significantly higher mortality ($P{\leq}0.05$) and it was observed that the onset of mortality due to NE was quicker in those chicks as compared to non-*Salmonella* infected chicks. Body weight gains were significantly lower ($P{\leq}0.05$) in *Salmonella* infected controls as compared to the probiotic treated chicks. In addition, total ileal *C. perf* was quantified by RT-PCR and it was found that the probiotic fed chicks had significantly lower ($P{\leq}0.05$) *C. perf* colony forming units (CFUs) as compared to the non-treated chicks. In summation, our preliminary studies indicate that a *Salmonella* infection at an early age followed by an *Eimeria* challenge accentuates clinical signs of NE and results in acute mortality. A *Salmonella* infection in young birds is capable of producing intestinal lesions, which may be facilitating in the early pathogenesis of *C. perf* and an *Eimeria* challenge is the perfect window for clinical NE to occur. Additionally, it is well known that an *Eimeria* infection co relates with a corresponding increase in *Salmonella* numbers in the gut (2,4,5). The effect of probiotics in ameliorating NE has been promising in these studies. A further analysis of mechanisms of action and identification of new *Bacillus* isolates will be part of future studies and also include investigations on cytokine responses.

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EFFECT OF SELECTED ORGANIC ACIDS AND PROBIOTICS ON *SALMONELLA* ENTERITIDIS (SE) INFECTION IN BROILER CHICKS

A. D. Wolfenden, G. Tellez, and B. M. Hargis

SUMMARY

The effect of an organic acid mixture (OA) and a lactic acid bacterial-based probiotic culture on *Salmonella* Enteritidis (SE) infection in broiler chicks was evaluated. In experiments 1 and 2, chicks were SE challenged, held in chick boxes for 2 h, and randomly assigned to either untreated control, probiotic, OA, or probiotic +OA. After 24 or 48 h, crop and cecal tonsils were cultured for the recovery of SE. After 24 h, probiotic or probiotic +OA significantly reduced SE recovery from the crop as compared to controls. All

treatments reduced SE recovery from the cecal tonsils at 24 h. At 48 h, SE recovery from probiotic and or probiotic +OA groups was significantly lower than the controls in the cecal tonsils. These data suggest that a combination treatment with the selected OA and *Lactobacillus*-based probiotic culture is more effective than individual treatment for *Salmonella* reduction in chicks.

INTRODUCTION

Increased pressure by consumers and regulatory agencies for reducing or even eliminating the use of antibiotics in food producing animals has created a need to find alternatives to maintain healthy and productive animals. The use of certain lactic acid bacteria as probiotics has been proposed for many years. These probiotic bacteria have been shown to prevent enteric disease, as well as, improve the overall health of poultry (3). Another alternative to antibiotics is the use of certain organic acids. Direct acidification of the water with organic acids could significantly reduce the amount of recoverable *Salmonella* on the carcasses or in the crops and cecal tonsils when used during the pre-slaughter feed withdrawal period (1,2).

In the present study a commercially available *Lactobacillus*-based probiotic culture and water acidifier were used in combination to reduce *Salmonella* Enteritidis in the crop and cecal tonsils of broiler chicks.

In experiment 1, chicks were challenged by oral gavage with SE, held in chick boxes for 2 h, and randomly assigned to untreated control or continuous OA treatment in the drinking water. Crop and cecal tonsils were cultured at 48 h and 5 d post-challenge for recovery of SE. In experiments 2 and 3, chicks were challenged with SE, held in chick boxes for 2 h, and randomly assigned to untreated control, probiotic, OA, or probiotic +OA. After 24 or 48 h, crop and cecal tonsils were cultured for the presence or absence of SE. These data suggest that combination treatment with the selected OA and *Lactobacillus*-based probiotic culture is more effective than individual treatment for *Salmonella* reduction in chicks.

In experiment 1, treatment with OA in the drinking water reduced (*P*<0.05) SE recovery from the cecal tonsils when compared with controls (OA treated = 55% vs. controls =100%). Also, treatment with OA lowered (*P*<0.05) SE recovery from the crop (75%) as compared to controls (100%).

In experiment 2, treatment with probiotic or probiotic +OA significantly reduced (*P*<0.05) SE recovery from the crop at 24h as compared to controls (75%, 40%, and 100%, respectively). All treatments reduced (*P*<0.05) SE recovery from the cecal tonsils at 24h (control: 60%, probiotic: 25%, OA: 45%, probiotic +OA: 13%). While no significant differences were observed in SE recovery from crop at 48 h, SE recovery from probiotic and probiotic +OA groups was significantly lower than the controls in the cecal tonsils, by 20%, 20%, and 100%, respectively.

In experiment 3, probiotic or probiotic +OA caused reduced cecal tonsil SE recovery as compared to controls at 24 h (5%, 15%, and 75%, respectively), and at 48 h (40%, 21%, and 100%, respectively) and probiotic +OA treatment again reduced SE recovery incidence in crops at 48 h as compared to controls, 42% *vs.* 100%, respectively (table 2). When SE recovery was evaluated after 24 h, >2.5 $\log_{10}$ reductions of SE recovery were observed from crop of probiotic or probiotic +OA treated chicks and >1 $\log_{10}$ reductions of SE recovery were observed from crop of chicks treated with only OA When SE recovery was evaluated only from samples that were crop positive following enrichment after 48 h, >1.5 $\log_{10}$ reductions of SE recovery were observed from crop of probiotic +OA treated chicks.

These data suggest that combination treatment with the selected OA and *Lactobacillus*-based probiotic culture is more effective than individual treatment for *Salmonella* reduction in chicks.

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LITERATURE REVIEW OF ANTIMICROBIAL DRUG USE IN CANADIAN BROILERS FOR THE THERAPY OF *ESCHERICHIA COLI, CLOSTRIDIUM PERFRINGENS, STAPHYLOCOCCUS AUREUS,* AND MISCELLANEOUS DISEASES

Agnes Agunos, Sheryl Gow, David Léger, and Richard-Reid Smith

Laboratory for Foodborne Zoonoses, Public Health Agency of Canada

SUMMARY

Over the last decade, broiler production has met the genetic potential of the prevalent poultry strain and hence improved overall farm production efficiencies; however, bacterial diseases remain a threat to the Canadian poultry industry. Antimicrobial use (AMU) is important in the management of clinically relevant bacterial infections and in promoting animal health and welfare but emerging antimicrobial resistance (AMR) has limited therapeutic options and is substantial animal and human health concern. The objectives of this literature review were: 1) to develop a listing of antimicrobial drugs investigated by various authors through *in vitro*, *in vivo*, and pharmacokinetic studies for use in broilers; 2) to compare this listing of antimicrobial drugs with the current Canadian drug listings; and 3) to review relevant guidelines and regulations on AMU in broilers in Canada. Antimicrobial use for the pathogens *Escherichia coli, Clostridium perfringens,* and *Staphylococcus aureus* will be described because of their public health, economic, and animal welfare importance. In addition, AMU for paratyphoid *Salmonella* (PT), though rarely implicated in clinical infections in broilers, will be discussed due to its public health significance and potential for AMR dissemination.

MATERIALS AND METHODS

Literature searches of *in vivo* (e.g., animal/clinical trials), *in vitro* (e.g., based on antimicrobial sensitivity testing methods), and pharmacokinetic studies in broilers were conducted from March to May, 2009 using electronic databases (e.g., Pubmed, USDA National Agricultural Library digital repository) and poultry-specific on-line journals, such as *Avian Diseases* and *Poultry Science* Journal. In addition, general poultry listings for antimicrobials (1,2) and proceedings of poultry/avian diseases meetings/ conferences (e.g., American Association of Avian Pathologists, Western Poultry Disease Conference) were searched for supplementary information. The Veterinary Drug Directorate (VDD) drug listing was consulted for the drug classifications used in the construction of the summary antimicrobial drug listing. For review of available guidelines, the Canadian Association of Veterinary Medicine Prudent Use Guidelines (CVMA-pg) 2008 and the 2009 Compendium of Veterinary Products and Compendium of Medicating Ingredient brochure online were reviewed to determine applicable guidelines and policies regarding veterinary drug use in Canada.

RESULTS AND DISCUSSION

The listing of antimicrobials used or investigated for use in broilers is presented in Table 1. The literature review yielded the following general results:

- Some antimicrobial drugs used in the therapy (e.g., prophylactic and therapeutic use) of the clinically-relevant broiler pathogens are (or belong to classes that are) essential for serious life-threatening infections in humans. The Veterinary Drug Directorate (VDD) ensures safety, quality, and effectiveness and sets standards for veterinary drug use (http:// www.hc-sc.gc.ca/dhp-mps/vet/index-eng.php) to reduce both animal and public health risks.

- Available veterinary drugs in poultry medicine are largely broad-spectrum in nature, targeting most of the pathogens that commonly occur in Canadian broiler flocks. Given that non-antimicrobial alternatives are currently limited, targeting mainly enteric infections with lower efficacy for systemic diseases (e.g., probiotics, prebiotics, organic acids), it is important to preserve the remaining effective antimicrobials.

- A valid veterinary-patient-client relationship (VPCR), prescription only medication, extra-label drug use considerations (ELDU), compliance with the CMIB (3) (e.g., in-feed use), and consultations with the Canadian Global Food Animal Residue Avoidance Database (CgFARAD) were cited by various authors as components of prudent anti-microbial drug use practices intended to reduce public health hazards.

This review will provide a summary of the current knowledge regarding broiler AMU in Canada and guidance in the development of a proposed national broiler farm AMU/AMR surveillance framework.

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(Please send email to agnes_agunos@phac-aspc.gc.ca, for complete reference list. The full article will be published in the World's Poultry Science Journal.)

Table 1. Summary of antimicrobials used for the therapy of common broiler pathogens.

VDD Category	*E. coli*	*C. perfringens*	*S. aureus*	**Paratyphoid (*PT*)** *Salmonella* [b]
I	Ceftiofur [a] *Enrofloxacin* *Flumequine*			Ceftiofur [a] *Enrofloxacin*
II	Amoxicillin *Ampicillin* Apramycin[a] Gentamicin[a] Lincomycin-Spectinomycin[a] Neomycin Ormethoprim-sulfadimethoxine [a] Spectinomycin Tetracycline-neomycin Trimethoprim-sulfadiazine[a]	Amoxicillin *Ampicillin* Lincomycin Neomycin Penicillin Spectinomycin Streptomycin Trimethoprim-sulfadiazine[a] Tylosin Virginiamycin	Erythromycin Ormethoprim-sulfadimethoxine[a] Penicillin Spectinomycin Streptomycin Tylosin	Amoxicillin Gentamicin[a] Lincomycin-Spectinomycin[a] Virginiamycin Trimethoprim-sulfadiazine
III	Chlortetracycline *Doxycycline* Florfenicol *Fosfomycin* Oxytetracycline Sulfamethazine[a] Sulfaquinoxaline[a] Tetracyclines	Bacitracin[a] Chlortetracycline Oxytetracycline Tetracycline	Tetracycline	Bacitracin Trimethoprim
IV		Narasin *Other coccidiostats that have anti-clostridial activity:* Lasalocid, Maduramicin, Monensin Salinomycin		
Unknown		*Novobiocin*		

[a]Extra-label drug use (ELDU) applies. Italicized drugs are not available, have never been used, or have been withdrawn for poultry use in Canada.

[b]Treatment for PT *Salmonella* is not routine in broilers.

USING ELISA TITERS TO COMPARE THE EFFICACY OF AVIAN ENCEPHALOMYELITIS (AE) VACCINATION

Randall T. Bishop

Cornwallis Veterinarians Ltd, 273 Belcher Street, Kentville, Nova Scotia, Canada

Three commercially available avian encephalomyelitis (AE) vaccines were given via drinking water and course spray to 10 week old single comb commercial leghorn pullets. Idexx ELISA titers to AE were compared at 15 and 18 weeks of age.

POOR HATCHABILITY AND INCREASED CULL CHICKS ASSOCIATED WITH WHITE CHICK SYNDROME AS EXPERIENCED IN EASTERN CANADA IN 2009

Randall T. Bishop

Cornwallis Veterinarians Ltd, 273 Belcher Street, Kentville, Nova Scotia, Canada

Reduced hatchability and increase in number of cull checks with white down and green livers were experienced in four breeder flocks in Nova Scotia in 2009. This paper describes test results in trying to determine a cause for this syndrome.

SUSPECT YEW TOXICITY IN BACKYARD TURKEYS

Marina L. Brash and Alma Mujakovic

Animal Health Laboratory, University of Guelph, Guelph, Ontario, N1H 6R8, Canada

Sudden death was reported in a fourteen week old turkey that was part of a small group of turkeys and dual purpose chickens being raised together on a small rural property. One other turkey was noted to be depressed and passing loose yellow droppings. Blackhead (histomoniasis) was suspected as the cause of death because of the history of being raised with chickens and the passing of yellow droppings by the clinically sick turkey.

At necropsy, the turkey was in good body condition. The liver was firm and the spleen was enlarged. The crop contained a large amount of grain and some plant material including pieces of grass and evergreen twigs with short stems and slender flat green leaves resembling yew (*Taxus* spp.) clippings.

Bacterial culture of liver and spleen produced no bacterial growth and histological findings were nonspecific. Virus isolation was conducted on lung/trachea and spleen with negative results.

The diagnosis of suspect yew toxicity was based on the history of sudden death, lack of other identifiable causes of death and the presence of the plant in crop content.

Yew toxicity in birds is seldom reported and birds are generally considered to be less sensitive to the toxicity of the taxines. With the increasing interest in backyard urban and rural production of small groups of poultry, the opportunity to come in contact with yew is increased so inadvertent exposure to yew should be added to the list of causes of sudden death.

PRELIMINARY INVESTIGATIONS ON *ENTEROCOCCUS CECORUM* IN CANADIAN CHICKENS

Marina Brash[B], Patrick Boerlin[A], Vivian Nicholson[A], Durda Slavic[B], and Babak Sanei[C]

[A]Dept of Pathobiology, Ontario Veterinary College, Guelph, Ontario, N1G 2W1, Canada
[B]Animal Health Laboratory, University of Guelph, Guelph, Ontario, N1H 6R8, Canada
[C]Ontario Ministry of Agriculture, Food and Rural Affairs, Dept of Pathobiology, Guelph, Ontario, N1G 2W1, Canada

Enterococcus cecorum was first described in 1983 as a *Streptococcus* species. Relatively little is known about this organism and the medical and veterinary literature is very sparse on its ecology and significance in animals. *E. cecorum* is part of the normal intestinal flora of chickens, but it can also be found in a variety of other animal species. The prevalence of *E. cecorum* in the intestinal flora of chickens seems to increase with age, to become a dominant part of the enterococcal and streptococcal flora from egg layers and from breeder birds aged 12 weeks and older. *E. cecorum* has been described on several occasions in the literature as a cause of bone and joint lesions in broilers, including in the form of outbreaks. An increase in isolation of *E. cecorum* has been reported in the past in Europe, possibly because the organism might have remained an unrecognized pathogen for a long time as a consequence of its peculiar characteristics. *E. cecorum* infections have also been increasingly reported in North American chickens and it has become the cause of concern in the poultry industry. In Ontario, infections with *E. cecorum* were diagnosed for the first time in 2008 and cases have now continued to be identified on broiler and broiler breeder farms across the province. It is not yet clear what has caused the increase in diagnostic cases. It may be the result of improvements in diagnostic procedures and increased awareness. However, it is also possible that this increase corresponds to a real increase of the disease frequency in the field. A new virulent *E. cecorum* strain may have penetrated the North American poultry population, thus causing a spike in disease frequency and severity. Alternatively, changes in management may also have led to either increased exposure and challenge of birds by the organism, or to a higher susceptibility of the birds.

In order to be able to better assess the situation of *E. cecorum* in Canada and Ontario in particular, the objectives of the study were: 1) to collect *E. cecorum* isolates from chickens in Canada; 2) to assess identification criteria; and 3) to develop a macrorestriction typing method (PFGE) for *E. cecorum.*

E. cecorum isolates from clinical infections were recovered in pure culture using blood agar and/or Columbia agar with colistin and nalidixic acid (CNA). Isolates from ceca of healthy birds were isolated using CNA agar. Isolates from α-hemolytic colonies were further identified using biochemical tests (bile-esculin, PYR, 6.5% NaCl in BHI, growth at 45ºC in BHI, mannitol, sorbose, arginine, sorbitol, and raffinose) and using API20S galleries. 16S rRNA sequencing was further performed on selected isolates to confirm their identification. Based on protocols used for other enterococci and related species, PFGE was tested for readability of restriction profiles (i.e. number and size distribution of fragments) and discrimination for a small subset of strains using the endonucleases *Apa*I, *Asc*I, *Sbf*I, *Sma*I, and *Xma*I.

The most reliable criteria for the successful presumptive identification of *E. cecorum* were α-hemolysis, positive bile-esculin test and negative PYR test. Growth in 6.5% NaCl and at 45ºC were generally less reliable and needed long incubation times to be evaluated correctly. The sugar fermentation test results were not consistent across strains. API20S gallery profiles were variable but as described in the literature. Although the isolates tested appeared relatively similar, the highest discrimination with adequate number and size distribution of bands on PFGE gels was obtained with the restriction endonucleases *Sma*I and *Sbf*I. Preliminary results suggest that *E. cecorum* isolates from clinical cases are less diverse than cecal isolates, thus supporting the hypothesis that most infections are caused by a subset of *E. cecorum* strains.

In conclusion, our results provide a simple profile for the rapid isolation and identification of *E. cecorum* from clinical samples and from mixed contaminated samples such as intestinal content. A molecular typing method has been developed, which can be used to assess the diversity of *E. cecorum* isolates from different sources. A collection of isolates from *E. cecorum* isolates from a variety of clinical infections and from the normal intestinal flora of healthy chickens has been set up to investigate the epidemiology of this organism and associated disease in Ontario and Canada.

EFFECTIVENESS OF TYLVALOSIN (AIVLOSIN®) IN AN EXPERIMENTAL DUAL CHALLENGE WITH *ORNITHOBACTERIUM RHINOTRACHEALE* AND A *MYCOPLASMA SYNOVIAE*-TYLOSIN RESISTANT STRAIN IN BROILER CHICKENS

R. Cerdá[A,B], J. Origlia[A,] J. Uriarte[A], D. Gornatti[A], E. Spinsanti[A], and A. Mockett[B]

[A]National University of La Plata, CC296, 1900, La Plata, Argentina
[B]ECO Animal Health, London, England

INTRODUCTION

Ornithobacterium rhinotracheale (ORT) and *Mycoplasma synoviae* (MS) have been recognized as primary respiratory pathogens in broiler chickens (1,4,5,6). Both bacterial species have a worldwide distribution and are associated with respiratory disease and high economic losses (4,5). In a recent study (1) their pathogenic synergism has been demonstrated using a dual infection study in broiler chickens. Different antibiotics are used to prevent or reduce the lesions produced by these organisms. Tylvalosin, the active ingredient in Aivlosin®, (ECO Animal Health, UK), has shown good *in vitro* activity against both bacterial species (2,3). The aim of the present study was to evaluate the *in vivo* efficacy of Tylvalosin for the prevention of respiratory lesions in broiler chickens inoculated with either ORT, MS, or MS and ORT. The MS strain was resistant to tylosin.

MATERIALS AND METHODS

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

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

***Mycoplasma synoviae* inoculum.** The MS strain used was a recent field isolate from an outbreak of infectious synovitis in a laying hen farm in Buenos Aires province, Argentina. The strain was selected after a MIC (minimum inhibitory concentration) study showed it to be resistant to tylosin ($\geq$ 12 µg/mL) and sensitive to tylvalosin (0.75 µg/mL). The culture was prepared using Frey (Gibco) broth supplemented with 12% pig serum and contained 10^8 color changing units/mL (CCU/mL) of *Mycoplasma synoviae*.

Experimental design. Seventy one-day old chicks were randomly allocated to seven experimental groups such that each group contained 10 chicks. At two weeks of age the birds were shown to be free of maternally- derived antibodies to ORT and MS using a commercial enzyme-linked immunosorbent assay (ELISA) (IDEXX) and a rapid serum agglutination test (RSA) (Intervet Schering Plough, The Netherlands), respectively. At this time all the chickens received a ND-IB (B1-Massachusetts) vaccination, via eye drop. At three weeks of age, one group of chickens was allocated as a control group. An ORT culture (0.3 mL/bird) was inoculated intratracheally into two groups of chickens. The MS culture was given by aerosol (created using a nebulizer in a closed chamber for three min) to two groups of chickens. The ORT culture and MS aerosol was given in combination to two groups of chickens. One of each of the challenged groups remained unmedicated while the other group was medicated using 25 mg tylvalosin/kg BW for five days via the drinking water.

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

RESULTS

The lesion score results are shown in the histogram. Total reduction in air sac lesions was observed in the ORT/MS medicated group compared to the ORT/MS unmedicated group. A reduction of about

50% in air sac lesions was observed in the medicated ORT group and the medicated MS group, compared to their respective unmedicated groups. Complete prevention of lung lesions was observed in the ORT/MS and MS medicated groups compared with their respective unmedicated groups and about 86% reduction in lung lesions was observed between the ORT unmedictated and medicated chickens.

DISCUSION

An unexpected lack of synergism between MS and ORT with regards to respiratory lesion scores was observed in the present study -in contrast to a previous one (1). The only variable was the use of the B1 Newcastle vaccine instead of the LaSota. Another interesting finding, also difficult to explain, was the different effectiveness of the treatment. Tylvalosin showed good efficacy against both the tylosin- resistant strain of MS and the ORT, when given singly. However, greatest efficacy was observed with the dual challenge. Further studies will be carried out in order to confirm or clarified the results obtained in the present work.

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EVALUATION OF THE EFFICACY OF AIVLOSIN® 625MG/G GRANULES FOR USE IN DRINKING WATER FOR CHICKENS FOR THE CONTROL OF DISEASE ASSOCIATED WITH *CLOSTRIDIUM PERFRINGENS* UNDER COMMERCIAL BROILER REARING CONDITIONS

J. McKelvie[A], A. Walker[B], R. Cerdá[C,D], and A. Mockett[C]

[A]Evita Services, Gilford, Northern Ireland
[B]Slate Hall Veterinary Practice Limited, Unit 7, Cambbs, England
[C]ECO Animal Health, London, England
[D]Faculty of Veterinary Medicine, National University of La Plata, La Plata, Argentina

INTRODUCTION

Clostridium perfringens infection (clostridiosis) in growing chickens may be associated with diarrhea, mortality, and production losses (1). At processing, chickens which have been infected with *C. perfringens* can be rejected due to hepatitis. A study was conducted to determine the effectiveness of tylvalosin in water, compared to Lincospectin™ in chickens naturally infected with *Clostridium perfringens* based on clinical, pathological, and performance variables.

MATERIALS AND METHODS

This study was a partially masked, randomized block design. One-day-old healthy commercial

chickens (unsexed Ross 308) were randomly assigned to one of two treatment groups at placement; TVN-TVN, two houses, 19,680 birds (Aivlosin® 625mg/g granules, ECO Animal Health, 25mg/kg bodyweight (BW) daily for the first three days of life followed by 25mg/kg BW daily for three days starting on day 11, two days prior to anticipated clinical signs) or LIN, two houses, 19,920 birds (150mg antibiotic activity lincomycin (33.3%)/spectinomycin (66.7%)/kg bodyweight for the first three consecutive days of life, Pfizer Animal Health). Treatments were administered in water. Chickens were fed *ad libitum* on the same crumb or pellet feed. The chicks received the normal program of vaccinations, supplements and anti-coccidials used on the farm.

Mortalities were recorded daily. Body weight (100 chickens/house), necropsy results (10 chickens/house), water content of fecal material, enteric lesions, bacteriology) and litter quality were recorded weekly. The total feed consumption, the number of rejects/part-rejects, variation in body weights, and incidence of hepatitis was recorded at processing (day 35). Effectiveness was assessed via clostridial disease variables (mortality due to clostrial disease, litter quality, clostridial lesions in the enteric tract, isolated clostridial numbers, enteritis, water content of fecal material at necropsy) and production variables (weekly live weight gain, feed intake, feed conversion ratio, percentage of rejects at slaughter, variation in mean bird weight immediately before killing). Safety was assessed via mortality.

Chicken enteric tract mean lesion score, mean weight of sampled chickens, and cumulative mortality was analyzed with a normal based linear model. A significance level of $P{\leq}0.05$ was used to determine statistical differences for all analyses. Isolated clostridial species, processing rejects, variation in body weight at processing, and feed consumption and conversion were summarized descriptively.

RESULTS

There was little evidence of clostridial disease. There were no mortalities considered due to clostridiosis and litter quality and water content of fecal material was similar for both groups. No *C. perfringens* were isolated, although there were fewer clostridial species isolated from the TVN-TVN group (8) than the LIN group (11). However, the tylvalosin group had significantly lower mean enteric lesion scores (21 days of age; P=0.0147), and had less processing rejects (0.71% vs 0.94%).

Both groups had a similar feed consumption (0.087 vs. 0.086 kg feed/chicken/day, TVN-TVN vs. LIN). However feed conversion appeared more efficient for the tylvalosin group (1.574 vs. 1.614 kg feed/kg weight gain, TVN-TVN vs LIN). At processing, the mean body weight was not significantly different (P=0.8047), but body weights showed greater uniformity in the group treated with tylvalosin (% group ± 200g of median weight range, TVN-TVN vs LIN: 72% vs. 69%).

CONCLUSION

In conclusion, tylvalosin at a dosage of 25 mg TVN/kg body weight for the first three days of a chick's life followed by 25 mg/kg body weight for three consecutive days starting at 11 days of age is safe and effective at reducing enteric clostridial lesions in three week old chickens. This may have contributed to the improved feed conversion, overall weight gain, less rejects, and more consistent processing weights observed in tylvalosin treated birds.

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IMMUNOGENICITY OF AN OIL EMULSION VACCINE CONTAINING A VIRAL STRAIN OF NEWCASTLE DISEASE VIRUS MODIFIED BY MOLECULAR BIOLOGY AGAINST A HOMOLOGOUS CHALLENGE WITH THE UNMODIFIED STRAIN

Donají García López, Efigenia Merino Cortés, Raúl F. Montalvo, J. Andrés Morales Garzón, and Eduardo Lucio Decanini

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

SUMMARY

An immunogenicity test was performed with a Newcastle recombinant strain (P05) in eight-week-old light birds. The test was performed with two different formulations (Groups A and B) containing the antigen and vaccinating birds with one dose of 0.5 mL under isolation conditions in Horsfal Bauer units. The birds were bled each week (7, 14, 21, and 28 days post vaccination) for the determination of antibodies by HI for La Sota, P05, and recombinant P05 antigens. The unmodified P05 Newcastle virus was used as a challenge strain at a concentration of 200,000 EID per each 0.2 mL four weeks post vaccination.

Also, the response of the P05 strain was compared with a group of birds vaccinated with a La Sota strain vaccine against Newcastle (Group C). The parameters to be evaluated were response to HI after four weeks post vaccination, protection against challenge, and quantity of viral particles in tracheal and cloacal swabs at three and seven days post challenge.

The results of HI show that the birds vaccinated with recombinant P05 have a greater response with the homologous recombinant antigen and a low response to the La Sota strain NC antigen. There were no differences regarding protection to challenge since the three evaluated groups were satisfactory, all control group death between three to five days post challenge.

Regarding viral recovery in tracheal swabs three days post challenge, there was a difference of more than three logarithms less in the three vaccinated groups being group B (vaccinated with P05) the one that had a lower recovery of the virus. Similarly, Group B vaccinated with an oily emulsion in water was the one that showed less viral excretion in cloacal swabs after three days post challenge.

The conclusion of this study is that the recombinant P05 strain is immunogenic, it provides 100% protection against a Newcastle pathogenic strain, and it decreases viral excretion in vaccinated birds.

RESULTS

Titers of the HI test with the recombinant P05 antigen show geometric means of 197 and 160 for Groups A and B, 61 to La Sota group, and 10 to control group at four weeks after vaccination. There are differences regarding viral excretion, especially in samples of cloacal swabs, principally with the group vaccinated with formula B where there was a difference of more than two logarithms with respect to the group vaccinated with La Sota, and of more than three with respect to the positive control at three days after challenge. In the same way, Groups A and B had a difference of more than two logarithms with respect to the group vaccinated with La Sota in samples of cloacal swabs at seven days after challenge.

DISCUSSION

These results coincide with the ones obtained by Miller and cols (1) where it is mentioned that vaccines elaborated with strains homologous to the challenge virus reduce significantly viral excretion than heterologous vaccines do and therefore reduce transmission of the virus in birds.

CONCLUSIONS

The experimental emulsified vaccines elaborated with a recombinant strain Newcastle virus provide protection and reduction of the viral excretion to challenge with a homologous virus.

The immunogenicity test for the vaccines formulated with the P05 strain Newcastle virus was satisfactory.

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Table 1. Individual bird titers of viral particles of NC P05 strain detected by rt PCR test seven days after challenge (dpc) in cloacal swabs.

Bird No	Group 70/30	Group 60/40	Group LS	Control
1	Negative	3.88×10^3	8.9×10^5	ND
2	1.61×10^5	4.18×10^3	5.86×10^5	ND
3	2.04×10^6	3.24×10^4	4.6×10^6	ND
4	5.48×10^5	2.36×10^4	1.854×10^6	ND
5	Negative	5.66×10^4	1.304×10^6	ND
6	6.92×10^5	8.04×10^4	5.60×10^5	ND
7	2.94×10^6	1.2×10^4	1.056×10^6	ND
8	9.04×10^5	2.68×10^4	6.06×10^6	ND
9	1.258×10^3	6.16×10^4	1.124×10^6	ND
10	8.24×10^3	1.35×10^4	1.646×10^6	ND
GM	$10^{4.2}$	$10^{4.3}$	$10^{6.16}$	-

ND = Not determined because of nonsurvival between three to five days after challenge.

VACCINATION OF SPF CHICKENS WITH A RECOMBINANT HVT EXPRESSING THE HA FROM H5N1 HIGHLY PATHOGENIC AVIAN INFLUENZA PROTECTS AGAINST LETHAL CHALLENGE

Darrell R. Kapczynski[A], Moto Esaki[B], Mark W. Jackwood[C], and Kristi M. Dorsey[B]

[A]Southeast Poultry Research Laboratory, United States Department of Agriculture, Athens, Georgia, USA
[B]CEVA Biomune, Lenexa, Kansas, USA
[C]Department of Population Health, University of Georgia, Athens, Georgia, USA

Vaccination is an important tool in the protection of poultry against avian influenza (AI). For field use, the overwhelming majority of AI vaccines produced are inactivated whole virus formulated into an oil emulsion and to a lesser degree recombinant vectored vaccines (e.g., virus expressing AI genes). In these studies, protection against lethal challenge with a H5N1 highly pathogenic AI (HPAI) isolate was determined in SPF chickens vaccinated with either cell-free or cell-associated forms of a recombinant herpes virus of turkeys (rHVT) expressing the hemagglutinin (HA) gene from a recent H5N1 HPAI virus. All vaccinated birds were protected from clinical signs of disease and mortality. In addition, oral and cloacal swabs taken from challenged birds demonstrated that vaccinated birds had lower incidence and titers of viral shedding compared to sham-vaccinated birds. Taken together, these studies provide support for the use of rHVT vaccines expressing HA to protect poultry against HPAI challenge.

ASSESSMENT OF THE EFFICIENCY OF FOUR AIR BIO-SAMPLERS AFTER AEROSOLIZATION OF *ENTEROCOCCUS FAECALIS* SUSPENSIONS: A PRELIMINARY STUDY

Y. Zhao[A], A. J. A. Aarnink[A], P. W. G. G. Koerkamp[A,C], M. C. M. de Jong[B], P. Doornenbal[D], T. T. T. Huynh[A], and W. J. M. Landman[D,E]

[A]Livestock Science Department, Animal Sciences Group of Wageningen UR, Lelystad, the Netherlands
[B]Quantitative Veterinary Epidemiology Department, Animal Sciences Group of Wageningen UR, Wageningen, the Netherlands
[C]Farm Technology Group of Wageningen UR, Wageningen, the Netherlands
[D]Animal Health Service (GD), Deventer, the Netherlands
[E]Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands

SUMMARY

A major challenge for the biosecurity within and between livestock farms is the air transmission of pathogens, which plays a key role in the spread of some infectious diseases. In order to study the airborne transmission of diseases efficient air samplers are required. The objective of this study was to investigate the sampling efficiency of four bio-samplers (Andersen six-stage viable bio-sampler "AVB", All Glass Impinger "AGI-30", OMNI-3000, and Airport MD8 with a gelatine filter) using polydisperse aerosols of *Enterococcus faecalis* generated in a HEPA isolator. However, prior to studying their efficiency using aerosols of *E. faecalis* and other bacteria (*Escherichia coli*, *Campylobacter jejuni*, and *Mycoplasma synoviae*), the tracer used and the influence of air sample processing on the survival of the bacterial involved should be determined. Preliminary experiments were performed to test 1) the effect of uranine (as a physical tracer) on the survival of bacteria in their suspensions, 2) the recovery efficiency of bacteria and uranine by rinsing agar plates used in the Andersen bio-sampler, and 3) the recovery efficiency of bacteria and uranine from gelatine filters used in the Airport MD8. *E. faecalis* suspensions with uranine, *E. faecalis* suspensions without uranine, and uranine suspensions were subsequently aerosolized in duplicate and sampled with four bio-samplers. Results showed that the survival of *E. faecalis*, *E. coli* and *M. synoviae* was not significantly influenced by the addition of 0.02% uranine to the bacterial suspensions after an incubation period of two hours, while *C. jejuni* was affected by this tracer. The recovery efficiency by rinsing agar plates ranged between 79.8% to 123.6% for bacteria, and 33.1% to 60.1% for uranine. The recovery rate from gelatine filters after five min exposure to ambient air varied between 62.7% to 171.7% for bacteria and 114.2% to 123.2% for uranine. Results of the preliminary aerosolization tests showed that the Airport MD8 with a gelatine filter had the highest physical efficiency. The physical efficiency of the AGI-30 and the OMNI-3000 was about 69.8% and 49.4% relative to that of the Airport MD8. The biological efficiency of all air bio-samplers was not significantly different from 100%, indicating that significant losses of *E. faecalis* during sampling did not occur. Half-life time of airborne *E. faecalis* was on average 8.6 min at 21 to 23°C and 80 to 85% RH.

INTRODUCTION

Airborne transmission of pathogens from livestock poses infection risks to surrounding farms and people living in the vicinity (1). Measurements of pathogen concentrations and emission from livestock houses have been increasingly performed for hazard evaluation. In order to get precise assessments, bio-samplers with high sampling efficiency are required. However, the sampling efficiency of common air bio-samplers has not been well established so far.

The sampling efficiency of a bio-sampler is determined by both, its physical and biological efficiency. The physical efficiency is determined by how well particles are aspirated by the device's inlet and transported to the collection medium, and how well the bio-sampler retains these particles (2,3). Biological efficiency refers to the ability of a bio-sampler to maintain the microbial culturability and to prevent cell damage during sampling (2), e.g. loss of viability due to impaction (4), impingement (5,6,7), and dehydration (8).

Studies on sampling efficiency have been carried out by comparing the performance between bio-samplers in the same environment (9,10,11). However, in these studies the efficiency of bio-samplers was not compared to defined aerosol concentrations, therefore only the relative efficiency between bio-samplers was calculated. As a solution, Thompson and others (12) developed a system to investigate sampling efficiency

by aerosolizing bacterial suspensions of known concentrations in isolators. Sampling efficiency was separately determined by investigating physical efficiency, which was the difference of the particle numbers in the upstream and downstream air of a bio-sampler monitored by an aerosol spectrometer, and biological efficiency, which was the difference of ratio (viable bacteria counts towards total particle counts) in the aerosol suspensions and the air samples. However, the losses of micro-organisms in the aerosolization process and in the air sampling processes were not evaluated and excluded from calculation of efficiency.

Sampling efficiency can also be determined by aerosolizing microbial suspensions containing inert tracer compounds. The amount of inert tracer collected by bio-samplers indicates the physical efficiency and the differences of ratio of viable micro-organisms towards tracer in aerosol microbial suspensions and in air samples indicates the biological efficiency. It is important to ensure that the used tracer is harmless to micro-organisms both in suspension and in the aerosol state. Among the tracer compounds used for the detection of airborne virus, uranine (sodium fluorescein) remains popular because it was reported as virus-friendly in suspensions and was detectable at tiny concentrations (13,14,15).

The present study is part of a broader project aiming to investigate the sampling efficiency of four bio-samplers (Andersen Viable Bio-sampler "AVB", All Glass Impinger "AGI-30", OMNI-3000 high volume bio-sampler, and Airport MD8 with a dissolvable gelatine filter) on four species of animal-associated airborne bacteria (*Enterococcus faecalis*, *Escherichia coli*, *Campylobacter jejuni*, and *Mycoplasma synoviae*) by collecting bacterial aerosols containing uranine as the physical tracer in an isolator. Analysis of both bacteria and uranine requires that the air samples are in liquid form. The AGI-30 and OMNI-3000 already collect the bacteria directly into liquid media, however for the other two bio-samplers (the Andersen collects bacteria on agar plates and the Airport MD8 on gelatine filters) further procedures are needed to transfer the samples into liquid. Transfer of samples from the latter two devices is performed by rinsing the surfaces of bacteria-loaded agar plates with buffered peptone water (BPW) for the AVB, and by dissolving gelatine filters into 37°C BPW for Airport MD8. The recovery efficiency of bacteria and uranine of these devices has to be assessed.

The objectives of this preliminary study were to investigate 1) the effect of uranine (as a physical tracer) on the bacteria survival in their suspensions and in aerosol state, 2) the recovery efficiency of bacteria and uranine by rinsing agar plates used in the AVB, 3) the recovery efficiency of bacteria and uranine from gelatin filters used in the Airport MD8, and 4)

sampling efficiency of the bio-samplers to detect airborne *E. faecalis*.

MATERIAL AND METHODS

Bio-samplers. The AVB (16) bio-sampler consists of six stages in each of which a plate with agar is put under a screen with 400 holes. The number of holes is the same for each stage but the diameter of the holes becomes smaller in each stage following downwards direction. When taking samples at the airflow rate of 28.3 L min^{-1}, the air speed in AVB increases from the first stage to the sixth stage (the first stage with lowest air speed, and the sixth stage with highest air speed) due to the hole size. The particles are impacted according to their aerodynamic diameter (largest particles are retained on the plate of the first stage, and smallest particles on the plate of the sixth stage). In this experiment, the bacteria loaded agar plates were rinsed three times (see 2.5), and the rinsing-off fluid was used for bacteria counting following an international standard (17).

The AGI-30 (7540, Ace glass Inc., US) impinges airborne micro-organisms into a liquid collection medium. The airflow of AGI-30 is 12.5 L min^{-1}. After sampling, decimal dilutions are made from the collection liquid, which are pipetted onto agar plates for incubation.

The OMNI-3000 (Sceptor Industries Inc., US) operates at a high airflow rate of 300 L min^{-1}. The collection fluid consisting of 10 mL Phosphate Buffered Saline (PBS) is sucked from a cartridge into a contactor where PBS rotates and is exposed to incoming air. After sampling, PBS containing the collected airborne micro-organisms is drained back to the cartridge automatically. Bacterial counts are subsequently performed on a sample from the cartridge.

The Airport MD8 (Sartorius, Goettingen, Germany) collects micro-organisms on a gelatin filter (17528-80-ACD, Sartorius, Goettingen, Germany) by filtration. The loaded filter is dissolved in liquid medium, which is then used to make decimal dilutions and inoculate agar plates for bacteriological analysis.

Isolator. A stainless steel negative pressure HEPA isolator (Beyer and Eggelaar, Utrecht, the Netherlands) of 1.94 m in length, 0.75 m in width, and 0.95 m in height (1.38 m^3) was used as aerosolization space. Ventilation and temperature were controlled electronically. The ventilation was turned off during the experiment. The air inlet was left open for air access to compensate the negative pressure due to sampling. A temperature and humidity sensor (HygroClip2, ACIN Instrumenten BV, Rijswijk, the Netherlands) installed in the middle of the isolator was

used to monitor the climatic conditions during aerosolization.

Aerosolization, sampling and sample processing. A Walther Pilot I spray head with a nozzle of 0.5 mm diameter (Walther Spritz- und Lackiersysteme, Wuppertal, Germany) was used for the aerosolization of bacterial suspensions. The aerosol spectrum of the spray-head was characterized previously by laser diffraction (Mastersizer-S long bed; Malvern Instruments, Malvern, UK) and the volume median diameter D(v;0.50) was about 10 µm (Figure 1). The Walther Pilot I spray-head was connected to an air compressor (Mecha Concorde type 7SAX, 1001, SACIM, Verona, Italy), which was set at an air pressure of two bar.

Twenty mL of bacterial suspension with 0.02% uranine (4000 µg, or 2899 µg m^{-3} assuming no wall loss) was aerosolized in about 1.2 min (16.7 mL min^{-1}) in the isolator. All bio-samplers were positioned in duplicate inside the isolator before aerosolization. Four bio-samplers (one of each kind of bio-sampler) took air samples directly after aerosolization, and the others took samples 20 min after aerosolization. An open plate containing 20 mL BPW was used to evaluate the loss of bacterial viability during aerosolization. A polytetrafluoroethylene (PTFE) filter was mounted on the side wall of the isolator and. It was expected to have a high efficiency for even tiny airborne particles (18). The sampling air flow rate of the PTFE filter was 7 L min^{-1}. Sampling time of all bio-samplers was 2 min. During the experiments air temperature and relative humidity were 21 to 23°C and 80 to 85%, respectively. The isolator was ventilated for at least 2 h between two aerosolizations. Inlet of the isolator was kept open to allow the entrance of air in order to compensate for the negative pressure induced by the bio-samplers during sampling.

Liquid samples were processed for bacteriology and uranine analysis without further treatment. Samples from the AVB and Airport MD8 were transferred into liquid form according to the procedures described in section 2.5 and 2.6. Uranine was analyzed by a fluorescence detector (HP 1046 A, HP, USA), which had a detection limit of 0.002 µg mL^{-1}.

Calculation of the sampling efficiency and the half-life time. The bacteria survival during aerosolization and present in the aerosol (N), the physical efficiency (E_p), the biological efficiency (E_b) and the sampling efficiency (E) were calculated using the equations below.

Equation 1

$$N = \frac{C_{plate,viable} / C_{plate,tracer}}{C_{viable} / C_{tracer}} \times 100\%$$

Equation 2

$$E_p = \frac{C_{bio-sampler,tracer}}{C_{ref-sampler,tracer}} \times 100\%$$

Equation 3

$$E_b = \frac{C_{bio-sampler,viable} / C_{bio-sampler,tracer}}{C_{viable} / C_{tracer} \cdot N} \times 100\%$$

Equation 4

$$E = E_p \times E_b$$

C_{tracer}: The tracer concentration in the aerosol bacterial suspension;

C_{viable}: The viable bacteria count in the aerosol bacterial suspension;

$C_{plate,tracer}$: The tracer concentration in the air sample from the open plate;

$C_{plate,viable}$: The viable bacteria count in the air sample from the open plate;

$C_{bio-sampler,tracer}$: The tracer concentration measured by a bio-sampler;

$C_{ref-sampler,tracer}$: The tracer concentration measured by PTFE filters;

$C_{bio-sampler,viable}$: The viable bacteria concentration measured by a bio-sampler.

Equation 5 was used to calculate the half-life time, $t_{1/2}$ (19).

Equation 5

$$t_{1/2} = \frac{(\log_{10} 2) \times T}{\log_{10}(C_0 / C_{20})}$$

T: Time interval in min. In this experiment, T=20 min;

C_0: Bacteria concentration at 0 min after aerosolization;

C_{20}: Bacteria concentration at 20 min after aerosolization.

Due to the diluting effect of entering air during air sampling, C_{20} had to be corrected before calculation of half-life time. Assuming the dilution was linear without wall loss, the correction was calculated with Equation 6.

Equation 6

$$C_{20} = C_{bio-sampler,viable} \times \frac{V_{isolator} + V_e}{V_{isolator}}$$

$V_{isolator}$: Volume of the isolator (1.38 m^3);
V_e: Extracted amount of air (0.76 m^3).

Assessment of the effect of uranine on the survival of bacteria in suspension. Bacterial suspensions were prepared in different media, i.e. *E. faecalis* and *E. coli* in BPW, *C. jejuni* in Heart Infusion Broth (HIB), and *M. synoviae* in Mycoplasma Experience Broth (MEB). Two suspensions of 10 mL were prepared for each species, one contained 0.02% uranine, while the other did not. These suspensions were kept at 4°C. Viable bacteria were counted in the eight suspensions just after adding uranine and 1 h and 2 h later. All countings were preformed in duplicate.

Assessment of the recovery efficiency of bacteria and uranine by rinsing agar plates. Due to the fact that all air samples had to be in liquid form for bacteriology and uranine analysis, the recovery efficiency from agar plates (used in AVB) was investigated by pipetting bacterial suspensions with 0.02% uranine on them and rinsing the plates with 2 mL of BPW three times consecutively. Sheepblood Agar (SBA), MacConkey Agar (MA), Charcoal Cefoperazone Deoxycholate Agar (CCDA) and Mycoplasma Experience Agar (MEA) were used in AVB bio-sampler for *E. faecalis*, *E. coli*, *C. jejuni* and *M. synoviae*, respectively. 0.1 mL of a bacterial suspension of known concentration with 0.02% uranine was pipetted onto the corresponding agar plate. After five min at room temperature, the bacteria-loaded agar was rinsed three times consecutively with 2 mL BPW by carefully scraping its surface with a plastic scraper. The rinsing-off liquid samples were mixed together for bacteriology and uranine analysis. A fourth rinsing was performed in order to assess the amount of remaining bacteria and uranine. The test was done twice for all bacterial species.

Assessment of the recovery efficiency of bacteria and uranine from gelatin filters. The recovery efficiency of bacteria from gelatine filters was performed as described earlier (20). Briefly, 0.5 mL of a bacterial suspension of known concentration was added to a sterile gelatin filter in a petri dish. After five min, the gelatine filter was dissolved in 20 mL BPW kept at 37°C. The liquid sample was pipetted into a small flask containing 30 mL BPW (final sample volume was 50 mL). From the final sample decimal dilutions, bacterial counts and uranine analysis were performed. The test was done twice.

Assessment of the efficiency of the bio-samplers after aerosolization of *E. faecalis*. A preliminary study was performed in duplicate to assess the efficiency of four air bio-samplers at detecting airborne *E. faecalis* after aerosolizing 20 mL of either *E. faecalis* suspensions (in BPW) with 0.02% uranine, *E. faecalis* suspensions without uranine, or 0.02% uranine solution only (without *E. faecalis*). The aerosolization protocol, air sampling and sample processing were performed as described before.

Data analysis. All the statistic analyses were performed with the SAS program (SAS 9.1.3 Service Pack 4, SAS Institute Inc., Cary, NC, USA). The effects of the factors (uranine, time and their interaction) on the bacteria concentrations (in log scale) in suspensions were estimated with the general linear model procedure (GLM procedure). The physical efficiency of the AVB, AGI and OMNI was compared to that of the Airport MD8 by Analysis of Variance. The biological efficiency was compared to 100% using the T-test. Half-life time of *E. faecalis* aerosolized with or without uranine was compared also by means of the T-test.

RESULTS

Assessment of the effect of uranine on the survival of bacteria in suspension. Statistical analysis showed that there was no significant effect of uranine on *E. faecalis*, *E. coli* and *M. synoviae* (P=0.55–0.94), neither was there a significant interaction effect between uranine and time (P=0.10–0.50). Concentrations of *C. jejuni* in HI Broth after 0, 1, and 2 h were within 1 $\log_{10}$ (6.5-7.1 $\log_{10}$ CFU mL^{-1}). However, *C. jejuni* concentrations notably decreased from 7.0 $\log_{10}$ CFU mL^{-1} at 0 h to 5.2 $\log_{10}$ CFU mL^{-1} at 2 h after adding 0.02% uranine. Statistical analysis showed that there was a significant effect of uranine (P=0.01), time (P<0.01) and the interaction between both (P=0.01) on the survival of *C. jejuni*. There was also a significant effect of time on the survival of *E. coli* (P=0.01), but not of *E. faecalis* and *M. synoviae*.

Assessment of the recovery efficiency of bacteria and uranine by rinsing agar plates. Most bacteria (79.8% to 123.6%) were recovered by the rinsing procedure. Recovery of *C. jejuni* (123.6%) was higher than 100%. An additional (fourth) rinsing step recovered 0.4% *E. faecalis*, 0.6% *E.coli*, 4.4% *C. jejuni* and 1.0% *M. synoviae*. The uranine recovery (33.1% to 60.1%) from all the four types of agar plates was lower than the bacterial recovery.

Assessment of the recovery efficiency of bacteria and uranine from gelatine filters. Bacterial recovery ranged from 62.7% to 171.7%. However, uranine recovery was constantly higher than 100% (114.2% to 123.2%).

Assessment of the efficiency of the air bio-samplers after aerosolization of *E. faecalis*. Table 1 shows the concentration of airborne bacteria and uranine after aerosolization of *E. faecalis* with uranine, *E. faecalis* without uranine, and suspensions with only uranine. All bio-samplers collected similar amounts of *E. faecalis*, about 9 $\log_{10}$ CFU m^{-3} at 0 min after aerosolization. Twenty min after aerosolization, the *E. faecalis* concentrations decreased 1 $\log_{10}$ CFU m^{-3}. There were large variations in recovery of airborne

uranine by different bio-samplers. Concentrations of uranine ranged from 190 to 1680 µg m^{-3} at 0 min, and from 36 to 223 µg m^{-3} at 20 min after aerosolization.

In Table 2, the efficiency of bio-samplers and PTFE filter are given relative to the Airport MD8 with a gelatin filter. Statistical analysis showed that the physical efficiency of all air bio-samplers was significantly lower than the efficiency of the Airport MD8 (all P-values <0.05).

Survival during aerosolization was measured by comparing the bacteria/uranine ratio in an open plate to the ratio in the aerosol suspension (Equation 1). It was found that 88.1% of *E. faecalis* were alive just after aerosolization with a standard error of 29.1%. The T-test showed that the loss of *E. faecalis* bacteria was not statistically significant (P=0.75).

The biological efficiency of all bio-samplers, calculated by Equation 3, was above 100%. The efficiency of the AVB was 2052.3%, while that of the other bio-samplers ranged from 117.9% to 151.0%. The P-values of the different samplers were >0.05, indicating that their biological efficiency was not different from 100%.

Table 3 shows the half-life time of airborne *E. faecalis* aerosolized with our without uranine. All bio-samplers gave similar half-life time results (7.7 to 11.3 min). P-value >0.05 indicates that there was no significant effect of uranine on the survival of *E. faecalis*.

DISCUSSION

Uranine was added as a tracer to the aerosol fluid in order to assess the efficiency of the bio-samplers. A prerequisite was that the tracer itself should not affect the bacteria, either negatively or positively, during the experimental period. Our results show that uranine had no effect on *E. faecalis* (P=0.55), *E. coli* (P=0.69) and *M. synoviae* (P=0.94), however, it did negatively affect on *C. jejuni* (P=0.01). Therefore, *C. jejuni* can not be aerosolized together with uranine.

Most of the bacteria (79.8% to 123.6%) could be recovered from agar plates by the rinsing method. A recovery of *C. jejuni* higher than 100% was explained either by bacterial growth during rinsing or by the variation in analysis of bacterial count or both. The fourth rinsing recovered 0.4% to 4.4% of bacteria, which indicates that the number of bacteria remaining after three rinsing steps was low. Therefore, a fourth rinsing step was considered redundant. The recovery of uranine was lower than that of bacteria. This was explained by the fact that uranine probably binds much stronger with the agar, which was confirmed by the fact that after the fourth rinsing step 7.5% to 10.1% uranine could still be recovered from the agar plates.

Most bacteria (62.7% to 171.7%) could be recovered from the gelatine filters. The recovery of uranine was constantly higher than 100%. An explanation for this could not be found.

Concentrations of *E. faecalis* aerosolized with or without uranine were similar at both 0 min and 20 min (Table 1). There was no effect of uranine on the survival of *E. faecalis* in aerosol state. Therefore, *E. faecalis* could be aerosolized with uranine. All four air bio-samplers recovered comparable uranine concentrations from aerosols of *E. faecalis* with uranine and from aerosols of uranine alone, both at 0 and 20 min (e.g. 190 vs 194 µg m^{-3} at 0 min, and 38 vs 36 µg m^{-3} at 20 min by AVB). The air bio-samplers detected similar tracer concentrations with or without bacteria. Therefore, aerosolizing separately uranine suspensions and suspensions of bacteria could be an option to investigate the sampling efficiency by avoiding the negative effect of uranine on bacteria in aerosol state, especially for micro-organisms which are vulnerable to uranine like *C. jejuni*.

The Airport MD8 with gelatine filters showed the best recovery of uranine (1590 to 1680 µg m^{-3} at 0 min, and 197 to 223 µg m^{-3} at 20 min; Table 1), indicating that it had the highest physical efficiency. Burton (18) found that both, the gelatine filter and the PTFE filter could recover more than 94% of airborne particles. However, our results show that the amount of uranine collected with PTFE was about half compared to that collected by the Airport MD8. This might be due to the non-uniform distribution of uranine aerosols in the isolator. Uranine recovery from AVB was low (190 – 194 ug m^{-3} at 0 min and 36 – 38 ug m^{-3} at 20 min; Table 1). This was probably caused by the fact that uranine binds strongly to the agar surface, making it difficult to rinse it off. When taking air samples, the droplets are impacted on the agar in AVB at a high speed, causing the uranine to be bound on the agar surface even more firmly. In total 4000 µg uranine was aerosolized in the isolator, i.e. 2899 µg m^{-3}. The maximal uranine concentration measured by MD8 was 1680 µg m^{-3}. This indicated that wall loss occurred during aerosolization.

The biological efficiency of the AGI-30, OMNI-3000 and Airport MD8 at sampling *E. faecalis* was not significantly different from 100%, i.e. all the four air bio-samplers could recover the airborne *E. faecalis* without significant loss of viability. The efficiency of AVB was extremely high. The high recovery of *E. faecalis* and low recovery of uranine by the rinsing procedure were probably the cause of this high value.

The half-life time of *E. faecalis* in the air ranged from 7.7 to 11.1 min at 21 to 23°C and 80 to 85% RH. This result was in agreement with earlier research by Landman and van Eck (21), who reported a half-life time for airborne *E. faecalis* of two to 15 min.

The highest physical efficiency was obtained for the Airport MD8, while the biological efficiency of all devices was not significantly different from 100%. Although based on these data the Airport MD8 is, under these circumstances, the best performer, it should be noted however that variations in efficiency of 50% for instance, imply a difference in detection of micro-organisms of merely 0.3 $\log_{10}$. This is irrelevant at least in a heavily contaminated environment (e.g. 10^7 *versus* $10^{6.7}$ CFU m^{-3}). Subsequently, other factors such as portability, user friendliness, low purchase, maintenance and sampling costs, and so forth will determine the choice of air bio-sampler. Considering these factors the Airport MD8 ranks high as well.

ACKNOWLEDGEMENTS

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Figure 1. Droplet size distribution measured with a laser diffraction particle size analyser of an aerosol of water generated by the Walther Pilot I spray-head.
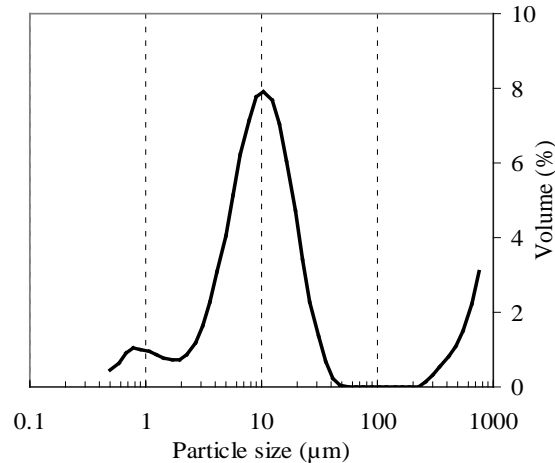

Table 1. Concentration of airborne *E. faecalis* and uranine collected by the air bio-samplers at 0 and 20 min after aerosolization (each value is the mean of a duplicate test).

BIO-SAMPLER	*E. FAECALIS* WITH URANINE				*E. FAECALIS* WITHOUT URANINE		URANINE (μG M^{-3})	
	E. FAECALIS (LOG$_{10}$ CFU M^{-3})		URANINE (μG M^{-3})		*E. FAECALIS* (LOG$_{10}$ CFU M^{-3})			
	0 MIN	20 MIN	0 MIN	20 MIN	0 MIN	20 MIN	0 MIN	20 MIN
AVB	9.3	8.4	194	36	9.4	8.5	190	38
AGI-30	9.0	8.1	930	148	9.3	8.4	1000	173
OMNI-3000	9.0	8.2	783	86	9.1	8.2	766	107
MD8	9.2	8.2	1590	197	9.4	8.4	1680	223
PTFE	-	-	-	-	-	-	845	150

Table 2. Physical and biological efficiency of air bio-samplers.

BIO-SAMPLER	PHYSICAL EFFICIENCY ($\pm$ SE, %)	P^1	BIOLOGICAL EFFICIENCY ($\pm$ SE, %)	P^2
AVB	13.9 ($\pm$ 1.0)	0.00	2052.3 ($\pm$ 1321.6)	0.38
AGI-30	69.8 ($\pm$ 4.7)	0.00	151.0 ($\pm$ 98.8)	0.70
OMNI-3000	49.4 ($\pm$ 4.0)	0.00	117.9 ($\pm$ 53.4)	0.79
MD8	100 ($\pm$ 6.4)	-	128.3 ($\pm$ 75.6)	0.77
PTFE	56.5 ($\pm$ 6.0)	0.00	-	-

[1] Probability that the physical efficiency of bio-samplers is not significantly different to that of the Airport MD8 (a *P*-value <0.05 means there is significant difference).

[2] Probability that the biological efficiency of bio-samplers is not significantly different from 100% (a *P*-value <0.05 means there is significant difference).

Table 3. Half-life time of *E. faecalis* aerosolized with uranine and without uranine (each value is the mean of a duplicate test).

BIO-SAMPLER	HALF-LIFE TIME (MIN)		P[1]
	E. FAECALIS WITH URANINE ($\pm$ SE)	*E. FAECALIS* WITHOUT URANINE ($\pm$ SE)	
AVB	7.7 ($\pm$ 0.6)	8.7 ($\pm$ 0.0)	0.24
AGI-30	7.7 ($\pm$ 1.4)	11.3 ($\pm$ 4.3)	0.51
OMNI-3000	8.2 ($\pm$ 1.1)	9.3 ($\pm$ 1.9)	0.68
MD8	7.6 ($\pm$ 0.1)	7.8[2]	0.55

[1]Probability that the survival of *E. faecalis* is not affected by the uranine in aerosol state.

[2]Half-life time obtained from one sample. The other sample was contaminated with bacteria other than *E. faecalis*.

EFFECTS OF OREGANO ESSENTIAL OIL ON PERFORMANCE AND LIVABILITY OF LARGE BROILERS IN A COMMERCIAL SETTING

M. A. Mellencamp[A], R. Smith[A], R. Dvorak[A], and T. S. Cummings[B]

[A]Ralco Nutrition, Marshall, MN, USA
[B]Mississippi State University, Mississippi State, MS, USA

SUMMARY

The poultry industry is investigating natural compounds to improve commercial broiler performance. Oregano essential oil (OEO) is a natural feed flavor that improves broiler performance on farms with enteric challenges. This study investigated the effects of OEO on broiler performance on a commercial farm. Two houses received OEO (Regano®) and two control houses received a chemical program (24,400 birds per house). OEO birds were within 1.5% of control birds for final weight (8.569 lb vs. 8.649 lb), average daily gain (0.134 lb vs. 0.135 lb), and feed conversion (2.106 vs. 2.080). Livability was similar (97.08% vs. 96.85%). Clinical coccidiosis was not observed in OEO or control houses. Some intestinal lesions were seen in OEO birds at d 21 and d 28, but no lesions were seen on d 37. As expected, control birds showed very few lesions. Overall, OEO birds remained healthy and performed well compared to control birds fed an aggressive anti-coccidial program.

INTRODUCTION

The poultry industry is investigating natural products, including plant-derived essential oils, because of their proven anti-microbial and anti-oxidant properties. Oregano essential oil (OEO) extracted from Greek oregano plants is an all natural feed additive that enhances appetite and supports intestinal health. University and independent research studies have shown that OEO and its active components, carvacrol and thymol, have potent anti-microbial activity against a wide variety of bacterial and fungal pathogens. OEO is environmentally friendly and leaves no harmful residues in manure, litter, or groundwater. OEO has a very high level of anti-oxidant activity due to its phenolic nature. Anti-oxidants protect tissues and cells from oxidative damage. Oregano is far more potent that blueberries and other foods that are advertised as protective anti-oxidants.

To meet the nutritional needs of high performance birds, chicks need to develop a healthy, well-functioning gut. Dietary OEO can maximize intestinal health by supporting beneficial gut microflora that, in turn, helps to prevent common intestinal diseases. The objective of this trial was to determine the effects of OEO on performance of large (8+ lb) broiler robustness by measuring growth, liveability, and feed conversion. Post mortem examination was performed at three, four, and five weeks post-placement to assess intestinal health.

MATERIALS AND METHODS

A typical farm with four tunnel-ventilated houses was selected for the trial, which took place in the southern US in the spring of 2008. Two houses were used for each dietary treatment. Each house was stocked with 24,400 birds on day 1 of age. All birds were on built-up litter. Birds were fed the farm's usual five phase broiler diet, supplemented with either OEO or the farm's usual health control additives from day 1 to day 48 (Table 1). On day 49 to day 59, both groups received Stafac® in their feed. Plain feed was fed from days 60 to 63. Birds were harvested on day 64. Feed grade OEO was supplemented with OEO liquid in water (3 oz/gallon at 1:128) for the first five days of the trial and for days 23 to 27.

RESULTS AND DISCUSSION

Results showed that survival in both treatment groups was >96%. Mean weights were 8.65 lb and 8.57 lb for control and OEO-fed birds, respectively. Average daily gains were 0.135 and 0.134 for control and OEO-fed birds, respectively. Feed conversion was 2.08 for control birds, compared with 2.106 for the OEO-fed birds, which was 1.5% higher than the control group. This increase may have been related to the high level of OEO that was fed from days 39 to 48. The farm was unable to make a diet change midway

through their four diet phase. Our current recommendation is to remove OEO on day 38 and replace it with a product containing prebiotic fiber to protect gut health.

Gross and microscopic examination of intestines showed that the OEO group had mild lesions consistent with subclinical coccidiosis on weeks 3 and 4 post-placement. By week 5, lesions had disappeared, suggesting that the OEO-fed birds had developed immunity to coccidiosis. Despite the presence of mild lesions and oocysts in the intestine of OEO-fed birds, the disease was sufficiently controlled so that clinical signs were not present and the birds developed immunity at the same time as the control birds. As expected, the birds fed the control program had very few intestinal lesions.

Overall, these results demonstrate that OEO–fed birds performed as well as birds on a chemical and ionophore program. Production results in the OEO houses were comparable to control, with a modest reduction in weight gain and feed conversion but slight increase in livability by the OEO group. There was no evidence of clinical coccidiosis over the entire trial period in both groups. Necropsies showed that *Eimeria* parasites were present in the intestine of the OEO-fed birds, but this mild infection resulted in development of immunity by week 5 that cleared the pathogen from the intestine.

Table 1. OEO and control diet additives.

Diet	Days 1-14	Days 15-28	Days 29-48	Days 49-59	Days 60-63
Control	Nicarb® 90g BMD® 50g 3-Nitro 22g	Robenz® BMD® 50g	Biocox® 60g BMD® 25g 3-Nitro 34g	Stafac® 10g	Plain feed
Oregano essential oil	0.88 lb./ton[1]	0.75 lb./ton[1]	0.66 lb./ton	Stafac® 10g	Plain feed

[1]OEO was administered in the water (6 oz/gallon at 1:128) on days 1-5 and days 23-27.

Table 2. Effects of OEO or control diets on growth and livability of large broilers.

House	Diet	Number of birds		Age (d) at harvest	Survival (%)
		Placed	Sold		
1 & 2	OEO	48,800	47,363	64	97.07
3 & 4	Control	48,800	47,263	64	96.85

WATER SUPPLEMENTATION WITH AVI-LYTE™, AN INNOVATIVE VITAMIN, ELECTROLYTE, AND DIRECT FED MICROBIAL COMBINATION, SIGNIFICANTLY IMPROVES LIVABILITY OF LAYERS

M. A. Mellencamp, R. Smith, and R. Dvorak

Ralco Nutrition, Marshall, MN

SUMMARY

A healthy gastrointestinal tract is critical for optimum productivity of layers, breeders, and broilers. A healthy gut allows maximum nutrient absorption and acts as the first line of defense against disease. In this field trial, we investigated the effects of Avi-Lyte™, a unique water-soluble product containing electrolytes, vitamins and avian-derived beneficial bacteria on livability of replacement pullets. The test group (n=2,423) received Avi-Lyte for days 1-5 post placement according to manufacturer's instructions. The control group (n=10,260) received the farm's usual water-based vitamin protocol for the same period. Both groups were in the same house. Results on day 14 showed that the control group had 4.59% mortality compared with 1.98% for the Avi-Lyte group ($P<0.001$). Odds ratio analysis showed that the Avi-Lyte-fed birds were 2.4 times more likely to survive than control birds ($P<0.05$; 95% CI, 1.76 to 3.21). These results demonstrated that Avi-Lyte improved survival over the farm's usual vitamin protocol and provided measurable savings to the producer.

INTRODUCTION

Water and feed consumption after placement are key for development of healthy and robust chicks. Water and feed intake promote development of the intestine. The gut microflora is established at this time. A healthy, balanced microflora favoring *Bifidobacterium* and *Lactobacillus* spp. (i.e. the "good" bacteria) is critical for pathogen exclusion, epithelial cell function, and maturation of the gut-associated immune system (1,4). To support gut health and combat stress and dehydration, water-based products are often given to young chicks. Ralco Nutrition, Inc. has developed Avi-Lyte, a unique water-soluble product designed for the newly hatched chick. Avi-Lyte contains electrolytes, vitamins, and avian-derived beneficial bacteria to promote water and feed intake after placement and support development of a healthy and balanced gut microflora. The objective of this trial was to compare the effects of a five day course of Avi-Lyte with the farm's water-soluble vitamin protocol on livability over the first two weeks post-placement.

MATERIALS AND METHODS

This trial was performed by a major US egg integrator. The flock selected for testing was a standard breeder flock at its peak production. The test and control groups were placed in a single house at the same time. The test group (n=2,423) was given Avi-Lyte according to manufacturer's directions (8 oz/gallon of stock solution, metered at 1:128) from days 1 to 5 post-placement. The control group (n=10,260) received the flock's usual water-based vitamin protocol for the same time period. Death loss was recorded daily for 14 days.

RESULTS AND DISCUSSION

Results showed a five day course of Avi-Lyte reduced death loss on day 14 by 56%, from 4.59% in the control group to 1.98% in the Avi-Lyte group ($P<0.001$) (2). Odds ratio (3) showed that Avi-Lyte birds were 2.4 times more likely to survive than control birds (95% CI, 1.76 to 3.21). The producer reported that Avi-Lyte-fed chicks were uniform, robust and healthy. The 56% reduction in death loss represented a major savings for this producer.

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Figure 1. Effects of Avi-Lyte™ and a custom vitamin pack on cumulative death loss of layer chicks on day 14 post-placement.
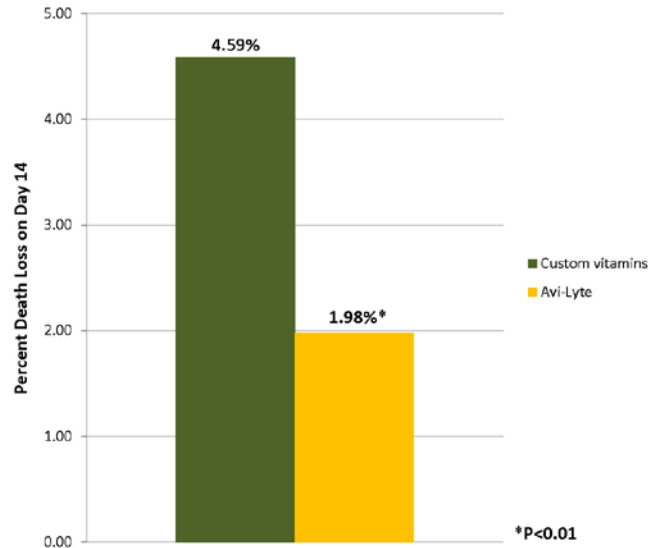
USE OF AN ORGANIC ACID (ACTIVATE® WD) AS FIELD INTERVENTION TO REDUCE *SALMONELLA* SPP. AND *CAMPYLOBACTER* SPP. IN BROILER CHICKENS

Andres F. Montoya[A], Suzanne Young[B], Charles Hofacre[C], Hugh Cholick[A], Marco Quiroz[A], Julia Dibner[A], Roy Berghaus[C], and Stephan Thayer[C]

[A]Novus International Inc. 20 Research Park Drive St. Charles, MO 63304
[B]Keystone Foods 6767 Old Madison Pike, Bldg 5 Huntsville, AL 35806
[C]Poultry Diagnostic and Research Center 953 College Station Road Athens, GA 30602

INTRODUCTION

Salmonella and *Campylobacter* have been recognized as leading bacterial causes of human gastroenteritis in the United States. In 2008, the Centers for Disease Control and Prevention (CDC) reported 7,444 cases of *Salmonella* and 5,825 cases of *Campylobacter* human food borne illness. Epidemiological data suggest that contaminated products of animal origin, especially poultry, contribute significantly to these cases. With the perception that antibiotics should no longer be used as animal growth promoters, there has been widespread interest in natural methods of inhibiting detrimental bacteria. The objective of this field trial was to evaluate the performance of organic acid water treatment during the first seven days and the last seven days of the growout phase as a preharvest intervention on the prevalence of *Salmonella* spp. and *Campylobacter* spp. in broiler chickens.

MATERIALS AND METHODS

A 16 house research farm was selected for this trial. A total of 352,000 broiler chickens were divided in two treatment groups. Eight houses were selected as control groups where birds received normal water and the other eight houses were selected as the treatment groups where birds received the water treatment. Before placement all houses received a water line treatment with citric acid for 24 hours. Activate® WD was administered to the treatment houses twice during the growout. First, at placement through seven days of age and then during the last full week of the growout. The water treatment program was monitored daily during treatment to ensure a pH of approximately 5.0 was reached the first week of grow out and a pH of 3.5 was reached the final week. Seven days prior to processing and just prior to water treatment started all houses were environmentally sampled for *Salmonella*. Four boot sock samples were collected per house (4 samples X 16 houses = 64 samples per sample day). Just prior to catching (after feed withdrawal), the following samples were taken from each house: 30 carcass rinses feather on, 10 crops, and 10 cecae. A total of 50 samples per house were collected for *Salmonella* testing and 209 samples were collected at the processing plant for *Campylobacter* testing and sent to the Poultry Diagnostic and Research Center at University of Georgia.

RESULTS

Results showed 88% positive *Salmonella* isolates in the boot sock samples obtained prior to treatment for the Activate WD treatment groups compared to 66% positive *Salmonella* isolates on the boot socks samples on the Control treatment groups. A reduction of *Salmonella* isolates in the crop (Control 19% vs. Activate WD 13%), and cecae (Control 15% vs. Activate WD 11%) can be observed in Figure 1. No statistical differences were found among the treatment groups.

Results also showed a reduction of *Salmonella* on carcass rinses (feathers on: Control 4.32 $\log_{10}$ MPN organisms/mL average culture-positive samples vs. Activate WD 3.66 $\log_{10}$ MPN organisms/mL average culture-positive samples) for the Activate WD treatment groups compared with the control groups (Figure 2). *Campylobacter* reduction was also observed in the carcass rinses (Control 6.99 $\log_{10}$ MPN organisms/mL average positive samples vs. Activate WD 4.93 $\log_{10}$ MPN organisms/ml average positive samples) for the Activate WD treatment groups compared with the control groups (Figure 3).

CONCLUSION

In conclusion, Activate WD aided in the reduction of *Salmonella* spp. and *Campylobacter* spp. when administrated in the water during the first seven days and during the last seven days of the life of the birds.

Figure 1. Percentage of *Salmonella* positive isolates in the treatment groups.
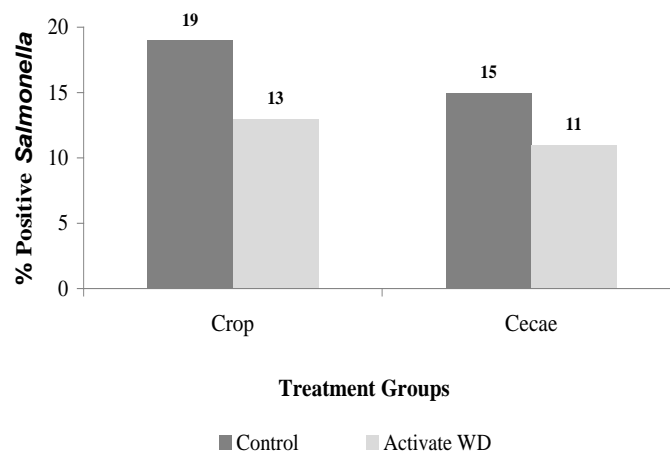
Figure 2. Log $_{10}$ *Salmonella* MPN values (organism/mL) on positive carcass rinses performed on the farm.
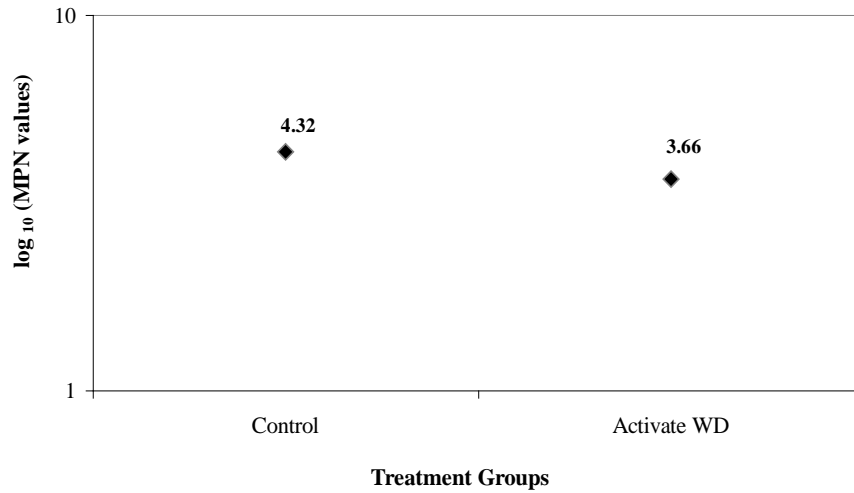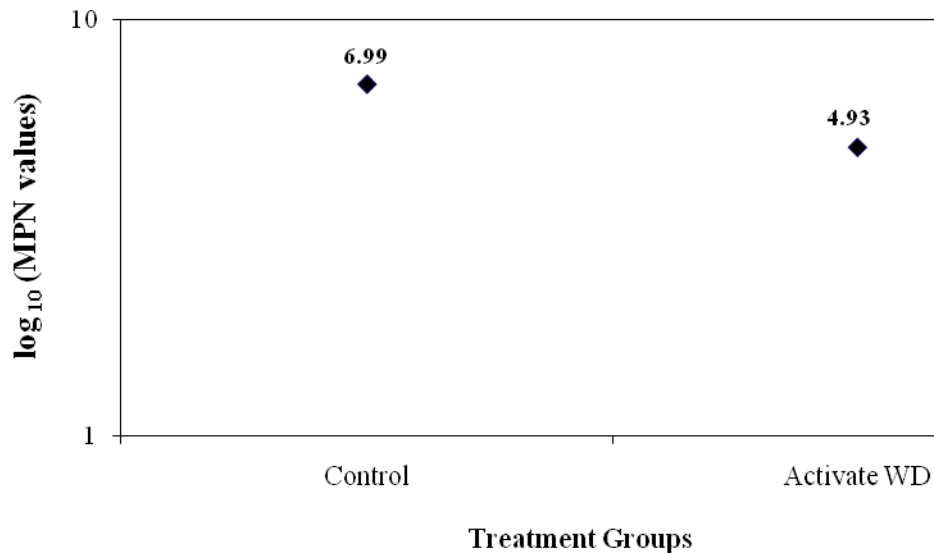

Figure 3. Log $_{10}$ *Campylobacter* MPN values (organism/mL) on positive carcass rinses performed on the processing plant.

EVALUATION OF A LIVE VIRUS EMULSIFIED VACCINE AGAINST CHICKEN ANEMIA VIRUS IN LEGHORN BIRDS

Andres Morales Garzon, Donaji Garcia Lopez, Raul Montalvo Ramirea,
Efigenia Merino Cortes, and Eduardo Lucio Decanini

Investigación Aplicada S.A. de C.V., 7 Norte 416 Tehuacan, Puebla, Mexico, 75700

SUMMARY

Ten 16-week-old SPF chickens were vaccinated with a 0.5 mL dose of a live chicken anemia virus oil-emulsion vaccine via subcutaneous injection. Another group of birds was maintained as the unvaccinated control. All birds were maintained through 11 weeks post vaccination and titers to CAV were tested by ELISA CAV100 (IDEXX, Inc.), at three weeks after vaccination.

INTRODUCTION

Vaccination against chicken anemia virus is a good method to prevent this disease in layer hens. There are several kinds of vaccines against CAV and could be administered by ocular, subcutaneous, or drinking water routes. However, there is not a live oil emulsion vaccine. In this paper we report findings with the use of a live CAV oil emulsion vaccine in Leghorn chickens that were given one dose by subcutaneous route

MATERIALS AND METHODS

Birds. Twenty 16-week-old Specific Pathogen Free chickens were maintained in wire cages**.** One group of 10 birds was vaccinated with 0.5 mL by subcutaneous route; the other 10 birds were maintained as unvaccinated controls.

Vaccine. Live oil emulsion chicken anemia virus Emulmax CAV® (Investigación Aplicada S.A. de C.V. Tehuacán, Puebla, México).

Feed and Water. Administered *ad libitum*.

Serology. A serum sample was collected from each group from 0 until 11 weeks after vaccination and ELISA CAV 100 was tested.

RESULTS AND DISCUSSION

The objective of this paper was to evaluate if a live oil emulsion vaccine against chicken anemia virus (CAV) could show seroprevalence in CAV- negative SPF birds. As can see in Table 1 CAV live oil vaccine started seroconverting from three weeks after vaccination and remained until end of trial at eleven weeks postvaccination with titers higher than recommended as protective to CAV (a titer of 1000 is considered protective). The unvaccinated group remained negative to antibodies to CAV during all trial long. Live CAV oil emulsion vaccine could be considered as an alternative to other vaccines for layer hens like drinking water or killed CAV vaccines because of its early seroconversion and prolonged time remained of antibodies.

Table 1. Titers measured by ELISA CAV 100 in birds vaccinated with a live oil emulsion CAV vaccine.

Age (weeks)	Weeks post vaccination	Group vaccinated with live CAV vaccine
16	0	999
17	1	999
18	2	999
19	3	4617
20	4	5612
21	5	5378
22	6	6104
23	7	6289
24	8	6292
25	9	6274
26	10	6757
27	11	4217

PHYLOGENIC ANALYSIS OF MYCOPLASMA SYNOVIAE STRAINS ISOLATED FROM JAPANESE COMMERICAL POULTRY FARMS BASED ON THE VLHA GENE, AND DEVELOPMENT OF REAL-TIME PCR FOR THE SPECIFIC DETECTION OF MS-H VACCINE STRAIN

Shoji Ogino[A], Yasuhisa Munakata[A], Shuichi Ohashi[A], Masato Fukui[A], Hiroshi Sakamoto[B], Yukio Sekiya[B], Amir H. Noormohammadi[C], and Chris J. Morrow[D]

[A]Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan
[B]Nippon Biologicals, Inc., Tokyo, Japan
[C]The University of Melbourne, Victoria, Australia
[D]Bioproperties Ltd, Victoria, Australia

INTRODUCTION

M. synoviae is often considered to occur as a subclinical infection in modern poultry systems and has a high prevalence, and except for infectious synovitis (now rare), infection with *M. synoviae* alone has not been associated with a poultry disease until recently. In 2004, Kraeger (1) reported *M. synoviae* as one of the precipitating factors in *Escherichia coli* peritonitis. In 2007, Feberwee *et al*. (2) demonstrated a causal relationship between eggshell apex abnormality and *M. synoviae* infection. Thus, it is now increasingly clear that similar to M. gallisepticum, *M. synoviae* is an economically important pathogen in the poultry industries. However, the pathogenicity of *M. synoviae* remains largely unclear, and its prevalence and the extent of damage have not been investigated in Japanese poultry populations. In the present study, we collected *M. synoviae* isolates from chickens across Japan and then analyzed their genetic characteristics by sequencing the MSPB region of *vlhA* in order to contribute to understanding the relevance of *M. synoviae* in the Japanese poultry population. Based on our findings, we also developed a novel method of distinguishing the vaccine strain and field isolates by applying cycling probe technology (CPT), which detects SNP, to this region of the *vlhA* sequence.

MATERIALS AND METHODS

Isolation of *M. synoviae* was attempted from 229 chickens at 18 farms across 11 prefectures in Japan. Total genomic DNA was extracted from tracheal dried swabs and cultured samples using QIAamp DNA Mini Kit. The DNA extracts were tested for the 16S rRNA gene of *M. synoviae* with specific primers (3) and SYBR Premix Ex Taq II (Takara Bio, Inc.). *M. synoviae* positive samples were then had *vlhA* DNA fragment amplified and sequenced with the forward (MS-Link) and reverse (MS Cons-R) PCR primers. Phylogenetic and alignment analyses of these sequences were performed using GENETYX-WIN software. A new PCR method was designed to specifically detect the vaccine strain MS-H based on the genetic variances found by the *vlhA* gene sequence alignment analysis of the Japanese field isolates and MS-H. PCR was performed using Cycleave PCR Core Kit (Takara Bio, Inc.).

RESULTS

By the phylogenic analysis of the 300- tp 400-bp sequence data, Japanese field isolates were grouped into nine genotypes (Fig. 1).

Group 1 contained five isolates (NZMSID1, NZMSID60, NZMSID19, NZMSID182 and NZMSID189) had the same sequence, although they were isolated at different farms. Similarly all five isolates in Group 2 (NZMSB10, NZMSID28, NZMSID171, NZMSID214 and NZMID229) were from different farms but had identical sequences. The isolates in Group 3 (NZMSN20 and NZMSID128), as well as those in Group 4 (NZMSID185 and NZMSID205) were also from different farms.

Based on our finding of A-to-G substitution at nucleotide 365 in the MSPB region in our field isolates, we designed a new method of specifically identifying MS-H by means of CPT, which is ideal for SNP typing. Melting curve analysis was performed to confirm that all tested isolates had the same melting temperature (Tm) value and that amplified *M. synoviae*-specific 16S rRNA sequence was detected (Fig. 2). Then, a second real-time PCR was performed using CPT to detect the MS-H-specific sequence containing the SNP site. As shown in Fig. 3, amplicons were detected only from the MS-H sample by this method.

DISCUSSION AND CONCLUSION

The first focus of our study was genetic analysis of Japanese field strains of *M. synoviae* based on the partial *vlhA* sequence. Based on the sequence homology of this gene region, we found that there were at least nine genogroups of *M. synoviae* in Japan.

The second focus of our study was to develop a method of distinguishing between field isolates and the vaccine strain, now that the live attenuated *M. synoviae* vaccine is commercially available in Japan. Our study revealed the significance of this fragment of the *vlhA* gene: this region contained a SNP site that was uniformly seen in the Japanese field strains when compared to MS-H. Based on this finding, we developed a new CPT-based PCR method that specifically targets this SNP site in MS-H.

Our method is based on SNP at the 365th nucleotide position of the MSPB region. In the Japanese strains examined in this study, adenine at this site was substituted with guanine.

This indicates that our method is not applicable to all field strains of *M. synoviae*. However, the method seems useful as the first detection step in countries where similar SNP is found in the genome of *M. synoviae*.

Vaccination against *M. synoviae* has led to lower mortality, increased egg production and productivity, lower incidence of egg abnormality and better feed conversion ratios, all of which indicate the impact of *M. synoviae* infection. However, the mechanism by which *M. synoviae* affects the poultry performance has not yet been fully explained.

Further studies to reveal any forms of association between the pathogenicity of *M. synoviae* and the *vlhA*-based genogroups may provide key information to the industry in terms of biosecurity.

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Figure 1. UPGMA-based phylogenetic tree using partial *vlhA* gene sequences of *M.synoviae* strains.
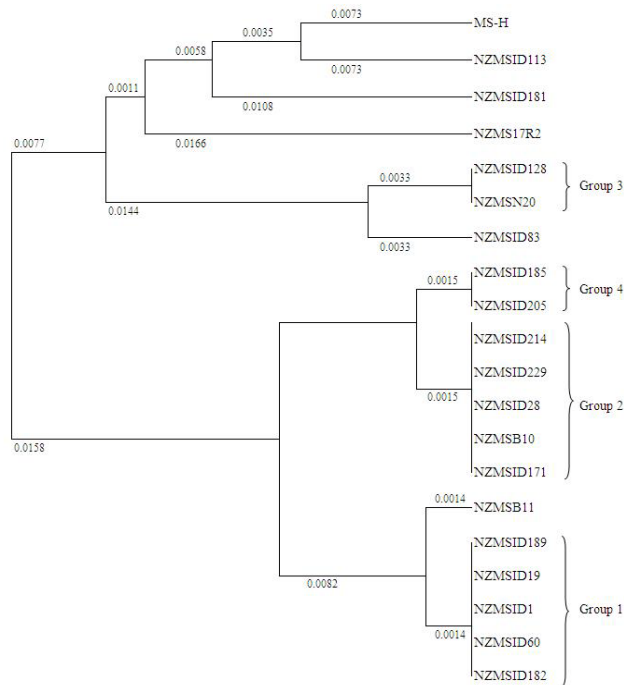
Figure 2. Melting curve analysis of *M. synoviae* strains using the 16S rRNA Lauerman primer set.
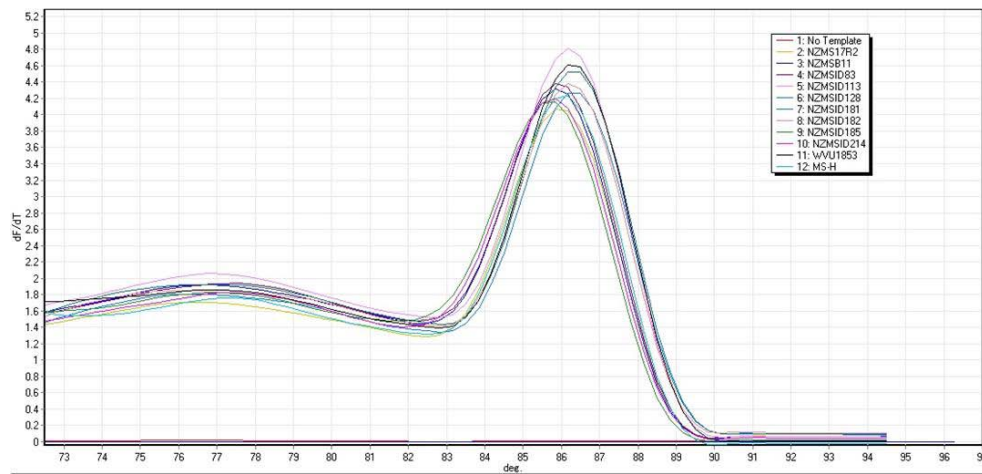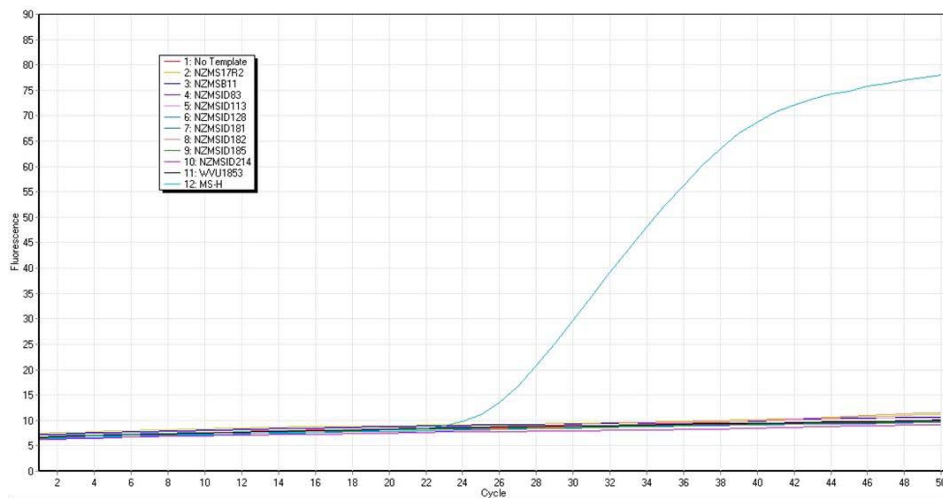

Figure 3. Detection of the *M. synoviae* MS-H strain using CPT.

EVALUATION OF BIOSECURITY MEASURES BASED ON VIDEO SURVEILLANCE IN POULTRY FARMS IN QUEBEC AND MAIN FAILURES

Manon Racicot[A], Daniel Venne[B], André Durivage[C], and Jean-Pierre Vaillancourt[D]

[A]University of Montreal, Canada, manon.racicot@umontreal.ca
[B]couvoir Scott ltée
[C]Psychologist, Professor at the University of Quebec in Outaouais
[D]Professor at the University of Montreal

SUMMARY

Biosecurity measures are designed to prevent the introduction of infectious diseases in flocks, and reduce the consequences of an infection. However, to be effective, biosecurity measures must be applied consistently by all. Poor compliance has been reported with all types of animal production, and many reasons have been given, such as the lack of understanding of biosecurity principles. It is essential to define strategies to improve the implementation of biosecurity measures. We are currently conducting a study on 24 poultry farms in Quebec to determine the impact of audits and of visible cameras on the level of biosecurity compliance. The effect of these two strategies will be determined in the short term (two weeks) and in the medium term (six months later). The targeted biosecurity measures are those required when getting in and out of poultry barns. Compliance is evaluated using hidden cameras. People filmed during the study will then be asked to complete a questionnaire designed to assess their personality in order to determine whether there is a relationship between certain personality factors (e.g. conscientiousness), and compliance. The first objective of the study is to determine whether audits or visible cameras increase biosecurity compliance compared to a control group (no intervention). The second objective is to determine whether a relationship exists between personality profiles and compliance. For this poster, descriptive data are presented and relate to the main compliance failures observed when entering and exiting poultry barns.

Results are presented for the short term assessment. Poultry production types were distributed as follows: 17 meat-type farms (chickens or turkeys), four commercial layers (two in the audits group and one each for the other two groups) and three breeder farms (one in each group). A total of 1635 visits were made by 180 different individuals (68 full-time employees, 28 part-time employees, 82 visitors, and two co-owners).

Required biosecurity measures: The entrance of all poultry barns included a "contaminated" and a clean area (i.e., an anteroom separated in two by a red line or a bench, with the area closest to the door giving access to the birds being considered "clean" or not contaminated). All visitors and employees were required to respect these two areas, although it was not mandatory for employees on one farm. All visitors and employees were required to change boots or put on plastic boots. In addition to changing boots, personal footwear had to be disinfected for visitors and employees on four farms. All visitors were also required to sign a logbook. Barn specific coveralls were necessary for visitors on 17 farms and for employees on 10 farms. Hand washing was also compulsory (at entry for visitors on 14 farms and on 12 farms for employees; when exiting on four (visitors) and three (employees) farms). Gloves were mandatory for employees handling birds on one farm. Wearing a hairnet was required for visitors on three farms and for employees on two farms. All required measures were clearly posted at the entrance of each barn.

A total of 58 different errors (non compliance) were noted. On average, three errors were recorded per visit. The maximum number of errors by one individual during one visit was 10. People observed over several visits made an average of six different errors. Only 89 visits (5.4% of the 1635 visits) were performed without error by 26 different people (15 full-time employees, two part-time employees, nine visitors). Only nine out 180 individuals made no mistakes during all their visits (one full-time employee, one part-time employee, and seven visitors). The 10 most frequent errors are presented in Table 1. The ranking is based on the number of individuals who committed the error, since in general, a participant repeat the same mistakes over successive visits. The percentage of erroneous visits is also presented.

Based on the nature and frequency of errors, there seems to be a lack of understanding of biosecurity measures, as already reported in several studies. Further analyses of this data will be performed considering variables such as ease of application of

biosecurity measures (anteroom design); the type of area separation (red line, bench, door, etc.); type of poultry production; function of individuals; type of measure requested; time of the visit (day of week and time of day); level of urgency (time taken to apply measures); duration of visit; presence of an observer (presence of the owner or another employee); number of entries during the same visit; etc. Results depending on the compliance control strategies (audits or visible cameras) will be presented later. However, for the short term assessment, the overall average compliance for the control group (without considering any of the above variables) was 36.7%. It was 47.0% and 60.8% for the audit and visible camera groups, respectively. At this point in this study, it is clear that the lack of biosecurity compliance is very prevalent. Similar results have been reported in other studies conducted elsewhere in Canada and the USA. But this current study offers a more detailed account of biosecurity errors.

Table 1. Most frequent biosecurity errors while entering or exiting poultry barns.

Rank	Failures	Number of individuals	Erroneous visits
1	Ignoring the separation between the contaminated and the clean areas	106/178 (59.6%)	622/1600 (38.9%)
2	Not wearing or changing boots	96/180 (53.3%)	466/1635 (28.5%)
3	Not washing hands or putting on gloves	79/99 (79.8%)	594/939 (63.3%)
4	Putting on clean area boots in the contaminated area or before entering the barn	54/178 (30.3%)	180/1600 (11.3%)
5	Removing boots in the clean area and walking with contaminated shoes in that area	47/178 (26.4%)	153/1600 (9.6%)
6	Going between the two areas (contaminated; clean) without respecting biosecurity measures because of specific triggers, such as getting equipment on the contaminated side to move it to the clean area.	46/178 (25.8%)	214/1600 (13.4%)
7	Not signing the log book	45/82 (54.9%)	55/124 (44.4%)
8	Not wearing coveralls	43/100 (43.0%)	197/699 (28.2%)
9	Put one or both plastic boots (or farm boots) after crossing the line to the clean area	41/178 (23.0%)	146/1600 (9.1%)
10	Removing boots in the contaminated area	41/178 (23.0%)	145/1600 (9.1%)

NUTRIGENOMICS: PRACTICAL APPLICATIONS EXPLAINING THE EFFECTS OF SELENIUM AT A MOLECULAR LEVEL ON HEN REPRODUCTIVE PERFORMANCE

Ronan Power and A.E. (Ted) Sefton

Center for Animal Nutrigenomics & Applied Animal Nutrition, Alltech Inc., Nicholasville, KY, USA
and Alltech Inc., Guelph, ON, Canada

SUMMARY

Principles of nutrigenomics were used to determine whether the reproductive benefit of dietary selenium (Se) in older broiler breeders extends beyond its role in antioxidant defense. Twenty-one broiler breeders (1 d old) were allocated to one of three treatment groups (seven birds per group). Treatments included a Se-deficient, torula-based control (0.02 ppm Se); and diets supplemented with sodium selenite or Sel-Plex® (Alltech Inc.) at 0.3 ppm Se. After 58 weeks of feeding, tissue from oviduct was harvested. Microarray analysis was performed and affected genes were assigned to biochemical pathways to visualize altered biological processes; treatment responses were compared. A total of 5,105 transcripts of the Affymetrix chicken genome array were affected in oviduct by at least one treatment ($P \leq 0.01$). The gene encoding glutathione peroxidase 3 (GPx3) was up-regulated by Sel-Plex ($P<0.01$); the gene encoding GPx4 was up-regulated by both sodium selenite ($P<0.05$) and Sel-Plex ($P<0.01$). In addition, dietary Se sources elicited a number of transcriptional shifts linked with fertility (e.g., key component genes of the follicle stimulating hormone pathway), with clear differences between Se sources, Sel-Plex having a much more marked and comprehensive effect. Many of these effects had not been linked to selenium previously and would have remained hidden without the use of a nutrigenomics approach.

INTRODUCTION

In the past 10 to 15 years, nutrition research has undergone a major shift in focus based on the realization that many micronutrients and macronutrients have the ability to act as potent dietary signals which can interact with DNA to switch genes on or off. Altering gene expression through nutrition, therefore, offers the potential to alter biological function in a beneficial way. Patterns of gene expression in response to particular nutrients or diets can be viewed as "dietary signatures" (1). Nutrigenomics is the study of these dietary signatures in an effort to understand how nutrition influences health and performance at a molecular level.

Selenium (Se) is an essential trace element most noted for its role in antioxidant defense mechanisms, in particular, the Se-dependent glutathione peroxidase family of enzymes which assist in detoxifying reactive oxygen species (ROS). Selenium supplementation also beneficially impacts female reproductive function, particularly in older animals; these benefits have generally been attributed to its protective effect against the negative effects of ROS on ovulation, oocyte development, implantation and embryo development (2,3).

The dietary form of Se also affects both male and female fertility. For example, Renema showed that older broiler breeders supplemented with an organic Se source (Sel-Plex, Alltech Inc.) had significantly improved total egg production and chick production compared with sodium selenite-supplemented and Se-deficient birds (4).

The objective of this study was to determine whether the reproductive benefit of dietary selenium in older broiler breeders extends beyond selenium's recognized role in antioxidant defense mechanisms.

MATERIALS AND METHODS

Twenty one broiler breeders (1 d old) were allocated to one of three treatment groups (seven birds per group). Treatments included a Se-deficient, torula-based control (0.02 ppm Se); and diets supplemented with sodium selenite or Sel-Plex (Alltech Inc.) at 0.3 ppm Se. After a 58 week trial, tissue from oviduct was harvested and microarray analysis (Chicken Genome Array, Affymetrix, Santa Clara, CA, USA) was performed (~38,000 probe sets on the array; ~14,000 unique transcripts on the array; duplicates and probe sets without a known Entrez Gene ID were removed.) Affected genes were assigned to biochemical pathways to visualize altered biological processes; treatment responses were compared.

RESULTS

Over 5,000 transcripts in oviduct were significantly affected by at least one selenium treatment ($P<0.01$), thus indicating how potently single micronutrients can act as modulators of gene expression. As expected, the activity of genes encoding important, selenium-dependent, antioxidant defense enzymes was significantly affected by selenium supplementation. For example, the gene encoding glutathione peroxidase 3 (GPx3) was significantly up-regulated by Sel-Plex ($P<0.01$) while the gene encoding glutathione peroxidase 4 was significantly up-regulated by both sodium selenite ($P<0.05$) and Sel-Plex ($P<0.01$), indicating increased levels of these key protective enzymes in the oviduct of Sel-Plex – supplemented animals in particular (Fig. 1).

In addition, sodium selenite significantly up-regulated a number of genes in oviduct that encode a variety of growth and transcription factors critical to reproductive function and embryogenesis, as did Sel-Plex which had a much more marked effect. Sodium selenite significantly up-regulated the gene encoding protein kinase A (PKA) relative to Se-deficient animals, but it had no significant effect on the expression levels of the other follicle stimulating hormone (FSH) pathway component genes. Sel-Plex significantly up-regulated all key component genes in the FSH pathway. Selenium elicited a significant effect on many genes involved in translation in oviduct, relative to Se-deficient animals; Sel-Plex had the most pronounced effect. Sel-Plex up-regulated most of the principal genes involved in translation in oviduct, an effect not previously associated with Se.

In summary, dietary Se sources elicited transcriptional shifts linked with fertility, with clear differences between Se sources, Sel-Plex having the most marked and comprehensive effects. Many of these effects had not been linked to selenium previously and would have remained hidden without the use of a nutrigenomics approach.

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Figure 1. Effect of selenium supplementation on GPx gene expression profiles in oviduct.
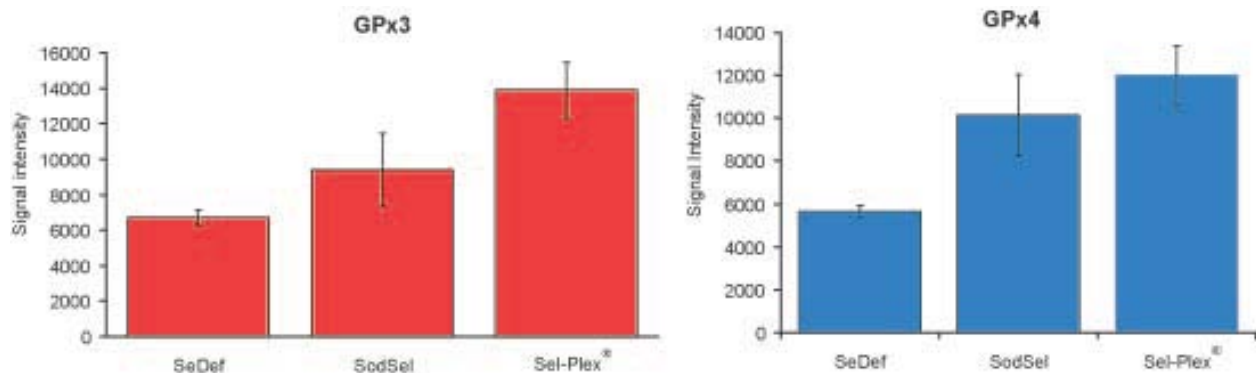
UNUSUAL CUTANEOUS FOWL POX SCRATCH-ASSOCIATED LESIONS IN BROILERS

C.G. Sentíes-Cué, B.R. Charlton, P. Woolcock, A.A. Bickford, G. Cooper, and M. Bland

Six live, eight week old broilers were submitted for laboratory examination with a clinical history of 15% condemnation due to skin lesions. Birds had multifocal, raised, rounded and longitudinal, black pustule-like lesions in several areas of the body associated with scratches. Some of the scratch lesions developed cellulitis consequently. The most common areas for fowl pox such as head and toes were not affected. Only one out of five houses was affected. Fowl pox was diagnosed by histopathology and confirmed by viral isolation and direct electron microscopy. Gross, microscopic lesions, and field investigation will be presented.

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