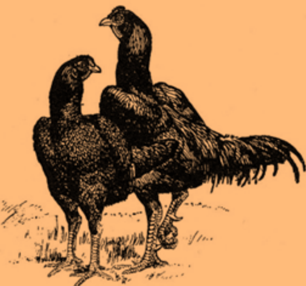
PROCEEDINGS OF THE SIXTY-FIFTH WESTERN POULTRY DISEASE CONFERENCE

April 24-27, 2016 Vancouver, BC, Canada



PROCEEDINGS OF THE SIXTY-FIFTH WESTERN POULTRY DISEASE CONFERENCE

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65TH WESTERN POULTRY DISEASE CONFERENCE

IN MEMORIUM

DUANE E. OLSEN

Duane E. Olsen, of Lacey, Washington, passed away on Oct. 24, 2014. Duane was born Jan. 7, 1941 to Donald W. (Pete) and Eleanor Jean Olsen at Chehalis, Washington. He grew up on the family's dairy farm on Newaukum Hill. Duane attended Napavine Schools and graduated in 1959. He attended and graduated from Western Washington (college) University, Bellingham, Washington and Washington State University's College of Veterinary Medicine where he earned his doctorate degree.

Duane was dedicated to his work. His professional career included veterinarian roles with the WSU extension in Puyallup, Fors Farms, Foster Farms, and finally as general manager of Briarwood Farms in Rochester. He consulted throughout the U.S. and Canada. He also contributed frequently in governmental matters concerning the poultry industry, including serving on President Reagan's task force for avian influenza. Duane was highly regarded in his field. He loved to teach and on occasion argue. Dr. Olsen was a walking encyclopedia relative to information about poultry – truly a gentleman and a scholar. He will be missed by all who had the pleasure of knowing and working with him.

65TH WESTERN POULTRY DISEASE CONFERENCE

IN MEMORIUM

Donald D. Bell

Don Bell was born on Dec. 17, 1933 in Santa Ana California, to Kenneth and Dorothy Bell and died on October 17, 2014. His childhood was spent in several California cities and early on, he showed a real talent for farming. As a teenager, he worked on the family dairy farm and raised cattle for 4H projects and at the age of 18, received the first of many statewide achievement awards for agriculture.

He graduated from Turlock Joint Union High in 1951 and soon entered the University of California – Davis. His intent was to pursue dairy production, but, by the time of his graduation in 1955, he had discovered a passion for poultry production, to which, he devoted the rest of his life. While at UC Davis, he also found the love of his life, Lucy Justice, meeting her at a Cal Aggie Christian gathering. They were married on Sept 2, 1956, soon after his graduation from Davis with a degree in Animal Science. He later went on to receive a Master's Degree in Avian Science from Colorado State University.

After a stint in the U.S. Army, Don began his career with the University of California as a Poultry Farm Advisor with the Extension Service. He began in Orange Co. and later moved to Riverside and served there for his remaining 42 year career. Along the way, he was appointed as Statewide Poultry Specialist and continued his service to the industry, even long after his retirement in 2000. As an Emeritus specialist, he continued to advise, teach, and encourage a new generation of producers through his interactions and his research.

During his career, his prolific writing and research projects provided much of the foundation for today's modern egg industry. In addition to two major textbooks, it is difficult to find any specific topic in poultry that his work did not touch upon at some point. As one of the leading researchers in the field, he was in demand as a keynote speaker and spoke at events around the world. He also consulted with key people in the worldwide industry and government officials, on improving production and welfare of animals. All who heard him speak knew of his commitment to this subject and his helpful nature made lasting friendships in the farthest corners of the world. The hundreds of awards and recognitions for his knowledge and service is now part of his legacy. The phrase "What does Don Bell have to say about it?" has become part of the language of a generation of producers and researchers. One of his last projects was to establish an Electronic Resource Library for the Egg Industry.

But, he was much more than just a researcher and teacher – he had a deep love of photography, art, food, history, and travel. He was also very active in the life of his church, serving as Deacon and Elder for the Riverside Calvary Presbyterian Church. His home was filled with mementos from his life and his travels. Many will remember him just humming a tune or whistling for the sheer pleasure of it as he worked.

65th WPDC CONTRIBUTORS LIST

(As of April 1, 2016)

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SPECIAL ACKNOWLEDGMENTS

The 65th Western Poultry Disease Conference (WPDC) is honored to acknowledge the many contributions and support to the Conference. The financial contributions provide support for outstanding presentations and to help pay for some of the costs of the Conference, thus helping us to maintain a relatively low registration fee for an international conference. More than 30 organizations, companies and individuals have once again given substantial financial support. Many companies and organizations, including some that also contribute financially, send speakers at no expense to the Conference. We thank all these people, and acknowledge their support and contribution.

Once again, the WPDC is forever grateful to our distinguished contributors and supports of the conference who are vital in making the conference a success. All our contributors and supporters are listed on the following pages. We greatly appreciate their generosity and sincerely thank them and their representatives for supporting this year's meeting of WPDC.

Many have provided special services that contribute to the continued success of this conference. For this year's meeting, the WPDC has contracted Conference and Events Services, of the University of California, Davis, for providing budgetary and registration support for the conference. We would like to thank Ms. Teresa Brown for her exceptional work with our conference.

We thank Dr. David Frame for editing and producing another outstanding proceedings of this meeting. Dr. Frame is indebted to Mr. Dana Frame for his meticulous proofreading and formatting the proceedings for publication. We express our gratitude to all authors who submitted manuscripts, and are especially appreciative of those who submitted their manuscripts on time. Once again, we acknowledge Bruce Patrick (Graphic Communications, Brigham Young University) for the front page cover design of these electronic proceedings.

SPECIAL 30th ANNIVERSARY NOTE

This year we had 109 printed manuscripts, which included invited speakers, oral, and poster presentations. I thank all authors for their timely submission of papers and for their patience in resolving any formatting challenges that may have occurred.

Reflecting back on the evolution of the WPDC Proceedings, it is indeed obvious much progress has been made over the years. I still have vivid memories of visiting Rosy at his home with WPDC paraphernalia scattered in seemingly endless and random (although well-organized for Rosy) piles all over his dining room and study. I'm sure none of us can fully appreciate the amount of personal expenses incurred and hours spent in mimeographing, typing, proof-reading, copying, and mailing those early proceedings.

A tremendous leap forward was made in 1986 by Dr. Marcus Jensen in making the printed proceedings available for distribution at the time of the actual conference. This was accomplished using a pioneering technique of collecting manuscripts through the infant World Wide Web. The added tasks of incorporating translated papers from ANECA and shipping the finished bound copies to Mexico made this accomplishment even more remarkable. Also during Dr. Jensen's tenure, the formatting and proof-reading of the Proceedings were generally formalized. Dr. Craig Riddell aptly continued these services for the next four years after the retirement of Dr. Jensen.

A 50th year anniversary CD was produced in 2001 containing all previous WPDC proceedings. In the wake of this accomplishment, a yearly CD was produced from 2002 through 2009 in addition to the traditional hard copy. Beginning in 2010 the CD was substituted with a removable flash drive. The last hard copy of the WPDC Proceedings was printed in 2013. We have discontinued producing hard copies of the proceedings because of the high costs of printing and mailing compared to current almost universal easy on-line access. At the 2014 combined 63rd WPDC and XXXIX ANECA meeting, all presentations were available on flash drive only. From 2015 onward the proceedings will be available through on-line access only.

Since 1986 it has been the firm resolve of the WPDC to provide finished proceedings at or before meeting time. This year's conference in Vancouver, BC marks the thirtieth anniversary of the WPDC Proceedings being accessible at the time of the meeting.

On a related note, with the gracious help of Bob Bevans-Kerr, Executive Director of AAAP, we are consolidating and migrating all WPDC Proceedings into electronic format. This has taken a tremendous amount of work by me, Rich Chin, and Bob in order to digitize and organize this material. The end result however, is that eventually all proceedings of the WPDC since its inception in 1952 will be available on-line.

It is indeed exciting to imagine what the next 30 years will bring to the WPDC. I have no doubt it will be even greater than anticipated.

- David D. Frame, WPDC Proceedings Editor

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65th WPDC PROCEEDINGS

Please note that the proceedings of the 65th 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.*

The proceedings of the 65th WPDC are available in electronic format only. They can be downloaded from the American College of Poultry Veterinarians website (www.acpv.info).

WESTERN POULTRY DISEASE CONFERENCE (WPDC) HISTORY

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

YEAR	PRESIDENT	PROGRAM CHAIR	DEDICATION	RECOGNITION
35th WPDC – 1986	D. W. Waldrip	Duncan A. McMartin	J. A. Allen	
11th ANECA	Jorge Basurto	Mario Padron	A. Tellez-G. Rode	
36th WPDC – 1987	D. A. McMartin	Marcus M. Jensen		
37th WPDC – 1988	M. M. Jensen	Barry Kelly	A. S. Rosenwald	
38th WPDC – 1989	B. Kelly	Masakazu Matsumoto		Louise Williams
39th WPDC – 1990	M. Matsumoto	Jeanne M. Smith		Dean Young
40th WPDC – 1991	J. M. Smith	Richard P. Chin	A. S. Rosenwald	
16th ANECA	Martha Silva M.	David Sarfati M.	A. S. Rosenwald	
41st WPDC – 1992	R. P. Chin	Rocky J. Terry	Marcus Jensen	Henry E. Adler * *(posthumous) R. A. Bankowski C. E. Whiteman
42nd WPDC – 1993	R. J. Terry	A. S. Dhillon	W. W. Sadler	Royal A. Bagley
43rd WPDC – 1994	A. S. Dhillon	Hugo A. Medina		G. B. E. West
44th WPDC – 1995	H. A. Medina	David D. Frame	W. M. Dungan* *(posthumous)	A. J. DaMassa Gabriel Galvan Walter F. Hughes W. D. Woodward R. Yamamoto
45th WPDC – 1996	D. D. Frame	Mark Bland	Don Zander	Pedro Villegas
21st ANECA	R. Salado C.	G. Tellez I.	M. A. Marquez	Ben Lucio M. Mariano Salem Victor Mireles Craig Riddell
46th WPDC – 1997	Mark Bland	James Andreasen, Jr.	Bryan Mayeda	Roscoe Balch Paul DeLay J. W. Dunsing Don Helfer D. E. Stover
47th WPDC – 1998	J. Andreasen, Jr.	H. L. Shivaprasad	W. J. Mathey	Marcus Jensen Duncan Martin
48th WPDC – 1999	H. L. Shivaprasad	R. Keith McMillan		
49th WPDC – 2000	R. K. McMillan	Patricia Wakenell	R. P. Chin	Ralph Cooper Robert Tarbell
50th WPDC – 2001	P. Wakenell	Ken Takeshita		Don Bell Art Bickford
51st WPDC – 2002	K. Takeshita	Barbara Daft	Hiram Lasher	Bachoco S.A. de C.V.
27 ANECA	J. Carillo V.	Ernesto P. Soto		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	B. Charlton	Rocio Crespo	A. S. Rosenwald* *(posthumous)	B. Daft
33rd ANECA	M. A. Rebollo F.	Maritza Tamayo S.	A. S. Rosenwald*	Ernesto Ávila G.
58th WPDC – 2009	R. Crespo	Victoria Bowes		G.L. Cooper
59th WPDC - 2010	V. Bowes	Nancy Reimers		
60th WPDC - 2011	N. Reimers	Larry Allen		John Robinson
61st WPDC - 2012	L. Allen	Vern Christensen		
62nd WPDC - 2013	V. Christensen	Portia Cortes	Víctor Manuel Mireles M.	A. Singh Dhillon

YEAR	PRESIDENT	PROGRAM CHAIR	DEDICATION	RECOGNITION
63rd WPDC – 2014	P. Cortez	Ernesto Soto	Hugo Medina	
39th ANECA	Néstor Ledezma M.	Ernesto Soto	Benjamin Lucio Martínez	
64th WPDC – 2015	Ernesto Soto	Shahbaz Haq	Bruce R. Charlton	David Willoughby
65th WPDC – 2016	S. Haq	Susantha Gomis		

MINUTES OF THE 64TH WPDC ANNUAL BUSINESS MEETING

Secretary-Treasurer, Dr. Richard P Chin, called the meeting to order on Monday, March 23, 2015, at 5:35 PM, at the Holiday Inn Capitol Plaza, Sacramento, CA. There were 23 people in attendance.

APPROVAL OF 63rd WPDC BUSINESS MEETING MINUTES

The minutes from the 63rd WPDC business meeting were discussed. Since a hardcopy of the proceedings was not produced, members of the Executive Committee reviewed the minutes during the Executive Committee meeting and recommended approval as written. A motion was made and carried to approve the minutes as recorded in the Proceedings of the 64th WPDC.

ANNOUNCEMENTS

Dr. Chin acknowledged all the contributors, in particular, Ceva Animal Health, which contributed at the Super Sponsor level, and the American Association of Avian Pathologists, which contributed at the Benefactor level. Finally, all the contributors were acknowledged and thanked for their generous support and donations.
The efforts of the current WPDC officers were acknowledged for their work and participation in the organization of this year's meeting.

We remembered those who passed away since the last WPDC, i.e., Dr. Bruce Charlton, Dr. Duane Olson, Dr. George West [note: Dr. George West, who had Lewy body dementia, passed away on April 2, 2014, during last year's joint meeting.] and Mr. Don Bell, with a moment of silence. All four were highly respected and contributed significantly to poultry medicine in the western region and nationally.

REPORT OF THE SECRETARY-TREASURER

Dr. Chin presented the Secretary-Treasurer report. Unfortunately, he did not complete the budgets in time for the business meeting. Nonetheless, he reported that for the previous meeting in Puerto Vallarta in 2014, we had contributions totaling $26,550.00, with about $16,000 in expenses, leaving WPDC with about $10,000 net profit. During the Executive Committee meeting, ANECA suggested that rather than transfer monies back-and-forth between WPDC and ANECA, that WPDC just keep any profit. Following a discussion, a motion was made, voted upon and approved to accept this recommendation.

Dr. Chin reported that contributions for this year's meeting (the 64th WPDC) are surprisingly very good at $29,450. He estimated that WPDC should have a positive revenue this year even though hotel and travel costs were higher, and WPDC provided extra discounts to students in an effort to increase student registration.

REPORT OF THE PROCEEDINGS EDITOR

Dr. David Frame presented the Proceedings Editor report. There were 60 papers submitted for publication in the proceedings. He thanked the authors for their timely submissions.

Last year, at the combined meeting with ANECA, hard copies of the proceedings were no longer printed. The proceedings were only available in electronic format on a flash drive. Continuing with the advancement in technology, it was decided that this year's proceedings will only be available online. WPDC is grateful to the American College of Poultry Veterinarians for providing space on their website to host the WPDC proceedings. In addition, Dr. Frame has been working with Bob Bevans-Kerr, Executive Director for ACPV and AAAP, to have all published WPDC proceedings available online. Currently, the most recent WPDC proceedings are password-protected. There were a few complaints about having troubles with the password. A motion was made and approved to remove all passwords, and to provide all WPDC proceedings free-of-charge.

FUTURE MEETINGS

It was agreed to continue with the current rotation for meeting venues, with three different locations, i.e., Mexico, Canada, and a location yet to be determined. WPDC will continue to return to Sacramento every other year.

Therefore, in 2016, the 65th WPDC and ACPV Workshop will be in Vancouver, BC, Canada, April 24-27, 2016, at the Vancouver Marriott Downtown Hotel.

In 2017, the 66th WPDC and ACPV Workshop will be in Sacramento, CA, March 19-22, 2017, at the Holiday Inn Capitol Plaza.

After a brief discussion on possible locations for 2018, by a majority vote, Salt Lake City was approved. Dr. Chin will begin looking into venues in Salt Lake City later this year.

WPDC EXECUTIVE COMMITTEE

Dr. Chin reported that the WPDC Executive Committee nominated Dr. Gabriel Senties- Cué for Program Chair for the 66th WPDC in 2017 (in Sacramento, CA.) There were no other nominations and Dr. Senties- Cué was elected unanimously as program chair-elect. Dr. Chin nominated the following officers for 2015-2016:

Program Chair: Dr. Susantha Gomis
President: Dr. Shahbaz Haq
Past-President: Dr. Ernesto Soto
Contributions Chair: Dr. Yan Ghazikhanian
Proceedings Editor: Dr. David Frame
Secretary-Treasurer: Dr. Richard Chin
Program Chair-elect: Dr. Gabriel Senties- Cué

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

NEW BUSINESS

Dr. Chin stated that CE credits will be sent to every registrant from ACPV (Bob Bevans-Kerr). There were no additional items for discussion.

Dr. Soto turned the presidency over to Dr. Shahbaz who acknowledged and thanked those who helped organize this year's meeting.

Dr. Shahbaz adjourned the annual business meeting at 6:05 PM.

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INNATE HOST RESPONSES FOLLOWING INFECTIOUS BRONCHITIS VIRUS (IBV) INFECTION IN CHICKENS

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ABSTRACT

Infectious bronchitis virus (IBV) causes severe infections in multiple body systems in chickens including respiratory system and has significant negative impact on the poultry industry. Control of infectious bronchitis (IB) depends on live attenuated vaccines, but vaccination failures are frequent. Consequently, a novel approach to mitigate effects of this disease is urgently required. Interactions between the host and IBV, as well as mechanisms that lead to either virus clearance or disease progression in chickens, are poorly understood. As a first step, new knowledge regarding the complexity of host-virus interactions, particularly host responses elicited against IBV, is required. In this study, we experimentally infected the specific pathogen free chickens and studied the induction of innate responses early following infection. We found that IBV infection results in an increase in macrophage numbers in trachea and lung suggesting an induction of innate host responses following IBV infection.

INTRODUCTION

There are a range of diseases that impact poultry worldwide, but IBV is still an issue in most countries due to the limitations of vaccines available to protect both meat and egg laying chickens. Live attenuated and inactivated vaccines that are used for the control of IB and, in general, provide protection against field strains. However, the emergence of variant strains becomes a challenge and these heterogonous IBV strains lead to IB outbreaks in vaccinated flocks, leading to subsequent production losses. In addition, attenuated strains used for vaccination can spread among individual birds within the flock, change virulence during the bird to bird passage, and lead to disease and resultant production problems, such as a decrease in egg quality and quantity and a reduction in the size of meat birds. The interaction between host and IBV and the mechanisms which lead to virus clearance and disease pathogenesis are poorly understood. *In vivo* and *in vitro* host-virus models have demonstrated that immune system cells, particularly macrophages, are known to play a pivotal role in many host-virus interactions. Although the role of alveolar macrophages in many viral-host interactions is known, specific information on the interaction of avian respiratory macrophages with IBV infection is scarce, and further research is required to provide a better understanding of how macrophages play a role in the pathogenesis of infectious bronchitis in chickens. The objective of the study was to determine what effect IBV infection has on macrophage numbers in the trachea and lungs.

MATERIALS AND METHODS

Specific pathogen free (SPF) chickens (six days old) were first infected with either IBV ($2.75x10^4$ EID_{50})/bird or PBS at the 12, 24, and 48 hr post-infection. Both trachea and lung were collected and immunofluorescence and qPCR assays were used to determine IBV infection. Any changes in macrophage numbers were determined via flow cytometry.

RESULTS AND DISCUSSION

As demonstrated using the established IBV infection model, IBV infection in chicken trachea and lung results in significant increase in the number of macrophages in these tissues, early following the infection peaking 24 hr post-infection. Further studies are necessary to optimize a technique of complete depletion of macrophages from respiratory tract and then to study the role of macrophages in the IB progression in trachea and lung of chickens.

(The manuscript will be published in a peer review journal.)

CHANGES IN PREVALENCE OF RESISTANCE TO ANTIMICROBIALS IN ENTERIC ORGANISMS FOLLOWING THE VOLUNTARY CHANGE IN ANTIMICROBIAL USE PRACTICES IN THE CANADIAN POULTRY INDUSTRY

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ABSTRACT

The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) tracks temporal and regional trends in antimicrobial use (AMU) and antimicrobial resistance (AMR) in selected species of enteric bacteria obtained at different points along the food chain and from humans. Chickens on farm at pre-harvest (>30 days of age), at the abattoir and at retail are routinely sampled for the detection of *Salmonella*, generic *E. coli,* and *Campylobacter.* Isolates are tested for resistance to antimicrobials used in both human and veterinary medicine. Following the May 2014 industry-led voluntary ban on the preventive use of Health Canada's Veterinary Drugs Directorate (VDD) Category I antimicrobials, which includes 3rd generation cephalosporins (3rd GC) and fluoroquinolones. CIPARS noticed a decrease in resistance to 3rd GC in chicken *E. coli* and *Salmonella* isolates collected at all stages along the food chain. CIPARS also detected a slight decrease in resistance to ciprofloxacin in chicken *Campylobacter.* The decrease in 3rd GC resistance mirrors the decreased use of ceftiofur at the hatcheries. The reason for the decrease in ciprofloxacin resistance is unclear since there are no reported uses of fluoroquinolones on the farms participating in CIPARS. The 3rd GC data are early indications of the poultry industry's success in implementing an intervention to reduce AMR.

INTRODUCTION

In Canada, antimicrobials are available to treat clinically relevant bacterial infections in poultry species (1, 2). Antimicrobials that are considered of very high importance to human medicine by Health Canada (3) and the World Health Organization (4) include the 3rd GC and fluoroquinolones, therefore emerging resistance to these antimicrobials in enteric pathogens have raised public health concerns. CIPARS has previously reported a strong correlation between the number of cases of 3rd GC-resistant *S.* Heidelberg in people and the prevalence of 3rd GC-resistant *S.* Heidelberg in retail chicken (5). Unlike *Salmonella*, monitoring of *E.* coli in humans is not yet included in routine CIPARS AMR surveillance however previous research has identified chicken meat as a source of extra-intestinal *E. coli* infections in humans (6). Research has also shown that resistance to ceftiofur in chicken *E. coli* has been associated with the use of ceftiofur at the hatchery (2). Similarly, fluoroquinolone resistance has also emerged in *Campylobacter* isolated from broiler chickens in Canada (7). To address AMR concerns, the industry gradually reduced the use of ceftiofur subsequent to initial surveillance and research findings (2,5) and in May 2014, the Canadian poultry industry formally eliminated the preventive use of antimicrobials considered highly important to human medicine in broiler chickens and turkeys (8). This policy was extended to include the broiler breeder sector in May 2015. This short paper summarizes AMR trends in *Salmonella, E. coli* and *Campylobacter* isolated from chickens.

MATERIALS AND METHODS

Retail. Sampling of chicken meat involved regular sample collection from randomly selected census divisions, weighted by population, in each participating province/region.

Abattoir. In the abattoir component, cecal samples are regularly collected in 23 participating chicken slaughter plants across the country.

Farm. At the farm, four pooled fresh caecal samples (equivalent to 10 individual droppings) from the four quadrants of the barn were collected for each

farm (n=93 to 143 farms). Approximately half of the flocks sampled were also visited at chick placement for sampling chick paper pads and the environment. Farm-level AMR results described below were adjusted for clustering to account for multiple samples collected per farm

Details regarding methods used for sample collection, culture, and antimicrobial susceptibility testing are available in the CIPARS annual reports (9).

RESULTS AND DISCUSSIONS

Bacterial recovery rates **across programs.** ***Salmonella.*** In 2014, the *Salmonella* recovery rate in chickens from the agri-food chain (farm to retail) was 34%. Overall, recovery rates have remained relatively stable over the last five years (29% to 34%). However, at the farm, the recovery rate at both stages of sampling decreased between 2013 and 2014: chick placement (2013:22%, 2014:12%) and preharvest (2013: 59%; 2014: 44%). The top four serovars detected on-farm at preharvest were *S.* Kentucky (34.5%), *S.* Enteritidis (23.3%), *S.* Schwarzengrund (8.0%) and *S.* Heidelberg (6.4%). The overall decrease in recovery on-farm is an early indication of the impact of farm-level interventions such as vaccination in breeders, and food safety programs; however, despite these efforts *S.* Enteritidis was still detected in some regions over the last two years of farm sampling.

Non-type specific E. coli. The overall recovery rate in chickens from farm to retail has remained stable over the last five years (92 to 96%). On-farm, the recovery rate at preharvest remained at 99% (2013, 2014) and at chick placement recovery was 80% in 2013 and 81% in 2014.

***Campylobacter*:** Across the production chain, the recovery rate in 2014 was 25% and the levels have remained stable over the last five years (24% to 30%). On farm, the levels slightly decreased from 20% in 2013 to 16% in 2014.

Antimicrobial resistance. The industry-led elimination of the preventive use of 3rd GC has resulted in a significant decrease in reported ceftiofur use at the hatcheries; this paralleled the observed changes in resistance to 3rd GC antimicrobials.

Salmonella. Resistance to 3rd GC, highlighted in Figure 1 shows a decreasing trend. On-farm, between 2013 and 2014, the percentage of isolates resistant to ceftriaxone decreased from 29% to 6% at chick placement (gray bars) and from 22% to 12% ($p \leq 0.05$) at preharvest (black bars). No remarkable increases in resistance to any other antimicrobials (ampicillin, gentamicin, nalidixic acid, streptomycin, tetracycline, trimethoprim-sulfonamide) tested were observed but the levels of resistance to streptomycin and tetracycline were relatively high in both years (>30%).

E. coli. Resistance to 3rd GC, presented in Figure 1 also shows a decreasing trend. The decrease in ceftriaxone resistant *E. coli* reflected the reported decrease in the use of ceftiofur at the hatcheries better than the *Salmonella* data. On-farm, between 2013 and 2014, the percentage of isolates resistant to ceftriaxone decreased from 39% to 25% ($p \leq 0.05$) at chick placement (gray bars) and from 32% to 24% at preharvest (black bars). No remarkable increases in resistance to any antimicrobials tested were observed but resistance to streptomycin and tetracycline were relatively high in both years (>40%).

Campylobacter. Resistance to ciprofloxacin slightly decreased overall. On-farm**, t**he percentage of isolates resistant to ciprofloxacin decreased from 16% to 9% (Figure 1). *Campylobacter* were not isolated from chick placement samples. Tetracycline resistance slightly decreased from 59% to 46%. In 2014, low level resistance to azithromycin (3%), a macrolide antimicrobial, and telithromycin (2%), a ketolide antimicrobial, were detected.

CONCLUSIONS

The industry-led intervention to eliminate the preventive use of VDD Category I antimicrobials resulted to a decrease in resistance to 3rd GC among generic *E. coli* and *Salmonella* isolated from chickens at the farm, abattoir and retail/grocery stores. Ceftiofur-resistant *Salmonella* and *E. coli* were also present in the barn environment and in chick meconium, suggestive of a self-perpetuating cycle of contamination with resistant strains within the farm, and vertical transfer from parent to progeny flocks, respectively. Breeder flock level data is yet to be collected to fill the knowledge gap in understanding the ecology of resistant organisms in the chicken sector and to identify other potential intervention points to contain antimicrobial-resistant organisms. CIPARS will continue to monitor trends in AMU/AMR in chickens.

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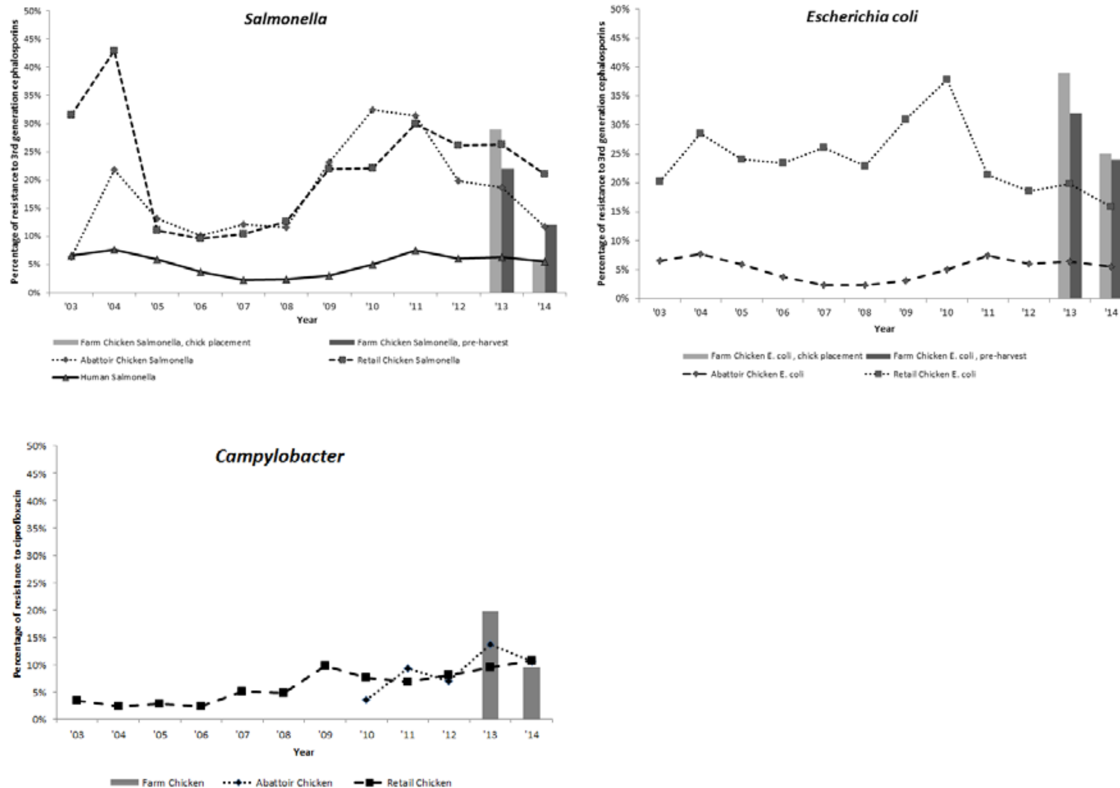
Figure 1.Resistance of *Salmonella, E. coli* and *Campylobacter* isolates from chicken (s).



FARM-LEVEL SURVEILLANCE OF ANTIMICROBIAL USE AND RESISTANCE IN SENTINEL TURKEY FARMS IN CANADA, INITIAL FINDINGS

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ABSTRACT

Turkey is the fourth most commonly consumed meat in Canada. While chickens have been extensively investigated for antimicrobial resistance (AMR) and antimicrobial use (AMU), little is known about the prevalence of AMR-organisms and AMU in Canadian turkeys. Data from retail sampling suggests that turkey meat is contaminated with AMR-bacteria that have resistance profiles distinct from those of chicken, demonstrating that chicken AMR data cannot be extrapolated to turkeys. In 2013 AMU and resistance surveillance was initiated in turkeys at the farm level through the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) in partnership with FoodNet Canada and in collaboration with the turkey industry. The sampling design was built on an existing surveillance framework developed for broiler chickens. This farm program monitors trends and patterns of AMR in generic *Escherichia coli, Salmonella* and *Campylobacter* and AMU administered at the hatchery and on-farm (e.g., water and feed). Overall, resistance of *Salmonella, Escherichia coli* and *Campylobacter* to most antimicrobials increased between 2013 and 2014; of note was the significant increase ($p \leq 0.05$) in resistance to ciprofloxacin among Campylobacter despite no reported use of fluoroquinolones. Antimicrobials from all of Health Canada's Veterinary Drugs Directorate's Categories (Highly important (I) to low importance to human medicine (IV)) were reported as being used.

INTRODUCTION

Surveillance of turkey meats is routinely included in national surveillance systems for AMR in the United States (US), some countries in the European Union (EU) (EFSA), and in Canada. In 2013, approximately 168.1 million kilograms of turkey (eviscerated weight) from 527 registered turkey premises was produced in Canada with 63.2% of production originating in Québec and Ontario (1). Turkey meat ranks fourth in terms of per capita consumption next to chicken, beef and pork (2); farm-level surveillance of AMU and AMR in enteric pathogens from turkeys is therefore an important complement to current retail sampling in Canada in order to further inform risk assessment activities, food safety interventions and antimicrobial prudent use guidelines.

Similar to chickens, food safety implications of consuming poultry meats contaminated with AMR organisms from turkeys have been reported. In the US, turkey contaminated with multi-drug resistant (MDR) *Salmonella enterica* serovar Heidelberg was linked to human outbreaks in 26 states in 2011 (6). In Canada, widespread turkey-meat related outbreaks of *S*. Heidelberg, similar to the US have not yet been reported, although a large increase in the proportion of *S*. Heidelberg isolates recovered from human blood samples occurred between 2012 and 2013 [10% (55/554) to 15% (63/418)] (3). The exact source of these clinical cases has not been determined, but previously a strong correlation was reported between ceftiofur-resistant *S*. Heidelberg in humans and retail chicken suggestive that broiler meat consumption poses a risk to humans for *S.* Heidelberg infection (4). The contribution of turkey meat products to *S*. Heidelberg infection in humans has not yet been assessed. Retail surveillance in this commodity was initiated in 2011 and therefore temporal trends for retail turkey have not been fully established. In comparison, retail surveillance in broilers, beef and pork was initiated in 2003. However, since ceftiofur was previously approved for use in turkey poults and since turkey meat is frequently contaminated with ceftiofur-resistant *S*. Heildeberg, it is likely that turkey meat also contributes to human infections.

Other than a study conducted in Québec turkeys in 2002/04 to investigate the emergence of ceftiofur resistance in *Escherichia coli* (5), AMU data in Canada for turkeys is very limited. This current project summarizes AMR (2013-2014 only) and AMU (2013-2015) results from sentinel turkey farms in British Columbia.

MATERIALS AND METHODS

AMU. Turkey farm data was collected through questionnaires administered by the poultry veterinarian (or designated practice staff) to the producer (or designated farm staff). The questionnaires utilized poult delivery receipts to obtain hatchery information on poult source, hatchery drug use and vaccine administration. The data collector verified this information via a telephone conversation with the hatchery. The farm-level portion of the questionnaire was answered by using feed delivery receipts, farm records, prescriptions and/or by asking the producer. Details were obtained on farm demographics (e.g., age and estimated weight of birds at the time of sampling, farm/barn/floor capacity), biosecurity and vaccination programs. Producers (or a designated farm person) were asked about AMU via feed and water. Data were collected on each diet fed to the flock, including medicated and non-medicated feeds. Information was collected on each type of feed fed including feeding duration, age of flock at the start and end of each ration, active antimicrobial ingredient(s), their concentration(s) in the feed, primary reason(s) for that AMU (growth promotion, disease prevention, or treatment) and diseases targeted. Data collected on exposure to antimicrobials though water was similar to those described for feed AMU. The producers were asked if a prescription was provided by a veterinarian or if the water medication was an over-the-counter purchase. Data were entered into a database and analyzed descriptively and quantitatively using either StataSE Version 13.1 (College Station, Texas) or SASv9.3 (Cary, North Carolina). Three years of data are presented.

AMR. For the recovery of organisms, four pooled (equivalent to ten individual droppings) fresh caecal samples were collected from the four quadrants of the barn for each farm. Details regarding methods used for sample collection, culture, and antimicrobial susceptibility testing are available in the CIPARS annual reports (6). Only two years of data are presented.

RESULTS AND DISCUSSION

Farm characteristics. Twenty-nine to 30 sentinel turkey flocks were sampled each year (n=88, 2013-2015). The flocks sampled represented different marketing weight categories including broilers, light hens, heavy hens, light toms, and heavy toms. Hybrid and Aviagen (Nicholas turkeys) were the strains raised. The poults/hatching eggs were sourced from British Columbia, Manitoba and the United States. Diseases were rarely reported, except for occasional yolk sac infections. Flocks were routinely vaccinated against Adenovirus (Hemorrhagic enteritis) and Newcastle Disease (1-3 times depending on the weight category).

Antimicrobial drug use (2013-2015; n=88 flocks). At the hatchery, only one flock used ceftiofur (Category I) in poults and embryonating eggs. Overall, gentamicin was the most frequently reported antimicrobial use at the hatchery in all years. At the farm, penicillin-streptomycin and tetracycline-neomycin were the only antimicrobials used in water. In feed bacitracin virginiamycin and monensin were the most frequently used drugs.

Bacterial recovery rates (2013-2014 data; n=58 flocks). From 2013 to 2014 recovery rates of *Salmonella* significantly decreased from 35% (39 isolates/112 samples) to 23% (27/116), *Campylobacter* decreased slightly from 79% (88/112) to 73% (85/116), and *E. coli* remained stable at 98% (110/112) and 100% (116/116). The top 4 *Salmonella* serovars in 2013 were *S.* Liverpool (43.6%), *S.* agona (10.3%), *S.* Albany (10.3%), and *S.* Cubana. In 2014, the top four ranking slightly changed: *S.* Agona (25.9%), *S.* Hadar (14.8%), *S.* Heidelberg (14.8%) and *S.* Liverpool (14.8). Among *Campylobacter* isolates, the most frequently isolated species was *C. jejuni* (92% in 2013, 68% 2014).

Antimicrobial resistance (2013-2014 data; n=58 flocks).

Salmonella. From 2013 to 2014 the percentage of isolates resistant to ceftriaxone increased from 19% to 42%. Gentamicin resistance also increased from 9% to 17%. No nalidixic acid resistance was detected. In both years >20% of isolates were resistance to either ampicillin, streptomycin, sulfisoxasole or tetracycline. Resistance levels to these antimicrobials were relatively higher in 2014 than 2013.

E. coli. From 2013 to 2014 the percentage of isolates resistant to ceftriaxone slightly increased from 5% to 9%. Gentamicin also slightly increased from 12% to 14%. Low level resistance to nalidixic acid was detected and the proportion of resistant isolates was relatively stable between years (2013, 2%, 2014, 3%). In both years >30% of isolates had

resistance to ampicillin, streptomycin, or tetracycline with slightly higher levels of resistance detected in 2014.

Campylobacter**.** Results are presented in Figure 1. From 2013 to 2014 the percentage of isolates resistant to ciprofloxacin significantly increased ($p \leq 0.05$). Tetracycline resistance remained stable (36%, 38%). All isolates were susceptible to all other antimicrobials tested (azithromycin, clindamycin, erythromycin, gentamicin, and florfenicol).

CONCLUSIONS

Enteric organisms resistant to antimicrobials were detected from turkey flocks at the farm. Resistance to most antimicrobials generally increased between 2013 and 2014. The reported antimicrobials used in turkey flocks were in categories that ranged from highly important to low importance to human medicine. CIPARS will continue to monitor trends in AMU/AMR in turkey flocks.

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Figure 1. Summary of antimicrobials used in turkey flocks, 2013-2015 (n=88 flocks).

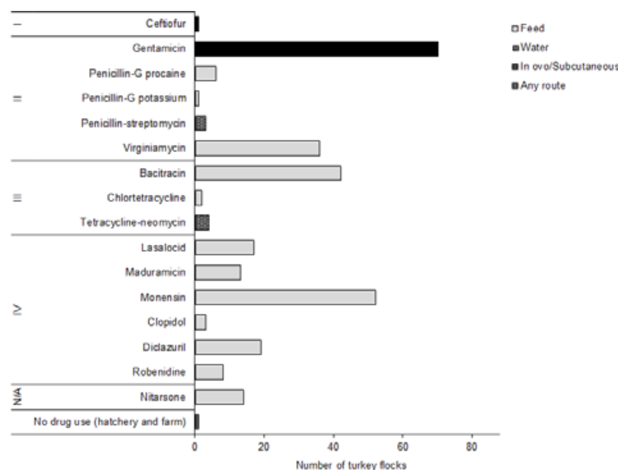
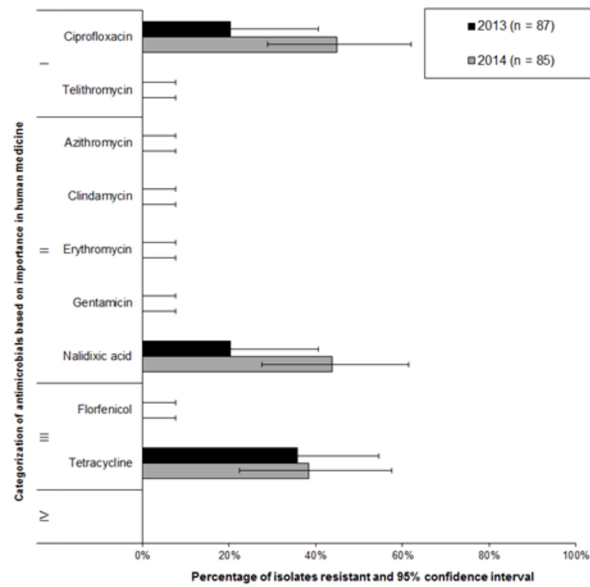


Figure 2. Resistance to antimicrobials in *Campylobacter* isolates from turkey flocks, 2013-2014 (n=58 flocks).



ANTIMICROBIAL USE MONITORING IN CANADIAN BROILER FLOCKS – RESULTS FROM THE CIPARS FARM SURVEILLANCE PROGRAM (2014 UPDATE)

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SUMMARY

The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) has been collecting data on antimicrobial use (AMU) and antimicrobial resistance (AMR) from sentinel broiler chicken farms across the major poultry producing provinces in Canada since April 2013. Producers, through their veterinarians voluntarily provided data and enabled collection of samples for bacterial culture and susceptibility testing. The objectives of CIPARS Farm Surveillance in broiler chicken are to provide data on AMU and AMR, to monitor temporal and regional trends in the prevalence of AMR, to investigate associations between AMU and AMR, and to provide data for human health risk assessments. In 2014, 143 sentinel farms provided data and samples; the total number of birds reared in these premises over six quota periods was equivalent to 7% of the national production. The flocks surveyed were largely conventional (n=126) in their production methods with only a few flocks indicating antimicrobial free (ABF, n=12) and organic (n=1) production practices. In Canada, ABF flocks are not exposed to any antimicrobials including ionophores and chemical coccidiostats from incubation/hatch to slaughter age. Broiler strains reared on participating farms included Ross (n=121), Cobb (n=42), and Hubbard (n=1). In 2014, antimicrobials used in broiler chickens largely belonged to Health Canada's Veterinary Drugs Directorate's Category's III to IV (High to no importance to human medicine) with very limited use of antimicrobials that are categorized as highly important to human medicine.

METHODS

General Surveillance Design. CIPARS monitors AMR and AMU through a framework of fifteen purposively selected sentinel poultry veterinary practices located in the major poultry producing provinces across the country. These practices implemented specific inclusion/exclusion criteria to enroll143 broiler producers. Allocation of flocks per province was proportional to the number of broiler producers or provincial flock populations. The exception to this approach was that in the three provinces where CIPARS collaborates with FoodNet Canada, a minimum of 30 flocks were sampled; regardless of broiler flock numbers or provincial flock populations. Samples for antimicrobial susceptibility testing and AMU data were collected one week prior to the end of grow/pre-harvest. In a subset of flocks samples were also collected at chick placement. Within a farm site only one randomly selected flock was sampled per year.

Farm data collection and analysis. Data on hatchery and flock-level demographics, farm and industry operational factors, biosecurity, AMU and flock health were collected by questionnaire at each sampling visit. Data were entered into a PostGreSQL Database and descriptive statistics were obtained with StataSE Version 13.1 (College Station, Texas) or SASv9.3 (Cary, North Carolina). Antimicrobial exposure from hatching egg/chick stage to end of grow were summarized by active ingredient or antimicrobial class (for feed antimicrobials). The reasons for use (e.g., treatment, prevention, growth promotion) and diseases targeted were also determined.

Quantitative estimates. Using methods adapted from the approach used in in Europe through the European Surveillance for Veterinary Antimicrobial Consumption (1), the quantity of antimicrobials in mg active ingredient adjusted for population and weight, expressed as mg/PCU (Population Correction Unit), was determined and compared to the national quantities of antimicrobials distributed for sale for use in animals provided by the Canadian Animal Health Institute (CAHI). Average weight at treatment used for calculating mg/PCU in broiler chickens and the national sales data were based on European standards to better harmonize and compare Canada AMU data internationally.

Details regarding methods used for data collection and analysis are available in the CIPARS 2013 Annual Report (2).

RESULTS AND DISCUSSION

Hatchery. Antimicrobials were administered to chicks or embryonating eggs at the hatcheries on 35% (50/143 farms) farms; the number of farms reporting AMU administration at the hatchery in 2014 was significantly lower compared to 2013 (58%, 57/99). Three antimicrobials were used: ceftiofur (6% of farms), gentamicin (5%) and lincomycin-spectinomycin (24%). The use of ceftiofur was documented prior to a voluntary change by the chicken industry to eliminate the preventive use of antimicrobials that are considered of very high importance to human medicine. The reported use of ceftiofur (0.10 to 0.20 mg/chick) significantly decreased from 31% of farms (2013) to 6% (2014) ($p \leq 0.05$) whereas reported use of lincomycin-spectinomycin (0.75 mg/chick, consisting of 0.50 spectinomycin and 0.25 mg lincomycin) and gentamin (0.20 mg/chick) remained relatively stable at 24% and 5% respectively.

Feed. Ninety one percent (128/141) of farms reported AMU in feed, similar to 2013 (93%, 90/97). The following antimicrobials were reported: avilamycin, bacitracin, oxytetracycline, penicillin, trimethoprim-sulfadiazine, tylosin, and virginiamycin. Ionophores and chemical coccidiostats were also frequently used. Except for trimethoprim-sulfadiazine, which was used for treatment of respiratory and/or systemic diseases, these antimicrobials were largely used to prevent enteric diseases (e.g., coccidiosis and necrotic enteritis).

Water. Medication via the drinking water was also reported on 14% (20/141) flocks; the number of farms that reported AMU in water increased from 2013 (7%, 7/97). The following antimicrobials were reported: apramycin sulphate, amoxicillin, neomycin-chlortetracycline, penicillin, sulfamethazine, sulfaquinoxaline and sulfaquinoxaline-pyrimethamine. These were mostly used for the treatment of neonatal and systemic diseases, and rarely reported used for disease prevention.

Quantitative analysis. In 2014, the overall quantity of antimicrobials used on farm, adjusted for population and weight, excluding ionophores, chemical coccidiostats and anti-protozoals (e.g., sulfaquinoxaline-pyrimethamine) increased from 151.5 mg/PCU in 2013 to 206.4 mg/PCU in 2014 while the national mg/PCU for total quantities distributed for production animals remained stable at 162 and 163 mg/PCU for 2013 and 2014, respectively. For the farm-level data, the increased number of flocks reporting AMU in water in 2014 contributed to the increase in mg/PCU. Despite the increased mg/PCU on farm, there was only a slight change to the ranking of antimicrobials used in chickens from the previous year. In 2013, the top three antimicrobials based on mg/PCU included bacitracin, streptogramins and trimethoprim and sulfonamides whereas in 2014 the top three in terms of mg/PCU were bacitracin, penicillin, trimethoprim and sulfonamides.

The relative quantities (kg) of antimicrobials distributed for use in in production animals (including ionophores and chemical coccidiostats) compared to broiler chicken on farms is presented in Figure 2. Compared to the other production animal species the spectrum of antimicrobial drug classes used in broilers is distinct. Of note is the relatively high proportion of tetracycline used in production animals (39%) compared to broiler chickens (3%).

Figure 2 represents the Canadian Animal Health Institute national sales data and figure on the right depicts overall usage in sentinel broiler chicken flocks participating in the CIPARS broiler farm surveillance program.

Note: For the CAHI data, Values do not include own use imports or active pharmaceutical ingredients used in further compounding. Production animals include horses.

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Figure 1. Percentage of broiler flocks reporting antimicrobial use at the hatcheries, 2013-2014.

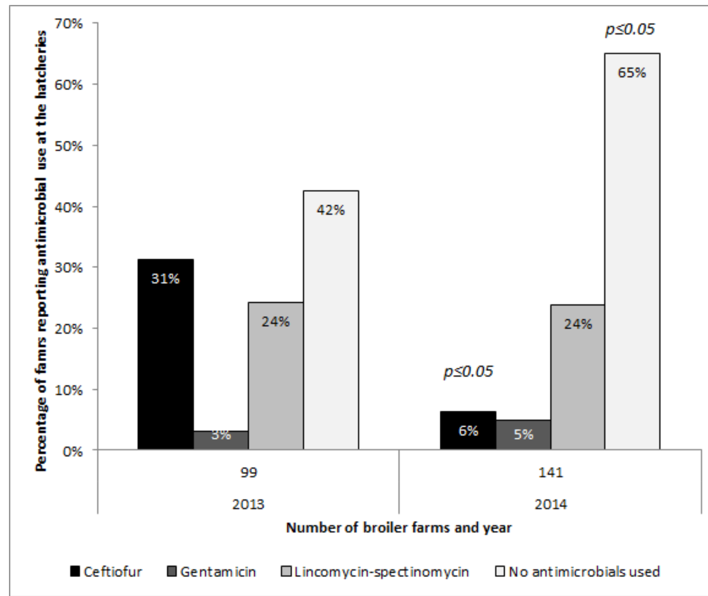
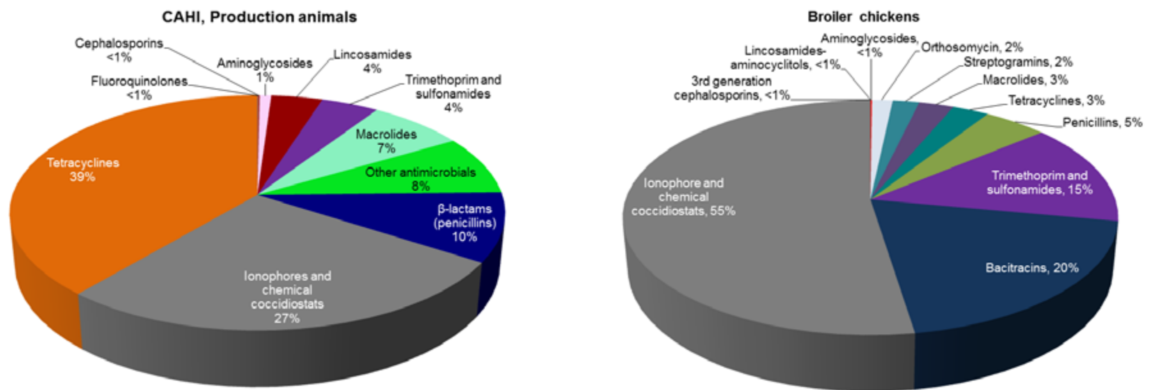


Figure 2. Distribution of antimicrobials used in production animals compared to broiler chickens, in kilograms active ingredient, 2014.



PROTECTION AGAINST INFECTIOUS BURSAL DISEASE AND PERFORMANCE OF COMMERCIAL BROILERS IMMUNIZED IN OVO WITH A RECOMBINANT HVT-IBD AND/OR 89/03 VACCINES

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SUMMARY

The purpose of this study was to determine the synergistic effect of the simultaneous *in ovo* administration of a recombinant turkey herpesvirus expressing the VP2 gene of infectious bursal disease virus (rHVT-IBD) and/or a live attenuated 89/03 vaccines on performance and protection against challenge. Eighteen hundred commercial broiler fertile eggs were divided in four treatment groups. Three groups were vaccinated *in ovo* before transfer with a rHVT-IBD, the 89/03 strain and both vaccines, respectively. Soon after hatching, chicks were allocated in floor pens using a completely randomized design. Performance parameters included body weight, feed conversion and carcass analysis. Protection against challenge was performed by moving randomly selected broilers from floor pens to isolation units. Oral challenge with an IBDV Variant E was performed at 7, 14, and 21 days of age. Protection against challenge was determined based on bursal body weight ratio and histopathological evaluation of bursa tissues. Histopathology lesions such as lymphoid depletion, necrosis, histiocytic infiltration and fibrosis were evaluated.

Adequate maternal antibody titers against IBDV were observed at day of age, with a geometric meant titer of 8,853 (IDEXX ELISA) and a coefficient of variation of 30.6%. Increased lymphoid depletion and histiocytic infiltration was observed at 14, 21, and 28 days of age in the bursas from all the challenged groups but the group vaccinated with both, the rHVT-IBD and 89/03 vaccines. When compared with the non-vaccinated/non-challenged group, an increase in body weight at 1, 14, and 19 days of age, as well as an improvement in carcass composition (breast yield: carcass and breast yield: live weight) was observed in the rHVT-IBD and 89/03 vaccinated group.

SCREENING FOR INFECTIOUS BRONCHITIS VIRUS (IBV) IN SHELL LESS EGG SYNDROME (SES) CASES IN WESTERN CANADA

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ABSTRACT

Shell-less egg syndrome (SES) is a condition characterized by sudden drop in egg production and shell-less eggs. SES outbreaks have been rising in the Western Canada layer operations for the last three years with economic losses. Currently the causative agent of the SES is not known and believed to be caused by a “variant” infectious bronchitis virus (IBV) owing to the fact that many of the SES outbreaks have been reported in IBV vaccinated flocks. Therefore, current study aims at detection of IBV from the flocks with SES history in Western Canada. Quantitative PCR (qPCR) was performed targeting the nucleoprotein (N) gene of the IBV genome for tissue samples collected from layer flocks with the history of SES in Saskatchewan and Alberta. We observed the association of IBV genome in the samples originated from SES and further investigations are underway for the characterization of the IBV isolates.

INTRODUCTION

SES is a condition characterized by sudden drop in egg production and shell-less eggs. SES outbreaks have been rising in the Western Canada layer operations for the last three years with economic losses. Currently the causative agent of the SES is not known and believed to be caused by a “variant” IBV owing to the fact that many of the SES outbreaks have been reported in IBV vaccinated flocks. Therefore, current study aims at detection of IBV from the flocks with SES history in Western Canada.

MATERIALS AND METHODS

Samples were collected from layer flocks with the history of SES raised in Saskatchewan and Alberta (Table 1).

Screening samples for infectious bronchitis virus (IBV). Viral RNA extraction was done using a Trizol based method using the tissue homogenates (Ambion, USA). Lungs, cecal tonsils, kidneys, and uterus were used for RNA extraction since the chances of virus multiplication is high in these tissues. A part of extracted RNA was immediately converted to cDNA using random primers to be used for downstream applications. Real-time polymerase chain reaction (rt-PCR) assay was used to amplify 200 base pairs (bp)-sized fragment of the IBV N gene (1). Further, positive samples were amplified on S1 (1760bp) using conventional PCR (2). This is the hyper-variable region in the IBV genome resulting in emergence of the new variants. The idea is to characterize the positives to identify the strain in order to differentiate field strains from vaccinal types.

RESULTS AND DISCUSSION

Sample collection from Saskatchewan and Alberta is ongoing viral RNA has been extracted from 91 tissue samples belonging to some of the farms in the above table. Two (n=2) samples were weakly positive for IBV based on the real time PCR assay (Cycle threshold= 33-34). All attempts of conventional PCR amplification of the real time PCR positive samples were failed. This could be due to low copy number of the starting viral genome material. Therefore, in order to increase the detection limits of the virus from tissues, virus propagation is being done on 9-11 days old specific pathogen free embryonated chicken eggs (3). Embryonated eggs were inoculated with tissue homogenates originating from tissue samples via allantoic cavity. Allantoic fluid was harvested upon infection in order to extract viral RNA. These inoculations have been conducted and we are in the process of analyzing allantoic fluid samples using PCR assays.

With a view of minimizing animal use (embryonated eggs), initially we screened the collected samples for the presence of IBV genome using highly sensitive real time PCR assay targeting a highly conserved viral gene, N. The plan was, then to go for egg isolation of the IBV from PCR positive samples. This approach yielded only two IBV positive samples of the screened 91 nucleic acid samples. Additionally, we have collected homogenate from tissue samples and inoculated them into the allantoic cavity to increase the IBV genome amount. We have isolated RNA to screen these allanoic fluids using real time PCR and conventional PCR before being submitted for sequencing targeting the S1 gene of the IBV.

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Table 1. Details of the samples collected from the poultry flocks with the history of SES in Western Canada.

Number of samples	Number of farms	Province of origin	Tissues received
Pooled	5	SK	Shell-less eggs, cecal tonsils, oviduct, kidney, lungs
25	7	SK	Cecal tonsils, kidney, oviduct, lungs, trachea
33	4	SK	Cecal tonsils, kidney, oviduct, lungs, trachea
5	1	AB	Moribund birds
33	8	SK	Cecal tonsils, kidney, oviduct, lungs, trachea

ISOLATION AND CHARACTERIZATION OF AVIAN REOVIRUSES BY PHYLOGENETIC ANALYSIS AND RESTRICTION ENZYME FRAGMENT LENGTH POLYMORPHISM (RFLP)

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ABSTRACT

Tendon tissues of birds suspected of avian reovirus (ARV) infection were collected from different farms of Saskatchewan to identify genotypes of ARV circulating in the region. A total of 28 viruses were isolated from tendon homogenates in cell culture using LMH cells. The σC gene encoding the variable σC protein was amplified by reverse transcription PCR (RT-PCR) and sequenced. Molecular classification and phylogenetic relationships of the isolates were examined by aligning the σC sequences with other reference genes published in the Genbank. Based on the phylogenetic tree, the isolates were grouped into four separate lineage groups. In addition, Restriction enzyme fragment length polymorphism (RFLP) was performed on the σC gene of a prototype virus from each group. The genes were digested with *Dde*I, *Hinc*II, *Taq*aI, *Bcn*I,or *Hae*III restriction enzymes. As expected, the DNA polyacrylamide gel electrophoresis results showed different cleavage patterns suggesting heterogeneity. Our study describes genotypes of ARVs that are not genetically well characterized yet, and a more detailed molecular characterization and study of pathogenicity will help in understanding the pathogenic reovirus strains circulating in the region, which would help in developing suitable vaccine.

INTRODUCTION

ARV's are grouped under the *Orthoreovirus* genus in the family *Reoviridae* (6). ARV is implicated as the major cause of tenosynovitis in chickens which is characterized by swelling of the hock joint. Depending on the degree of severity, affected birds may be unable to walk resulting in poor growth and poor production and sometimes death causing considerable economic losses (4).

ARV's are non-enveloped viruses with icosahedral double capsid containing ten segments of double stranded RNA (dsRNA) genome. The genomic segments are divided into three size classes (i.e. Large [L], Medium [M] and Small [S]) based on their electrophoretic mobility on a polyacrylamide gel (6). The ARV genome encodes four non-structural proteins (μNS, μNS, P10 and P17) and eight structural proteins (λA, λB, λC, μA, μBC, σA, σB and σC (1). The third open reading frame of the S1 gene encodes the σC protein. It is 326 amino acids long and is the most variable protein in the reovirus genome (4) and conatins both type specific and broadly specific epitopes and induces the production of neutralizing antibodies (5). Because of these reasons, the σC gene of reovirus is commonly used to classify different reovirus isolates into different distinct genotype groups. In recent years, there has been an increased incidence of reovirus associated tenosynovitis in poultry farms in Saskatchewan, Canada, regardless of reovirus vaccination programs using commercially available vaccine strains. Hence, the objective of this study was to isolate and characterize reoviruses from tendons of clinically sick birds, and classify the isolated strains into genotype groups to identify the new variants, which would help in designing more effective vaccine based on the circulating ARV strains.

MATERIALS AND METHODS

Cells and media. LMH cells (ATCC) were used for isolation and propagations of avian reo viruses. The growth medium for cell culture consisted of DMEM 12 with 10% calf serum, 20mM HEPES and 2mM L-glutamine (Life technologies) and 50mg/mL gentamicin. For propagation of virus stock the same media was used without calf serum.

Virus Isolation and propagation. Tendon tissues collected from chickens with arthritis/tenosynovitis in 2014 and 2015 from different farms of Saskatchewan were individually minced and homogenized in a bullet blender storm-24 and tubes with beads (Next Advance) for 10 min with maximum speed. The homogenate was centrifuged at 3000 rpm for 10 min and the supernatant was filtered through a 0.22um filter. Thereafter, the filtrate was diluted and added onto LMH cells freshly grown on six well plates. Subsequently, the cells were observed for the

appearance of reovirus specific cytopathic effect (CPE) over a period of seven days. Supernatants were collected from positive wells and passaged once and stored at -80°C freezer.

RT-PCR, sequencing and phylogenetic tree analysis. A segment of the σC gene of reo virus was amplified by RT-PCR using specific primers. Briefly, total RNA was purified from virus infected cells using RNA purification kit (Qaigen) as per the company's protocol followed by RT-PCR using a one-step RT-PCR kit (Qaigen). The PCR products were run on a 0.8% agarose gel and gel purified using a gel purification kit (Qaigen) as per the company's protocol. The DNA samples were then sequenced using σC forward and reverse primers (Macrogen, South Korea). Assembly and sequence analysis was performed using BioEdit software v7.2.5 and phylogenetic tree was constructed using MEGA 6 software.

Restriction enzyme fragment length polymorphism (RFLP). The reovirus isolates fell into different cluster groups after sequence and phylogenetic analysis based on the σC gene. One prototype reovirus isolate from each cluster group was selected for RFLP analysis. Briefly, RNA was purified from infected LMH cells and RT-PCR was performed using a one-step RT-PCR kit (Qaigen). The PCR products were purified from agarose gel using GenJet gel purification kit (Fermentas). The purified DNA fragments were digested with either *Dde*I, *Hinc*II, *Taq*aI, *Bcn*I,or *Hae*III restriction enzymes. The enzymes were selected based on published S1 gene sequences of ARV (2). The digested gene fragments were run on a vertical 10% DNA polyacrylamide gel, stained with ethidium bromide and imaged on a gel-doc (Biorad).

RESULTS AND DISCUSSION

Virus isolation, sequencing and phylogenetic tree analysis of reovirus isolates. An effort was made to isolate reovirus from tendons of broiler chickens that demonstrated clinical sign of lameness/tenosynovitis from poultry farms of Saskatchewan. The reovirus positive samples brought about a typical reovirus CPE with fusion of cells and formation of syncytia on cell culture. Overall, a total of 28 viuses were isolated.

The σC gene of avian reovirus is the most variable region in the reovirus genome (4). To classify and study the genotypic properties of the 28 reoviruses isolates, the σC gene of each isolate was sequenced. The phylogenetic tree analysis for conservation of the σC gene was performed with 30 reference strains retrieved from the GenBank. The results revealed that the isolates were grouped into four distinct genotypic clusters. Surprisingly, none of the isolates were clustered together with the reference vaccine strains. Among the isolates, 23.08%, 11.54%, 34.62% and 30.76% were clustered with groups III, Va, Vb and VI, respectively and they differ from the well described strains that are being used for commercial vaccines.

RFLP profiles of ARV isolates. To further classify and differentiate the ARV isolates, the PCR amplified σC gene fragments of the ARV isolates were digested with different types of restriction enzymes based on previously published S1 gene sequences of ARV. Only prototype ARV isolate from each cluster group (III, Va, Vb and VI) was selected based on the phylogenetic tree analysis. As expected, the DNA polyacrylamide gel electrophoresis results confirmed different cleavage patterns suggesting heterogeneity. *Bcn*I enzyme created only two cleavage patterns. On the other hand, *Hae*III, *Hinc*II, *Dde*I and *Taq*αI produced four different cleavage patterns. Except for *Bcn*I, the pattern produced by each enzyme was different for each prototype from each cluster group.

Our study described genotypes of ARVs that are not genetically well characterized yet, and a more detailed molecular characterization and study of pathogenicity will help in understanding the pathogenic reovirus strains circulating in the region, which would ultimately help in developing suitable vaccine.

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CHARACTERIZATION OF AN UPPER RESPIRATORY INFECTION IN YOUNG POULTS IN A COMMERCIAL TURKEY OPERATION

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CASE REPORT

Introduction. An increasing number of upper respiratory infections were diagnosed during the production year 2012-13 and continued during 2014-2015. Standard procedures of water sanitation and chlorination were instituted to try to control or prevent the spread of the infection. Standard procedures of vaccination for *Bordetella avium* were followed using previously successful protocols (1); however, vaccination seemed to become increasingly less effective.

Clinical signs, epidemiology, and effect on production. Initially, the disease manifested itself by wet nares, increased lacrimal secretion, a ragged yellow appearance of eyelids (Figure 1), infraorbital sinus swelling (Figure 2), and a persistent cough. The affected birds were apparently immunosuppressed and became susceptible to a variety of other localized and systemic infections.

The disease was characterized statistically by mortality patterns as well as seven-day, five-week and final body weights. Initially during the 2013-14 year, the first signs of infection manifested at two to three weeks of age and increased five-week mortality due primarily to other secondary infections occurred. Mortality during the initial week of life was not affected. However, during May of the 2014-15 production year, the disease began afflicting birds as young as three to four days of age. Hens seemed to suffer greater mortality than did tom poults. The seven-day tom mortality increased from 2.6% to greater than 5%. The greatest portion of the mortality was at five, six, and seven days of age. Hen poult mortality increased from 2.4% to greater than 5% as well. The days that hen poults died were similar to that of toms.

Five week mortality appeared to decline as the transition from 2014 to 2015 occurred, but increased abruptly in February preceding the increased mortality at day three to four of age. Tom mortality increased from 5 to 14% whereas hen mortality increased from 3 to 14%.

Body weights at seven days had been unaffected prior to the increased mortality seen at days five to seven of age, but when the infection was detected earlier in five to seven day old poults; it was accompanied by decreased seven-day and five-week body weights in both hens and toms. Tom weights at five weeks of age declined from 3.7 to 2.8 lbs. whereas hen weights declined from 3.1 lbs to 2.5 lbs. The growth at 136 and 84 days of age was also affected by the increased five to seven mortality as measured by average daily gain. The average daily gain of 136 day old toms declined from 0.31 lbs per day to about 0.27 lbs per day. The average daily gain for hens at 84 days declined from 0.18 lbs per day to 0.15 lbs per day.

Gross lesions. Gross lesions were restricted to the sinuses and trachea in the early stages; airsacculitis and occasional congested lungs were sometimes present during later stages if secondarily infected with *E. coli*. The most striking lesion, and the cause of substantial early mortality from suffocation, was a mucus plug within the tracheal lumen (Figure 3). Reddening of the tracheal lumen was also a consistent finding (Figure 4); however, only minor cartilaginous weakening was noted. Facial swelling is also present in many affected turkeys (see Figure 2).

Histologic characterization of tracheas. Tracheas of one, seven, 14, 21, 28, and 35-day-old poults were evaluated histologically. There was no significant microscopic lesions in one and seven-day-old turkeys; however in 14-day-old poults, clear evidence of an early tracheal inflammatory reaction was present. The inflammatory reaction is mostly heterophilic in the 14 and 21-day-old birds, and then becomes mainly lymphocytic. Tracheal mucosal epithelial cell injury started to become visible by 21 days of age and became significant by 28 days. At 28 days of age, epithelial hyperplasia started to become more pronounced (regeneration). By 35 days of age, most of the tracheas had recovered cuboidal epithelium and some had normal epithelial cells with long cilia as expected. However, lymphocytic inflammation, loss of glands and goblet cells, and epithelial hyperplasia were still prominent in most tracheas by 35 days of age.

Diagnostic investigation. Repeated serologic testing and virus isolation attempts confirmed no detection of infection by metapneumovirus, paramyxovirus, or avian influenza. *Mycoplasma gallisepticum* and *M. synoviae* were not detected either serologically or by PCR. *Bordetella avium* was isolated sporadically from the trachea, but then only during the early stage of clinical illness. Prevalent secondary bacterial isolations from the trachea included *Pseudomonas* spp., *Proteus* spp., *Enterobacter* spp., and *Escherichia coli*. Routine serologic flock surveillance showed an increase of *B. avium* ELISA titers in 2012-13 compared to earlier years.

Discussion. After extensive diagnostic investigation, it appears that the upper respiratory infection was caused primarily by *B. avium*. Anecdotal observations suggest that the current endemic strains may be becoming refractory to the classic temperature-sensitive (ts) vaccine strain 87 used in Utah for many years (1,2,3,4). The addition of contemporary local strains of ts *B. avium* isolates integrated into the vaccination program appears to be reducing the prevalence of this upper respiratory problem in the field.

Historically through the 1980s and 90s, clinical disease in Utah turkeys caused by *B. avium* only manifested itself in turkeys eight-week-old and older. The vaccination protocol employed between two and four weeks of age did an excellent job of eliminating clinical disease. However, apparent alteration(s) in the epidemiology, infectivity, and/or immunogenicity of current wild-type *B. avium* in Utah are allowing it to cause substantial clinical disease. It seems to be accompanied by increased earlier mortality as well as greatly suppressed body weights at five weeks and 84 days in hens and 136 days in toms. Further studies are needed to characterize the changes that may have occurred and what, if any, were additional ancillary causes of this upper respiratory illness.

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Figure 1. Lacrimal discharge and ragged yellowish appearance of eyelids.

Figure 2. Facial swelling.

Figure 3. Tracheal plug.

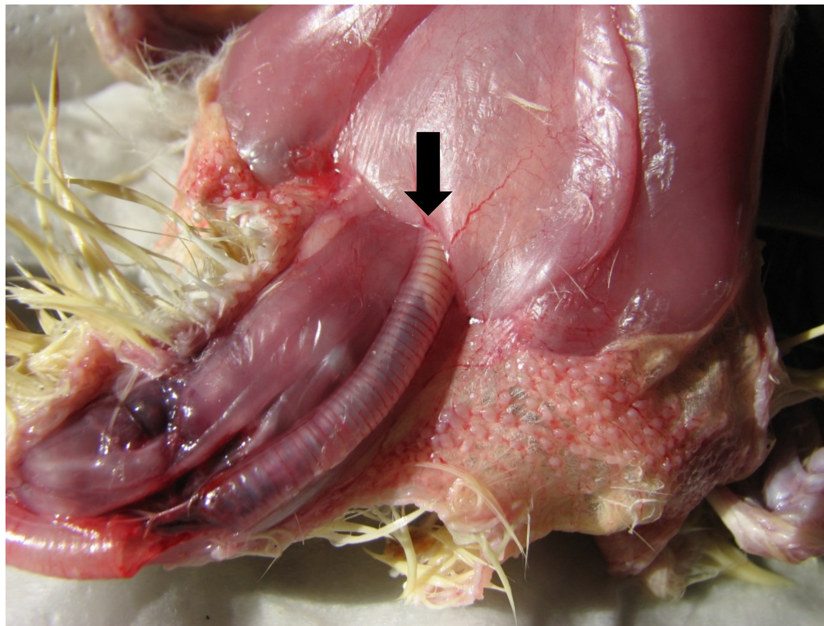
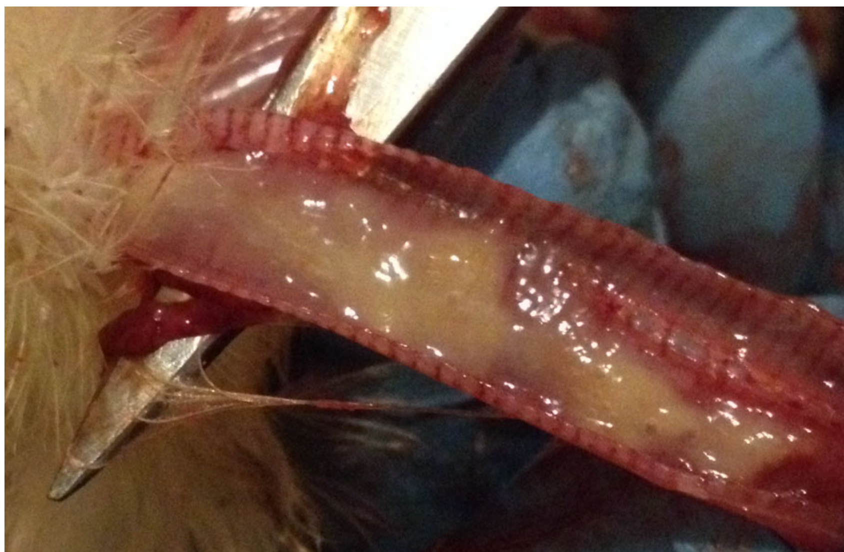


Figure 4. Reddened tracheal mucosa with lumen containing abundant mucus.



MULTI-PURPOSE MULTI-STRAIN PROBIOTIC/PREBIOTIC LACTIC ACID PRODUCING BACTERIAL PREPARATION

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INTRODUCTION

The principle of competitive exclusion is a natural process that is hindered by our modern poultry husbandry practices. In the past when the chick hatched under the setting hen, their intestines became colonized within a few hours with the adult hens normal health intestinal bacteria. With nest clean eggs, highly sanitized hatcheries and farms, today's birds are delayed or prevented from acquiring their mother's "protective gut flora". The history of competitive exclusion dates back to 1908 when longevity of Bulgarians was attributed to the daily consumption of yogurt. Then in 1973, Drs. Nurmi and Rantala found they could prevent salmonella infections by feeding chicks anaerobic cultures of normal intestinal adult chicken flora and referred to this process as competitive exclusion (1). Further work by Pivnick and Nurmi (2) demonstrated that introduction of intestinal flora of adult birds to newly hatched chicks resulted in immediate resistance to infectious doses of *Salmonella*.

In the USA, the original effective Competitive Exclusion, Aviguard (Bayer) culture was 'a non-defined multi-strain culture' and regulatory agencies had licensing issues with it, though the products were effective in reducing gut pathogen colonization (3, 4).

Effective microorganisms (EM) is a "defined competitive exclusion, multi-strain, multispecies microbial product with poly-functional properties." EM culture meets regulatory and GRAS approval.

WHAT IS EM?

EM is a term coined by its discoverer, the Japanese agricultural scientist and university professor, Prof. Dr. Teruo Higa. EM technology has been in use around the globe since 1982. EM-1® is a multi-strain microbial mixture primarily consisting of lactic acid and phototrophic bacteria, yeast and ferment-active fungi, most of which are used to manufacture foodstuffs or as ingredients. When this mixture of naturally occurring and non-genetically manipulated microorganisms comes into contact with organic materials, the microorganisms produce an abundance of natural substances such as vitamins, organic acids, mineral chelate compounds and various antioxidants.

EM can be applied as inoculants to shift the microbial diversity of intestinal and digestion functions, soil, environmental and plant micro-flora in ways that can improve animal health, soil quality, growth and yield of crops, and clean-up of environmental pollutants. The microorganisms comprising EM are neither toxic nor genetic engendered types; but are natural occurring species that have been isolated from natural environments worldwide and are selected for their specific beneficial effects and compatibility in mixed cultures.

Dr. Higa's research on the concept and use of EM began in the late 60s. For the past fifty years it has been directed largely towards resolving problems associated with livestock, human health, soil degradation, declining productivity in crop yields and intensive use of agricultural chemicals in monoculture cropping systems. Interest in the concept increased steadily and by 1982 EM had become a marketable product and considerable on farm testing has been done to demonstrate its merits. During the past decade the livestock, agricultural, environmental and consumer organizations in many countries have expressed an interest in EM as a possible means of reducing antibiotics and chemicals in livestock production, chemicals fertilizers and pesticides in their food production systems. EM has allowed many farmers to make a success transition from drugs and chemical based conventional agriculture to non-chemical, organic farming systems, and with considerably less risk. Consequently there are now more than 60 countries that are using EM to achieve a more sustainable agriculture environmental. Some countries have incorporated EM technology into their national agricultural research and development policies and agendas.

Among the most predominant types and numbers of microorganisms that comprise EM cultures are lactic acid bacteria, yeasts, and photosynthetic bacteria. Lacto-bacillus spp. and yeasts have long been used for processing fermented foods and beverages for human consumption. EM technology provides direct applications of beneficial microorganisms to agriculture, fisheries, forestry and

the environment; and indirect applications of EM metabolites or by-products to food processing sanitation, medicine and health. A number of countries have now granted permits for the production and registration of EM including the United States.

Microorganisms can be broadly classified into three types depending on their principal functions, i.e., synthesizing, decomposing and neutral types. EM are considered to be mainly of the synthesizing type, which impart beneficial effects to agricultural and the environmental processes by generating a wide array of bioactive substances. Many of these substances produced by EM cultures can function as antioxidants and bacteriocins

During normal metabolic reactions in living systems, oxygen atoms may lose electrons, thereby becoming free radicals; these are highly oxidative entities that can cause degradative reactions in cell tissues and membranes of both plants and animals. The usual defense against free radicals is the enzyme superoxide dismutase (SOD), which restores lost electrons and transforms free radicals back to normal oxygen atoms.

Antioxidants produced by EM greatly enhance the effectiveness of SOD by serving as electron donors and thereby providing a more efficient system for preventing the generation of free radicals and their degradative effects. Common antioxidants include vitamins A, E, and selenium.

Decomposing types of microorganisms function quite the opposite from synthesizing types. That is, they generate substances the promote oxidation and the production of free radicals. Neutral types of microorganisms are pivotal and can be transformed into synthesizing or decomposing types depending on the specific environmental conditions. In essence, the degradation of agricultural lands from erosion and the intensive use of chemical fertilizers and pesticides, as well as most disease are associated with oxidative processes.

Recent developments in EM technology indicate that the beneficial effects of EM can be extended considerably beyond agriculture and the environment largely because of the antioxidant potential of EM cultures. Based on research and development activities in many countries, EM is increasingly viewed as a means of providing solutions to problems of food production, depletion of natural resources, environmental pollution, food safety and nutrition, and human and animal health.

For example, EM inoculants have been used successfully to:

1) Improve soil quality and the growth, yield and quality of crops.

2) Suppress malodors associated with livestock production.

3) Enhance the growth and market weight of swine and poultry when used as a feed/water additive.

4) Improve the quality and shelf-life of fruits and vegetables.

5) Improve the process technology for sewage treatment and water purification.

6) Improve the process technology for composting municipal solid waste (i.e., garbage) into a high quality soil conditioner and biofertilizer.

7) Improve the process technology for recycling other waste materials including plastics, paper, rubber and textiles.

8) Enhance human and animal health through the use of EM by-products and metabolites.

9) Improve the process technology and quality of ceramics produced from waste materials, particularly ash from various combustion systems.

10) Improve the control of insect pest in both rural and urban communities.

All of these applications of EM technology have been cost-effective and acceptable to environmentalists and consumer.

Many of us are very confident that the EM technology offers environmentally-sound, economically-viable, and cost-effective solutions to problems of agriculture, the environment, industry, natural resources conservation and food safety and quality, Data presented will include field applications, procedures and results.

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MODELING INTER-PREMISES SPATIAL TRANSMISSION OF H5N2 HIGHLY PATHOGENIC AVIAN INFLUENZA IN MINNESOTA DURING THE 2015 OUTBREAK

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INTRODUCTION

Beginning in December of 2014, the U.S. was struck by an outbreak of highly pathogenic avian influenza (HPAI). The upper Midwest region of the U.S. was hardest hit, with the states of Iowa, Minnesota, North Dakota, South Dakota, and Wisconsin losing millions of birds during the outbreak. The current study analyzes the spatial transmission of HPAI H5N2 between poultry premises in Minnesota through the use of a spatial transmission kernel, which models the infection hazard faced by an uninfected premises located some given distance away from an infected premises. Spatial transmission kernels have been used to analyze implemented control measures and possible transmission pathways following HPAI outbreaks in the Netherlands, Italy, and U.S., respectively (1, 2, 3).

The control measures implemented in Minnesota involve the establishment of a ten km control area around each infected premises. Uninfected premises inside the control area require permits to transport product within or out of the zone, which are obtained by implementing product-specific biosecurity outlined in sector-wise business continuity plans and performing active surveillance via diagnostic testing and monitoring mortality. Additionally, uninfected premises located within ten km of the control area, designated the surveillance zone, must test for HPAI every seven days. Control measures are lifted 21 days after the last infected premise has started cleaning and disinfection activities with no new cases of HPAI having occurred.

MATERIALS AND METHODS

The spatial transmission kernel model used in this study was developed to analyze the spread of HPAI H7N7 during the outbreak in the Netherlands in 2003 (1). The transmission kernel quantifies the hazard rate for HPAI transmission from an infectious to a susceptible premises located some given distance away. Let i identify a specific infectious premises, j identify a specific susceptible premises, and d_{ij} be the inter-premises distance in kilometers. Let h_0, r_0, and α be unknown constants, where, in this case, h_0 represents the maximum possible daily transmission risk, α controls the rate of decline in the transmission risk over distance, and r_0 determines at what magnitudes of distance in kilometers substantial transmission risk still exists. The hazard rate is given below as a function of d_{ij}:

$$h(d_{ij}) = \frac{h_0}{1 + \left(\frac{d_{ij}}{r_0}\right)^{\alpha}}$$

In this model, the hazard rate depends only on inter-premises distance and infection status over time. Distance between premises in Minnesota was calculated from latitude and longitude locations present in the outbreak data. Infection status over time was approximated based on the date HPAI was first detected in case premises and the start date for disposal of the depopulated poultry carcasses. The three unknown parameters, h_0, r_0, and α, were estimated from Minnesota outbreak data using a maximum likelihood (1). The 95% confidence intervals for the mean parameter estimates were estimated using profile likelihoods.

Due to uncertainty in the day of infection and start of the infectious period for case premises, spatial transmission kernels were evaluated under two

different scenarios varying the assumptions governing changes in infection status. The scenarios are as follows.

Baseline scenario. Case premises are infected ten days prior to the day of detection. The infectious period begins on the same day and lasts up to and including the scheduled day of disposal of the depopulated poultry carcasses.

Latent period scenario. Case premises are infected eight days prior to the day of detection. The infectious period starts following a three day latent period, five days prior to the day of detection. The infectious period lasts up to and including the disposal start date.

RESULTS AND DISCUSSION

The mean transmission kernel parameter estimates and 95% confidence intervals for the baseline and latent period scenarios are given in Table 1. The results show that the transmission kernel estimates do not differ significantly between the two scenarios, providing evidence of robustness to changes in the assumptions governing the transitions in infection status. For comparison, parameters estimated from the 2003 HPAI H7N7 outbreak in the Netherlands under their default scenario, which assumes case premises become infected six days prior to the first rise in mortality, with the infectious period beginning two days after infection and lasting until the premises is depopulated; and from the 1999-2000 HPAI H7N1 outbreak in Italy under their basic model scenario, which assumes case premises become infected seven days prior to detection, with the infectious period beginning following a two day latent period and lasting until the premises is depopulated, are provided in Table 1 (1, 2). In addition, the transmission kernels as determined by the mean parameter estimates are plotted over distance in Figure 1. The likelihood of transmission during the Minnesota HPAI outbreak is clearly uniformly higher than the transmission risk during the HPAI outbreaks in the Netherlands and Italy. This outcome is most likely the result of differences in a combination of factors such as disease strain characteristics and implemented control measures.

The results indicate transmission risk was highest during the 2015 HPAI outbreak in Minnesota at close distances to infectious premises, which means the local contact structure between poultry premises (e.g, personnel visiting multiple premises) may have played a greater role compared to HPAI spread mechanisms that are relatively less distance dependent (e.g, movement of eggs and poultry). Therefore, future work in outbreak control should emphasize mitigating the effects of transmission pathways occurring locally to infectious premises. Spatial transmission kernels are used in risk assessments supporting business continuity plans for the event of a HPAI outbreak, for example the Secure Turkey Supply Plan, to estimate the total probability of a premises first becoming infected and then, following infection, moving undetected infectious product (4). In this way, the spatial transmission kernel estimated from the 2015 HPAI H5N2 Minnesota outbreak will improve understanding of the overall risk related to the managed movement of poultry products during an HPAI outbreak.

(A full-length article will be submitted for consideration to a peer-reviewed journal.)

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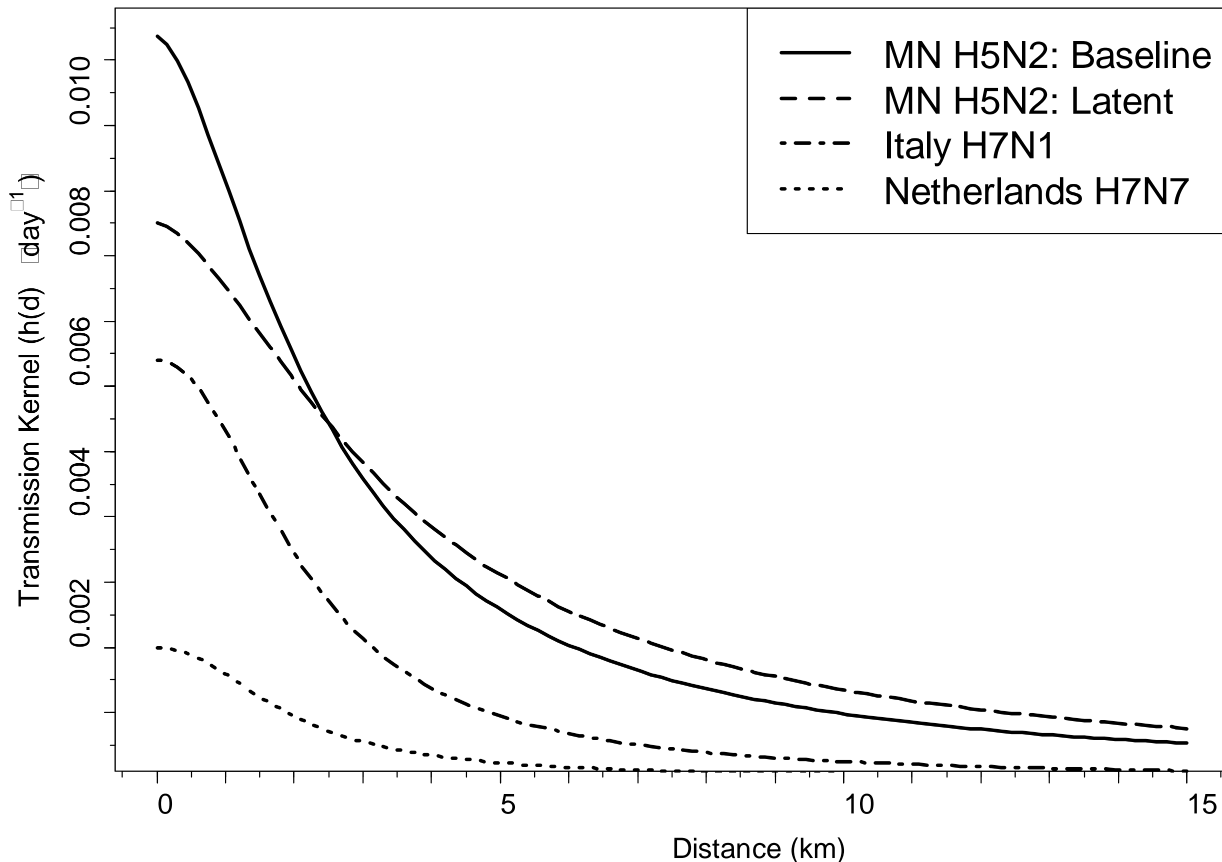
Table 1. Mean parameter estimates and 95% confidence intervals (in parenthesis) for the three unknown constants in the spatial transmission kernel model used in this study estimated from HPAI outbreaks in Minnesota, the Netherlands, and Italy. In this case, $\boldsymbol{h_0}$ is the maximum transmission risk per day, $\boldsymbol{r_0}$ influences the distances in kilometers at which notable transmission risk still exists, and $\boldsymbol{\alpha}$ controls the rate of decline in the transmission risk over distance. Each set of parameters was estimated under different assumptions about the daily transitions between infection statuses. See text for details on these various scenarios.

Description	h_0	r_0	α
Minnesota 2015 HPAI H5N2: Baseline Scenario	0.0113 (0.0047, 0.0323)	2.36 (0.75, 5.63)	1.63 (1.37, 1.97)
Minnesota 2015 HPAI H5N2: Latent Period Scenario	0.0085 (0.0034, 0.0220)	3.56 (1.26, 8.41)	1.61 (1.34, 1.95)
Netherlands 2003 HPAI H7N7: Default Scenario from Boender *et al.* (2007) (1)	0.0020 (0.0012, 0.0039)	1.9 (1.1, 2.9)	2.1 (1.8, 2.4)
Italy 1999-2000 HPAI H7N1: Basic Model Scenario from Dorigatti *et al.* (2010) (2)	0.0064 (0.0037, 0.0090)	2.15 (1.39, 2.91)	2.08 (1.87, 2.28)

Figure 1. The spatial transmission kernel model evaluated over distance under the mean parameter estimates given in Table 1 estimated from HPAI outbreaks in Minnesota, Italy (see Dorigatti *et al.* (2010) (2)), and the Netherlands (see Boender *et al.* (2007) (1)).



THE ROLE OF THE BRITISH COLUMBIA PROVINCIAL VETERINARY DIAGNOSTIC LAB IN NOTIFIABLE AVIAN INFLUENZA OUTBREAKS

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Since 2004, the British Columbia (BC) commercial poultry industry has experienced four Notifiable Avian Influenza (NAI) outbreaks: HPAI-H7N3 (2004), LPAI-H5N2 (2005), LPAI-H5N2 (2009) and HPAI-H5N2 (2014). The Animal Health Centre (AHC) is the BC provincial veterinary diagnostic lab and was responsible for the initial detection of avian influenza (AI) in all four events. The AHC played a key role in providing diagnostic support to the eradication effort lead by the Canadian Food Inspection Agency (CFIA). A significant lesson learned from the previous outbreaks was the vital need for local diagnostic support for the disease control effort as well as the need for timely AI testing to allow test-negative birds to proceed to scheduled slaughter and to allow on-farm egg collection. In the most recent outbreak (2014) the AHC performed >8500 PCR tests over a six week period that included clinical suspect, active surveillance, movement permit and wild bird samples.

Pre-outbreak. The AHC is one of three AAVLD-accredited diagnostic labs in Canada. The AHC is also accredited by the Standards council of Canada (ISO 17025) for specific test methods including tests for AI. The AHC is a member of the Canadian Animal Health Laboratory Network (CAHLN) and is accredited by the CFIA for AI testing. Currently, six laboratory technicians are certified by the CFIA to perform AI testing. This allows CFIA to have confidence in the accuracy of testing done by the AHC and will activate an immediate containment response based on an AHC test results, even while confirmatory National Centre for Foreign Animal Disease (NCFAD) test results are pending. The AHC maintains a Foreign Animal Disease (FAD) laboratory "at-the-ready" which is immediately activated upon NAI detection since the majority of outbreak samples will be processed through this enhanced biocontainment lab. A NAI-specific SOP has been developed and subsequently refined with guidance from each post-outbreak debriefing. It identifies key outbreak-specific roles for submission intake & coordination, sample processing and results reporting. An independent Emergency Laboratory Database System (ELDS) has been developed to capture all outbreak test data to the farm level, allow web-based result reporting and provide real-time aggregate summary reports. The AHC offers full service specialized diagnostic support to the BC poultry industry and will continue to provide early AI detection through passive surveillance. In collaboration with federal and provincial wildlife agencies the AHC continues to provide testing support to the National AI Wild Bird Surveillance Program.

Outbreak. Immediately after reporting a positive H5 or H7 test result to CFIA and the BC poultry industry, the AHC prepares for the inevitable surge in outbreak related submissions: the FAD lab is activated, supplemental supplies & reagents are fast-track ordered, all staff review the AI-FAD SOP, an outbreak-specific ELDS is activated with BC Poultry Premises ID data uploaded and roles assigned, and the Lab Emergency Operations Centre (dedicated meeting/break room) is defined. Once identified, the AHC coordinates with lab diagnostic representatives in CFIA and Industry. CFIA will define and coordinate outbreak surveillance testing and the industry will remain a critical component of coordinating negative "movement permit" testing. Daily sampling schedules provided by CFIA are vital to anticipating and managing AHC staffing and supply needs.

The AHC Lab Director (LD) plays a critical role as the liaison to provincial executives and as a credible media spokesperson. The LD also ensures that the AHC safely maintains its continued diagnostic service to other livestock industries and animal owners. Regular updates were provided to peripheral staff that may have felt marginalized by the intense focused activities of the AI diagnostic team. These updates were genuinely appreciated and served to unify the AHC.

During the outbreak the AHC tests all birds submitted to the lab regardless of clinical history. Cases with a high index of suspicion for AI (high mortality, dangerous contact) or those confirmed to be NAI positive are handled biosecurely, using full PPE. Multiple tissues from positive birds are harvested and forwarded to NCFAD for further

testing (IVPI, genetic sequencing, VI, IHC, etc.). In 2014 when the early connection to migratory birds as a source was made the AHC coordinated with wildlife agencies to significantly enhance wild bird field sampling and testing, with subsequent detections of a genetically related HPAI H5N1 in a Cooper's Hawk and HPAI H5N8 in a Widgeon.

The BC Ministry of Agriculture also provides outreach to non-commercial backyard poultry flocks, using the AHC as a point of contact for poultry-related inquiries. The 2014 outbreak potentially impacted thousands of small non-regulated poultry flocks due to the movement restrictions imposed within the Control Area (southern BC) and there continue to be significant outbreak communications challenges for this sector.

The primary role of the AHC is to efficiently accession submissions, track & test samples, verify results and enable results reporting using ELDS. The AHC testing capacity of 450 rRT-PCR matrix/H5/H7 tests per day generally exceeds the field sampling capability. The summary reporting feature of ELDS provides real-time diagnostic data for inclusion into the daily 3 p.m. situation reports provided to all key responders.

Post-outbreak. The official end of an outbreak response is always determined by CFIA, but it is usually 21days after the last positive test. At this point post-outbreak surveillance (POS) testing will be defined and initiated. The role of the AHC in POS has been variable in the last four NAI outbreaks but generally CFIA takes this over, although the AHC is prepared and equipped to support a full spectrum of POS. The AHC performs an internal debriefing to capture the experiences of the diagnostic staff in an effort to determine what went right, what went wrong and what could be done better. This information is then used to update and refine the working NAI SOP. The specialized expertise of the AHC will then engage in industry-led post-outbreak recovery initiatives to further manage on-farm AI risk and enhance early detection surveillance programs.

Table 1. The roles of the BC provincial veterinary diagnostic lab in NAI outbreaks.

Pre-Outbreak	Outbreak	Post-Outbreak
Achieve & maintain accreditation • AAVLD • SCC • CAHLN • FAD lab • AI tests & technicians	Make primary notifications • CFIA • Ministry Executives • BC Poultry Industry	Capability to continue with post outbreak surveillance (POS)
Provide poultry diagnostic expertise • Passive surveillance	Activate outbreak tools • FAD lab • AI-FAD SOP • ELDS • Assimilate BC Premises ID data with ELDS	Adopt suggestions from the AHC lab debrief • What went right? • What went wrong? • What could be done better?
Develop AI outbreak tools • AI-FAD SOP • Emergency Lab Database System (ELDS)	Assign AHC roles & coordinate with CFIA & Industry diagnostic representatives	Participate in multi-agency outbreak reviews
Ongoing wild bird testing • National Wild Bird AI Surveillance Program	Ensure adequate trained staff & supplies • Require sampling schedules	Provide specialized expertise to industry-led post-outbreak recovery teams • Rapid response • surveillance
Participate in tabletop exercises	Maintain full diagnostic service to all other livestock and animal owners & enhance general lab biosecurity	Adapt to capacity challenges posed by new surveillance activities
	Accession, test, verify, report	Continue wild bird AI testing
	Enhance wild bird testing capability if necessary	Continue outreach to small flocks
	Outreach to small flocks	
	Media	
	Provide ELDS summary reports to executive and official JEOC situation reports.	
	Harvest & prep tissues for NCFAD	
	Daily briefing Provide staff with regular updates	

ETIOLOGIC INVESTIGATIONS INTO WHITE CHICK SYNDROME IN ONTARIO

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White chick syndrome (WCS) has been recognized sporadically in Ontario broiler hatcheries for close to 30 years. In recent years, this syndrome has been more frequently observed, stimulating renewed interest in identifying the etiology which is now thought to be a chicken astrovirus based on results of recent European challenge studies. In the early summer of 2015, during the recovery phase of the Ontario HPAI outbreak, three major Ontario broiler hatcheries reported several cases of WCS. Clinical and pathologic features of the syndrome will be described and results of the etiologic investigations including viral genotyping will be reported.

USING NUTRIGENOMICS TO UNDERSTAND AVIAN GIT HEALTH AND ITS ROLE IN PRODUCTION AND PERFORMANCE

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INTRODUCTION

Nutrigenomics is the study of how nutrients and feeding strategies affect the animal genome. The use of "omics" technologies has allowed investigators to begin to understand how nutrition modulates gene expression, protein levels, metabolites and microbial profiles, and how this modulation relates to animal health and performance. These technologies generate vast amounts of data that can allow the rapid evaluation of nutritional strategies when the tools of bioinformatics are applied to decipher data in terms of their functional and biological relevance. Furthermore, nutrigenomics allows a systems biology approach to animal nutrition by elucidating how nutrients affect intestinal health by interacting with gastrointestinal tract (GIT) tissues and by interacting with the microbiota in the intestine.

USING NUTRIGENOMICS TO UNDERSTAND GUT HEALTH

The primary role of the avian GIT is to digest food and absorb dietary nutrients. Proper GIT function is essential for optimal health and growth, and disease and pathologies involving the gut can lead to decreased production. Nutrigenomics can be used to help identify the interplay between diet and gene function to maintain proper GIT health and elucidate modes of action for beneficial dietary additives such as probiotics and prebiotics. For example, although probiotics are used to help control pathogenic bacterial infections in chickens, the mechanisms behind their effects on the GIT are not well understood. By measuring GIT gene expression, it has been showen that the addition of specific *Lactobacillus* strains to broiler diets can improve performance in heat-stressed birds by increasing mRNA expression of the glucose transporters GLUT2, GLUT5, SGLT1, and SGLT4, thus enhancing the absorption of glucose to provide the bird more energy for growth (4). A study found that certain probiotics can directly attenuate gene expression changes caused by *Salmonella* exposure, including the down regulation of the *Salmonella*-induced pro-inflammatory cytokines (e.g., IL6, IL 4 and IL10) (6). A study evaluated global gene expression in the GIT to help explain the reduction in *Salmonella* after probiotics were added to broiler diet (5). Based on pathway analysis, they reported that genes associated with apoptosis were regulated by probiotic inclusion, suggesting that increased apoptosis is one mechanism whereby probiotics reduce pathogenic infection.

Similarly, other beneficial nutritional additives such as mannan oligosaccharides (MOS) have also been investigated using the methods of nutrigenomics to help elucidate their molecular mechanisms. For example, MOS was thought to improve GIT health indirectly primarily by binding pathogenic bacteria, thus altering the gut microbiome. However, recent nutrigenomics research suggests that MOS can also directly interact with receptors on the GIT cells and influence gene expression in those tissues. Feeding MOS products has been shown to increase the mRNA levels of mucin 2 (2, 3) an important component of the protective mucin layer in the gut, and also surprisingly to down regulate genes involved in cell turnover and proliferation. Down regulation of these genes and their processes can have an energy-sparing effect resulting in improved production (2).

MICROBIOMICS AND NUTRIGENOMICS: FEEDING THE GIT

The GIT of poultry contains a diverse, dense microbial population with concentrations of up to 10^{11} CFU/g in some gut segments (1). This microbial population plays a vital role in the bird's nutritional status by modulating nutrient biosynthesis, digestion, and uptake. Moreover, management of the microbiome through nutrition not only influences the bird's immune system and performance, but can also play a protective role in human health by potentially controlling pathogenic bacteria (7).

Until the past few years, analysis of the microbiome was limited to culture-based methods, so only a small proportion of the microbes present in the GIT were identifiable. New high through-put genomic sequencing has allowed for the identification of novel bacterial populations in the GIT and has produced large quantities of data on those populations (10). Current research characterizing the microbiome is focused on

differentiating "normal" and "abnormal" populations. Understanding the influence of nutrition on these populations can potentially lead to advances in nutritional strategies for the management of disease, improved methods for controlling human pathogens, and alternatives to antimicrobial growth promotants.

Although characterization of the microbiome is ongoing, studies have nevertheless begun to explore the influence of nutrition on microbial populations. One area offering great potential for application is the use of microbiomics to evaluate dietary strategies to modulate the GIT microbial populations to reduce pathogenic bacterial loads. For example, Shao et al. (8) evaluated the effects of supplemental zinc on the GIT microbiome and found that in *Salmonella*-challenged birds, total microbes and the number of beneficial bacteria were decreased, whereas zinc supplementation restored microbial populations to prechallenge levels, thus improving bird performance. Research is still needed to elucidate the mechanism underlying the beneficial effect of zinc on the GIT microbiome. In another example, microbial sequencing was used to effectively show that a specific feed additive could decrease *Campylobacter* levels while maintaining the normal GIT microbiome (9).

CONCLUSION

Optimal nutrition relies on the interplay between dietary nutrients and two genomes – that of the animal and that of the host-associated microbiome. Diet can directly and indirectly influence the genome of poultry, and researchers can use this information to develop precision diets to optimize animal health. By extending the methods of nutrigenomics to address how diet affects the GIT microbiome, we can develop a systems biology approach to craft strategies for optimizing poultry nutrition.

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USING RNA-SEQUENCING NEXT GENERATION SEQUENCING TO UNDERSTAND THE VARIABILITY IN GENE EXPRESSION IN *SALMONELLA* HEIDELBERG (SH) AFTER EXPOSURE TO DISINFECTANTS

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ABSTRACT

Next generation sequencing (NGS) such as RNA-sequencing technology allows for the rapid comparison of transcribed sequences (e.g. the ‘transcriptome’). This relatively novel technology allows for the development of a ‘transcriptional map’ of the chromosomal and plasmid RNA under different environmental conditions. In this study, we evaluated the transcriptomes of two strains of *Salmonella* Heidelberg (SH) isolated from a broiler processing plant in 2014 and 1992 after challenging them with cetylpyridinium chloride (CPC), acidified calcium hypochlorite (aCH) and peroxyacetic acid (PAA) for eight seconds to simulate dipping stations (CPC) or 90 min to simulate the chiller tank (aCH and PAA) at 4^0C. Using a publically available annotated SH reference genome with 4,623 genes, 65 genes were identified with functions related to virulence, pathogenicity, resistance and heat shock proteins (VPRH genes). Using an adjusted p-value of 0.05, upregulation of genes was most common in SH that was exposed to aCH (40/65 for the 2014 strain and 1992 strains respectively). In contrast, statistically significant up-regulation of VPRH genes when exposing the same SH strains to PAA was lower (3/65 for the 2014 strain and 8/65 for the 1992 strain). Results for CPC were in between aCH and PAA (31/65 for the 2014 strain and 33/65 for the 1992 strain).

This information combined with previously obtained mean inhibitory concentration (MIC) data which showed that the MIC for these SH strains exposed to acH is ineffective at 64x GRAS levels, suggests that not only is aCH ineffective at inhibiting SH but also that aCH creates resistance and increase virulence based on the RNA-seq results.

From a HACCP perspective, RNA seq is a new tool which should be considered in determining how to mitigate up-regulation of genes in zoonotic bacteria that are related to VPRH in a food processing environment.

INTRODUCTION

The current methodology for evaluating the efficacy of disinfectants requires identifying the inhibitory concentration and or log reduction of a disinfectant to bacteria. However, there is little interest in what happens to the surviving bacteria (1, 2). Only recently have studies looked at transcriptional differences in *Salmonella* strains after exposure to different stresses such as desiccation, cold and acid stress (3, 4). These among other studies have shown that *Salmonella* can express different genes under different environmental conditions that allow them to survive and can even make them harder to kill the next time they are challenged (4).

One approach that can be used to understand differential gene expression in zoonotic bacteria after exposure to commonly used disinfectants in the processing plant is Next-Generation RNA-sequencing. Up until recently, next generation sequencing technologies like RNA-seq were very expensive and time-consuming making them impractical for food safety applications. However, with recent advances in sequencing food safety system it is now less expensive (5, 6).

In this study we exposed two different field strains of SH to three commonly used disinfectants used in the chiller tank and dipping stations in poultry processing plants. The aim of this study was to identify differences in RNA gene expression with a focus on genes related to virulence, pathogenicity, resistance and heat shock proteins (VPRH genes).

MATERIALS AND METHODS

Two SH strains isolated from a commercial processing plant in 2015 and 1992 were used in this

study. Final concentrations (130 ppm, 20 ppm and 62.5 ppm for PAA, aCH and CPC respectively) of each disinfectant were determined by MIC values from a previous experiment (data not shown). The selected concentration was 0.5X below the MIC value. SH was challenged by splitting a mid-log culture into two groups, disinfectant and no treatment (control). Triplicates were done for each group. The CPC group was challenged for eight seconds at 4°C to simulate the dipping stations and the PAA and aCH group were challenged for 90 at 4°C to simulate the chiller tank. Total RNA was extracted based upon modified protocol of the RNeasy Protect Bacteria Mini Kit by Qiagen. After RNA extraction, the samples were further cleaned with the RNAClean. Next the RNA integrity was assessed using the Agilent Bioanalyzer. RNA integrity values were deemed acceptable if they were above five. (5). If samples were found to have low concentrations, they were concentrated using an RNA clean and Concentrator kit (Zymo). Once RNA concentration and integrity were acceptable, samples were sent to the UC Davis Core Facility for sequencing. RNA depletion was carried out using the Ribo-Zero rRNA Removal kit for gram negative bacteria (Illumina). Once libraries were validated using an Agilent Bioanalyzer, they were sequenced on an Illumina HiSeq3000 in single read 50bp mode**.** A publically available annotated SH genome was found with 4,980 genes. Genes were filtered down to 4,623 genes, with genes expressed at low levels taken out. Of those 4,623 remaining genes, 65 genes were associated to virulence (n=15), pathogenicity (n=3), resistance (n=35) and heat shock proteins (n=12). Gene expression comparisons were made in a pairwise fashion comparing the expression of the control to the challenged strain. Over and under-expression were determined using an adjusted p-values of 0.05.

RESULTS

Differential gene expression was most evident in the groups that were challenged with aCH. In both organisms, 61.5% of the genes associated with virulence, pathogenicity, resistance and heat shock proteins were up regulated. 10 out of the 12 genes associated with heat shock proteins were very consistent in that the same genes were upregulated by aCH in both organisms. Resistance genes also acted in a similar manner with 24 (68.6%) of the same genes being upregulated in both organisms. The aCH groups also had the largest percentage of up regulated resistance genes than any other group at 71.4% to 74.3% respectively. The effects of CPC were relatively more varied than PAA and aCH. For example of the three pathogenicity genes all three were down regulated in SH #20 whereas all three genes were up regulated in the historic strain.. The genes linked to heat shock proteins were relatively more consistent with 58.3% of those genes being upregulated in both organisms. PAA groups expressed the least statistically significant differential expression. 80% to 83.1% of the total genes for SH #20 and the historical strain showed no difference in expression compared to the control.

DISCUSSION

Differential RNA gene expression in disinfectant challenged field strains relative to non-disinfectant challenge was observed. For aCH and CPC, the genes associated with virulence, resistance and heat shock proteins were more likely to be up regulated than under regulated. Interestingly for PAA, there was less statistically significant differential gene expression.

Calcium hypochlorite has been shown to be ineffective in the complete control of *Salmonella* Agona (8) and in this study it has been shown that aCH is more likely to up regulate selected genes specifically those linked to resistance. Therefore, the potential exists for increased resistance when exposing SH to aCH. One possible explanation for the ineffectiveness of calcium hypochlorite is that the challenged bacteria are so effective at activating genes related to virulence, pathogenicity, resistance and heat shock proteins that the bacteria are able to survive the stress. This type of pre-adapation has been previously noted in other studies (4). CPC has been shown to be an effective disinfectant in other studies *(1, 6)* but as seen in this study it also induces up regulated expression of selected genes. PAA on the other hand has been shown to be more effective than CPC at inhibiting *Salmonella* growth in other studies (6). In addition, SH exposed to CPC had less differential gene expression implying that SH exposed to PAA would not increase resistance in viable SH. In the future, from a HACCP perspective, this approach could be used to determine critical food safety parameters in a way never previously investigated in a food system environment with the ultimate goal of identifying conditions in food production that mitigate transcription of genes associated with virulence and survivability. The work is a novel approach toward post-harvest control of pathogenic bacteria. It reflects the potential synergy between DNA based detection technologies and RNA based approaches, which allow for a greater understanding of how bacteria respond to its environment.

(The full-length article will be published in the *Journal of Food Protection*.)

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SAMPLING AND TESTING FOR INFLUENZA A VIRUSES IN POULTRY ENVIRONMENTS

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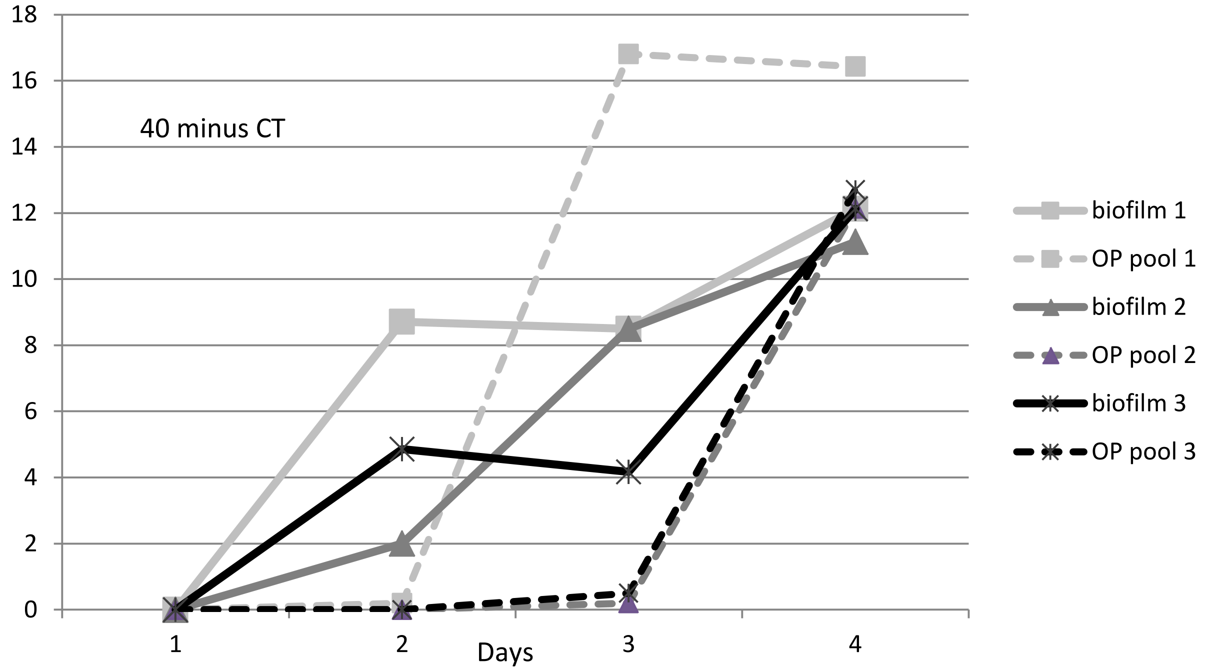
Sampling strategies for surveillance should be effective in detecting disease states including pre-clinical and subclinical states, and at less than 100% infection prevalence in a population. Based on this goal, we examined a variety of sampling strategies for detecting viral RNA from turkey flocks undergoing infections with influenza A viruses (IAV). We found that viral RNA was widely distributed in the barn including in both water and drinker biofilm samples. We found viral RNA concentrated in the biofilm and that RTPCR and virus isolation were similar in this sample type so subsequently pursued these samples for use in passive surveillance. Biofilm samples were effective in detecting infections and followed a similar pattern of progression as the concurrently collected oropharyngeal (OP) samples on a farm in which the two methods were directly compared. To evaluate the utility of biofilm sampling for the detection of highly pathogenic avian influenza virus (HPAIV), biofilm and pooled OP swabs were collected daily from negative turkey flocks on an HPAI positive premises. During the daily sampling of these four negative flocks, three became infected. The biofilm swabs were positive 1-2 days prior to positives appearing in the OP sample pools (Fig. 1). The drinker biofilm sampling strategy overcomes the difficulty of finding a subclinical infectious bird in a population by sampling a large number of individuals and collecting a sample in which a positive signal persists for several days to weeks. The sampling method is convenient for use in turkey barns and has been reliably used in both active and passive surveillance programs for LPAIV, avian metapneumovirus, Newcastle disease virus and HPAIV using RTPCR detection.

Figure 1. A comparison of drinker swab RTPCR CT values and oropharyngeal (OP) swab RTPCR CT values in HPAI infected flocks. Results are plotted as 40 minus CT value. All data are plotted from the day before the first positive test.



EVALUATION OF PERFORMANCE FROM A CONVENTIONAL VACCINATION SCHEDULE FOR IBD IN BROILERS

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SUMMARY

New technologies for vaccines have been developed trough years in the poultry industry in order to improve performance of the flocks. However, poultry companies keep using conventional vaccination programs to protect their birds from several diseases. The goal of this study is to analyze the efficiency of a conventional vaccination schedule against infectious bursal disease (IBD) on a broiler farm in west zone of Mexico. Also, this study objective is to assess vaccination days are appropriate to protect bird against IBD challenges.

INTRODUCTION

Virus of infectious bursal disease (IBDV) causes immunosuppressive disease in young chickens. The virus replicates in bursa and destroys lymphocytes B. The virus also causes a significant reduction in the function of lymphocytes T (1).

IBD is a highly contagious disease of young chickens, and is considered the leading immunosuppressive agent of birds. In addition, it is resistant to physical and chemical agents, persisting in the environment and in poultry facilities for long time (1).

The disease is highly contagious and occurs in two forms: clinic, which affects birds between 3-6 weeks of age; and subclinical in birds under three weeks. The disease is characterized by the destruction of immature B lymphocytes causing severe injuries to the bursa of Fabricius, mortality and varying degrees of immunosuppression that increase susceptibility to subsequent infections, favoring a poor post-vaccine response.

The disease control is done through biosecurity measures and vaccination programs of breeding and progeny.

MATERIALS AND METHODS

In a broiler farm in the west zone of Mexico, two flocks of 100K broiler each one was vaccinated with two Lukert vaccines at six and 17 days of age. Ten blood serum samples were collected per farm at ages three, 14, 28, and 35 days. Also, polymerase chain reaction (PCR) and image processing (IP) tests were used for this study collecting six bursas and six FTA cards samples.

RESULTS

Results show there was no significant damage in bursa in samples from three, 14, and 35 days of age in both flocks.

However, the IP test shows significant damage in both flocks at 28 days of age. PCR of flock 1 was found to have high sequence homology, 98.6%, with the IBD virus UK661 and 98.6%, with the IBD virus 03-10681 Canada isolate.

The results from PCR and IP will be on presented in the poster.

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AGE PROFILES OF HPAI H5N2 INFECTIONS OF TURKEY FLOCKS IN MINNESOTA

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SUMMARY

Age profiles of turkey flocks in Minnesota infected with highly pathogenic avian influenza (HPAI) virus H5N2 were evaluated. These data suggest an increase in infection incidence at 14 weeks of age in turkeys during the spring HPAI H5N2 outbreak from March–June in Minnesota. The numbers of infected commercial turkeys were matched with the total number of turkeys on each premise by county, flock size, and age for a total of 19 data points spanning 13 different counties in Minnesota. The data showed that 38% of commercial turkeys included in this analysis were never infected with HPAI virus H5N2. Out of the 62% that were infected, 35% of turkeys were infected at 14 weeks of age, 18% at 17 weeks of age, 16% were infected at nine weeks of age, with the remaining age groups infected at <10% for each age.

INTRODUCTION

During the spring outbreak of HPAI virus H5N2 from March–June 2015, 104 total turkey premises in Minnesota were infected comprised of 78 meat-type turkeys and 26 breeder turkeys (4). Of these, 58 were single age premises and 46 had flocks of multiple ages on site at the time of infection. The youngest index flock age at the time of infection was 28 days in meat-type turkeys, and the oldest age of an index flock was 686 days in breeder turkeys. The objective of this study was to assess the ages of turkeys infected with H5N2 during the HPAI outbreak. Some Influenza A virus (IAV) epidemics in the past have shown an age susceptibility characteristic in the pattern of infection (1). Although there is no available data specifically for turkeys related to age susceptibility, this characteristic is not limited to one particular species. There is evidence for the 2009 H1N1 outbreak in humans for a predilection of young adults. The median age of patients with pandemic influenza H1N1 is reported as 20-25 years from Europe and the United States (1). In ducks, pathogenicity varies with the age of the host and correlates with the level of viral replication in tissues (2). In 2010, experimental studies examined the effect of host age on the shedding of HPAI H5N1 virus in experimentally-infected Pekin ducks. The results show that susceptibility may be dependent upon the age of the bird at the time of infection (3). Gaining an understanding of the frequency of H5N2 infections in turkeys among different ages will provide insight for determining if there is an age susceptibility component to the turkey infections with H5N2 during the spring HPAI epidemic.

MATERIALS AND METHODS

Data were obtained from a large, integrative turkey company and analyzed using standard statistics (i.e. obtaining percentages of infected turkeys according to age).

RESULTS

Nineteen data points were collected covering 13 different counties in Minnesota to evaluate the possibility of age susceptibility in turkeys to HPAI H5N2. A ratio of infected flock size to the total number of turkeys on each premise was obtained for each data point. Eleven different ages were statistically evaluated to determine the percentage of birds infected within each age group (weeks). Out of the infected turkeys, 35% were 14 weeks of age, 18% were at 17 weeks of age, followed by 16% at 9 weeks of age. The remainders of the age groups were at 10% or less for total number of birds infected by HPAI H5N2. In our available data, the highest incidence of H5N2 infection occurred in 14 week-old turkeys.

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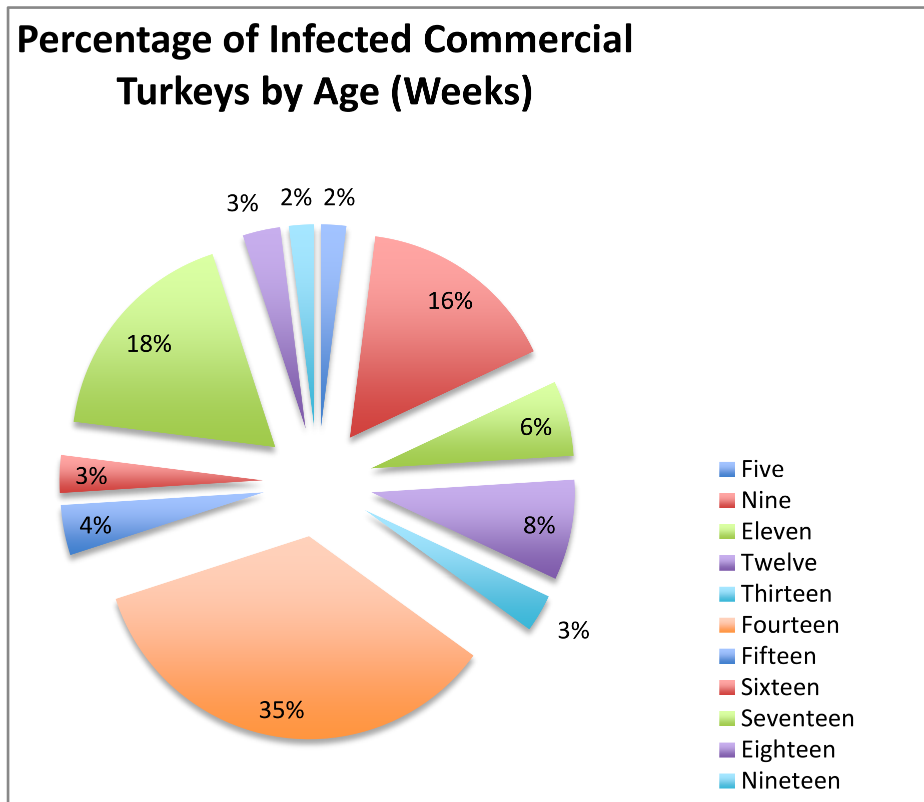
Figure 1. Pie chart of infected commercial turkeys by age (weeks) with HPAI H5N2 matched with each turkey premises by county, flock size, and age.



ANTIBIOTICS: POLICY CHANGES WON'T MAKE A DIFFERENCE

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INTRODUCTION

The contribution from antibiotic use in food-producing animals to the overall antibiotic resistance problem in human medicine has been greatly overstated (1, 2, 3). This is particularly true for the antibiotic growth promoters (AGPs) with a Gram positive spectrum of activity which have been the ones mainly used in chickens and turkeys in the USA. Several physicians and scientists from Europe have questioned the scientific basis for the ban of the AGPs (4) and the results derived from it (1, 2, 5). In the USA groups opposed to intensive food animal production have repeatedly overestimated the use of antibiotics in food animal production when compared to human use without consideration for the differences in the size of the populations and their respective biomass (6). Studies examining the relationship between antibiotic use and resistance patterns on specific bacteria on a farm basis are currently underway in the broiler and turkey industries in order to better understand and estimate the potential impact of antibiotic use in poultry on human health (7).

THE NEW POLICIES FOR ANTIBIOTICS

On April 23, 2012 the Food and Drug Administration (FDA) published a guidance in the Federal Register (FR) Guidance for Industry # 209 entitled "Use of medically important drugs in food-producing animals" (8). The guidance produced two important principles, the first one indicated that "the use of medically important antimicrobial drugs in food-producing animals should be limited to those uses that are considered necessary to assure animal health", and the second one indicated that "the use of medically important antimicrobial drugs in food-producing animals should be limited to those uses that include veterinary oversight or consultation".

As a result of this guidance, drug sponsors agreed to remove all indications for growth promotion and improvement of feed efficiency of medically important antimicrobials (MIAs) used in food-producing animals and only list on the label those uses indicated for disease prevention and control. What the guideline essentially does is eliminate all production performance enhancement uses of MIAs. All drug sponsors have agreed as well to change the labeling for all their MIAs by January 1, 2017.

On December 12, 2013 the FDA published a guidance in the FR Guidance for Industry # 213 entitled "Guidance for industry on new animal drugs and new animal drug combination products administered in or on feed or drinking water of food-producing animals: Recommendations for drug sponsors for voluntarily aligning product use conditions with guide for industry" # 209 (9).

The purpose of this guidance was to explain to the drug sponsors the steps needed to take in order to comply with the principles stipulated in Guidance for Industry # 209 and secure new indications for use for all their MIAs administered by feed or water to food-producing animals.

Finally, on June 3, 2015 the FDA published a guidance in the FR an amendment to the Veterinary Feed Directive (VFD) with the intent to improve the efficiency of FDA's VFD program while protecting human and animal health. It was felt that this action was required to accommodate the transition from over-the-counter to VFD status for all the MIAs administered by feed (10).

SCIENTIFIC JUSTIFICATION FOR POLICY CHANGES ON AGPs

There is little to no evidence that supports the belief that the use of antibiotics for growth promotion may impact human health significantly in a negative manner (1, 2, 3, 4, 11). In fact, there is growing admission that the main cause of the antibiotic-resistance problems confronted by the medical profession is antibiotic use in humans rather than animals (12, 13). In the USA practically all the AGP use was comprised by two antibiotics with a spectrum of activity limited to Gram positive bacteria: bacitracin and virginiamycin. Bacitracin is an antibiotic not considered medically important and therefore virginiamycin will be the main AGP affected by the new policies. The CDC lists *Salmonella*, *Campylobacter*, and *E. coli* as the foodborne pathogens of main concern, all of these bacteria are Gram negative and therefore not impacted by the activity of the AGPs. It is therefore not difficult to predict that the elimination of

virginiamycin as an AGP will have no effect on the resistance patterns of the main foodborne pathogens of human concern.

Others have expressed concern that the use of a streptogramin antibiotic like virginiamycin can foster the development of streptogramin-resistant *Enterococcus faecium* (SREF) and that this could ultimately impact human health adversely. However, two extensive studies set out to prove the transmission of SREF from chicken to people failed to do so (14, 15). Further proof of the lack of transmission of SREF from chickens to people is that an extensive sensitivity survey conducted with clinical isolates of *E. faecium* at the time the human streptogramin (quinupristin-dalfopristin [QD]) was introduced in the USA showed that QD was nearly 100% effective in spite of more than 20 years of virginiamycin usage in food-producing animals (16). Ultimately, risk assessments by the FDA and an independent risk assessor showed that the risk to human health from virginiamycin use as an AGP in food-producing animals was negligible (17, 18).

Similarly, in Europe the glycopeptide antibiotic avoparcin was banned as an AGP in 1997 over fears of generating cross-resistance to the human glycopeptide, vancomycin. Hospital data on nosocomial infections from the USA vs. Europe showed that in spite of avoparcin having been used for many years as an AGP in Europe and none in the USA, prevalence of vancomycin-resistant-enterococcal (VRE) infections was much higher in the USA than in Europe (4, 5). In addition, a more recent Danish study published in 2010 showed that when chickens were cultured with a media selective for VREs up to 47% of chickens carried VREs in spite of 15 years of no use of avoparcin in food-producing animals, the researchers stated that they had no explanation for the long persistency of VREs in Danish broilers (19).

SCIENTIFIC JUSTIFICATION FOR POLICY CHANGES ON OTHER ANTIBIOTICS

The use of enrofloxacin in poultry was prohibited by the FDA on September 12, 2005. The ban was imposed on fears that resistance increases seen on human isolates of *Campylobacter jejuni* could be related to enrofloxacin use in poultry. However, after the ban was imposed resistance to quinolones continued to increase in *C. jejuni* isolates from chickens, in fact, it increased from 15.1% in 2005 to 22.6% in 2011, without any use of enrofloxacin in poultry (20). The results from the human side are not any better, ciprofloxacin resistance increased from 18.1% in 2004 to 26% in 2007 after two full years of not using enrofloxacin in poultry and remains higher than it was when enrofloxacin in poultry was used (21).

Even though gentamicin has been widely and massively administered for many years *in ovo* to chicks, resistance in *Salmonella* isolates from chickens remains relatively low at 5.2% in the last year reported (20). Likewise, human isolates of *Salmonella* of importance in public health remain highly sensitive to gentamicin showing either no resistance or less than 3% resistance. The only exception was *Salmonella ser.* Heidelberg that in the last year tested had a resistance frequency of 7.3% (21).

The use of ceftiofur, a third generation cephalosporin that was administered massively *in ovo* to chicks was stopped by the FDA-CVM in 2012 since it was extra-label drug use. The last published report from the retail meat arm of NARMS is 2012 and resistance stood at 27.9% (20), it will be interesting to see if resistance decreases in the years to come, however, the most important parameter to monitor is resistance in human isolates and those were already low for nearly all the *Salmonella* serotypes of public health significance with the exception of *Salmonella* ser. Heidelberg which stood at 22% (21).

EUROPEAN EXPERIENCE

A manuscript published more than a decade after the ban of AGPs in Europe was implemented concluded that little had change in terms of resistance other than less detection of resistance to glycopeptides and streptogramins (by conventional methods) in enterococci isolated from fecal samples from animals and humans. However, no changes or an increased resistance trend towards glycopeptides and streptogramins in clinical isolates from humans was reported (2).

The three year moving average of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in humans from the latest data available in Denmark (22) shows that MRSA infections have increased from 50 per year in 1994 to 2,094 in 2013 although the latter includes people with clinical infections as well as people with colonization. Nevertheless, even when colonized cases are removed infections have increased from 50 in 1994 to 940 in 2013 for a 1,880% increase while the size of the population has remained essentially unchanged (5,569,077 inhabitants in 1994 vs. 5,614,000 inhabitants in 2013). It should be noted that the Danish report states that 20% of the infections were attributed to CC398, a clonal complex that emerged around 2003 and is associated with livestock, primarily swine. Even though 157 MRSA infection cases were identified as

livestock-associated (CC398), only four resulted in bloodstream infections and only two resulted in deaths, in both cases the patients had additional complications and neither one had any direct contact with swine.

A recently released report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food from 28 European Union members stated that "bacteria in humans, food and animals continue to show resistance to the most widely used antimicrobials" and warns that "resistance to ciprofloxacin is very high in *Campylobacter*" and that "multi-drug resistance Salmonella bacteria continue to spread across Europe." (23) This is not what was expected from eliminating the use of the AGPs and curtailing significantly the use of antimicrobials in food-producing animals. Nevertheless, the report continues to emphasize the concern about transmission of antimicrobial-resistant bacteria from food-producing animals to humans.

CONCLUSIONS

It seems clear that in spite of the ban of AGPs in Europe little has changed in regards to antibiotic resistance in humans. In fact, a just released report from the European Food Safety Authority and the European Center for Disease Prevention and Control warns about the continued increase of antimicrobial resistance in bacteria from humans, animals and food, the very high resistance to ciprofloxacin in *Campylobacter* and the spread of multi-drug resistant *Salmonella* across Europe (23).

In spite of these results, the USA appears to have adopted a similar "precautionary principle" policy and starting in 2017 will eliminate the use of MIAs for growth promotion and improvement of feed efficiency. Having veterinary oversight or consultation when MIAs are used in food-producing animals is definitely a positive change and one that the entire veterinary profession can support. It remains to be seen if the removal of the lower use levels of antibiotics in feed will result in an increased incidence of disease in food-producing animals and a higher use of antibiotics at therapeutic doses but regardless of whether this happens it seems evident that the antimicrobial resistance problems confronted by the medical profession are not likely to improve to any measurable degree.

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POTENTIAL OF AIR FILTRATION IN REDUCING RISK OF AVIAN INFLUENZA CONTAMINATION BY AEROSOL

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INTRODUCTION

The use of air filtration to prevent aerosol transmission in commercial poultry production was explored in the late 60s and early 70s but never resulted in widespread adoption. On the other hand, the knowledge acquired on the transmission via aerosols of Porcine Reproductive and Respiratory Syndrome virus (PRRSv) has resulted in rapid application to commercial swine production. Overall, air filtration led to an approximately 80% reduction in risk of introduction of novel PRRSv (2).

ProtairX (http://www.protairx.com/) has produced over the last year data demonstrating the efficiency of their 15 layer, 10 layer and a 6 layer pleated filter in blocking aerosolized low pathogenic avian influenza H9N2 virus. While the 15 layer filter has a filtration efficiency of 99.997%, the 10 layer filter 99.92%, and the 6 layer pleated filter 99.97%. However, contrary to PRRSv, there is no data available at this point in time to determine actual efficacy of air filtration in the field.

In this study we provide a quantitative characterization and estimate the reduction in probability of infection by avian influenza. We first evaluated the effect that filtration would have had on laboratory data by applying the reduction factors to the data of previous studies (1) on H5N1 virus, (4), and H9N2 (7). Secondly, we evaluated the potential risk reduction using the model of Ssematimba *et al.* 2012(13).

MATERIALS AND METHODS

Publication data relating the probability of successful infection to relative levels of virus exposure was used to estimate a dose-response model by logistical regression. For each set of data, the reduction factors for the 15, 10 and 6 layer filters were used. The logistic regression models were estimated using the GLM function with the binomial logit link function of the R software (5).

Ssematimba et al. 2012 (13) modelled the wind-borne spread of highly pathogenic avian influenza virus between farms. Equations 8 to 12 of Ssematimba et al. 2012 (6) were used to calculate the probability of infection given the highest level of farm dust deposited, as a worse case scenario, taken from Figure 1(6). Equation 9 was modified by adding a reducing factor to take into account the effect of filtration (6). The probability of infection of a flock as a function of time was calculated given four scenarios: no filtration, 15 layer filter, 10 layer filter, and the 6 layer pleated filter. Equations were programmed in the Julia (3) programming language.

RESULTS

The effect of filtration on the probability of infection is shown in Table 1. Overall the use of filtration would have reduced the probability of infection to very low levels, preventing contamination in most cases.

Figure 1 illustrates the effects of filtration vs no filtration on the probability of a flock breaking over time. Overall the average $\log_{10}$ reduction for the 15 layer, 6 pleated layer and 10 layer filters are respectively 3.66, 2.81 and 2.45 or expressed as % reduction, 99.978%, 99.844% and 99. 650%.

DISCUSSION AND CONCLUSIONS

Overall, applying the filter reducing factors measured under laboratory conditions to laboratory experiments involving exposure of chickens to Avian Influenza virus would have theoretically prevented infection. This remains to be validated by actual experimentation.

Under the conditions of a previous simulation, the results of the simulation would suggest that under field conditions the risk of contamination by aerosol would be reduced significantly (6).

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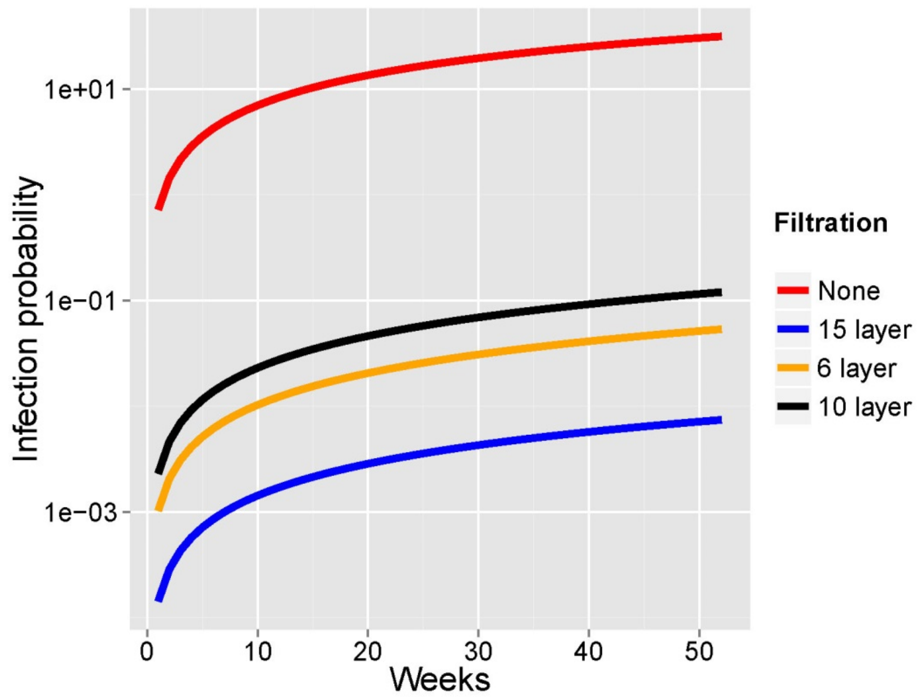
Table 1. Theoretical effect of air filtration on laboratory studies of aerosolized avian influenza infection.

Publication	Run	Probability of infection			
		No filtration	15 layer filter	10 layer filter	6 layer pleated filter
Agranovski	7	0.9999	8.19e-06	7.69e-02	5.26e-03
	6	0.9999	6.39e-09	6.50e-05	4.13e-06
	5	0.9903	1.99e-11	2.02^{e}-07	1.29e-08
	4	0.8522	1.12e-12	1.14e-08	7.26e-10
	3	0.5034	1.97e-13	2.01e-09	1.27e-10
	2	0.1518	2.22e-16	3.54e-10	2.25e-11
	1	0.0022	2.22e-16	4.40e-12	2.79e-13
Guan	1	0.9220	3.76e-04	9.75e-03	3.69e-03
	2	0.4032	2.15e-05	5.62e-04	2.12e-04
	3	0.0914	3.19e-06	8.36e-05	3.15e-05
Yao	1	0.9913	1.67e-03	5.30e-02	1.92e-02
	2	0.8998	1.32e-04	4.38e-03	1.54e-03
	3	0.4340	1.124e-0	3.75e-04	1.32e-04
	4	0.0688	1.08e-06	3.62e-05	1.27e-05
	5	0.0060	8.90e-08	2.97e-06	1.04e-06

Figure 1. Theoretical effect of air filtration on the probability of a flock breaking with avian influenza under the assumption of the model of Ssematimba et al. 2012 (13).



THE EFFECTIVENESS OF COMMON DISINFECTANTS USED IN POULTRY PROCESSING AGAINST *SALMONELLA* BIOFILM AND PLANKTONIC CULTURES

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SUMMARY

The effectiveness of antimicrobial agents (acidified hypochlorite [ACH], peroxyacetic acid [PAA], and cetylpyridinium chloride [Cecure™]) commonly utilized in commercial poultry processing facilities was investigated against non-typhoidal *Salmonella* in both planktonic and biofilm conditions. We measured log reductions, Minimum Inhibitory Concentrations (MIC), and Minimum Biofilm Eradication Concentrations (MBEC) following exposure to these disinfectants in order to characterize the bacteriostatic and bactericidal effects. Results showed that PAA and ACH were not bactericidal at Generally-Recognized-As-Safe (GRAS) concentrations, contact times, or temperatures against all strains. Cecure was the most bactericidal, but is concentration-dependent and ineffective against *S. e.* Heidelberg (SH) biofilm under conditions consistent with processing. Using MBEC/MIC assays in addition to log reduction experiments provides a greater suite of information than either technique alone. Thus, we propose using this novel and comprehensive approach for evaluating and quantifying new disinfectants, bacterial strains, and antimicrobial interventions in food production systems.

INTRODUCTION

Salmonella enterica consists of six subspecies and over 2500 serovars belonging to five serogroups, many of which are zoonotic pathogens (1). Poultry contaminated with *S. enterica* subsp. *enterica* is a major cause of foodborne illness in humans throughout the world, and therefore poses serious public health and economic burden. Previous studies have established the bacterium's ability to form biofilm on both biotic and abiotic surfaces (2, 3, 4), an adaptation which is important for bacterial survival in various environmental conditions, for enhancing antimicrobial resistance, and for providing protection against host immunity (5). When *Salmonella* biofilms become established in food processing environments, they can persist even in the face of cleaning procedures and chemical disinfection, which promotes cross-contamination between surfaces and poultry products (2, 6). The existence and resistance of biofilm in both host and non-host environments is especially important in food production systems because it becomes extremely difficult to eradicate (3, 7).

In the 2013-2014 outbreaks, serovar SH was implicated as the primary pathogen associated with the consumption of improperly prepared chicken. SH, a Group B host-unrestricted serovar lives symbiotically in poultry but causes gastroenteritis in humans via the ingestion of undercooked chicken. Over 600 people across 29 states were sickened with seven different strains of SH, which were linked to the consumption of Foster Farms brand chicken products from three processing facilities in California. Approximately 40% of consumers sickened during this outbreak were hospitalized and 15% developed bacteremia, almost double the normal rates associated with similar outbreaks (1). Although raw poultry is commonly found to have *Salmonella* bacteria present, the outbreak strains of SH were resistant to several commonly prescribed antibiotics (1, 8). Interestingly, these poultry facilities have historically met or exceeded all USDA Food Safety Inspection Service (FSIS) performance standards with respect to *Salmonella* (personal communication). This raises the question: if the prevalence of *Salmonella* from these facilities is relatively low, why did this outbreak occur, and how can we prevent future outbreaks?

The limited studies on SH biofilm control in food systems and intervention measures in poultry processing facilities warrant more context-specific strategies for food safety. It is well established that antibiotic efficacies are quantified using MIC and MBEC to evaluate and quantify bacterial susceptibilities and resistances (8). The Environmental Protection Agency (EPA) regulates the labeling, handling and efficacy of sterilants, disinfectants, and sanitizers to validate vendor claims and maintain quality assurance (9). Their testing is

performed by the Association of Analytical Communities (AOAC) International to determine bactericidal activity against American Type Culture Collection (ATCC) organisms, including various *Salmonella* spp. Though effective for many purposes, a major limitation of AOAC tests from a poultry processing standpoint is the lack of testing on biofilm, which studies have concluded to be more ubiquitous, robust and resistant than their planktonic counterparts in food production environments (10, 11).

To our knowledge, no previous study has characterized the relationship between *Salmonella* biofilm versus planktonic susceptibilities to disinfectants used during poultry processing via employing a combination of log reduction experiments and MBEC/MIC assays. Here, several strains were exposed to these agents under temperatures, concentrations and contact times consistent with the chiller tank and dipping stations. This approach allows for a more comprehensive understanding of the differences between planktonic and biofilm *S. enterica* serovars under simulated processing conditions with the goal of developing a rapid lab-based methodology to identify best practices for foodborne pathogen control in the processing plant.

MATERIALS AND METHODS

Field isolates of SH and two additional serovars (Ohio and Senftenberg), were acquired from a commercial poultry company. An additional SH isolate from 1992 was acquired from a diagnostic laboratory. Based on a review of the literature, these strains were tested against a known strong biofilm former (*Pseudomonas aeruginosa*), and a known weak biofilm former (*E. coli*). Isolates were stored at -80°C in glycerol stocks. Planktonic and biofilm cultures were grown in trypticase soy broth (TSB), while bacterial CFU counts and growth assessments were performed on trypticase soy agar (TSA). Biofilm growth curves were performed on each isolate prior to experimentation. Stock solutions of disinfectants were stored at 100X concentration, and diluted as needed. Disinfectants were tested at serial dilutions and at the average concentrations used by one poultry broiler company; PAA at 0.023% (GRAS < 0.05%), ACH at 0.005% (GRAS < 0.005%), and Cecure™ at 0.2% (GRAS < 0.8%).

Cultures of bacterial isolates were incubated overnight at 37°C. Planktonic cultures were grown to mid-log phase (3.5 hours) in tubes, while biofilm cultures were matured over 4 days in 96-well Biofilm Inoculator peg plates (Innovotech). For each replicate, 20 μL were serially diluted and spread-plated onto TSA for pre-treatment quantification. Biofilm and planktonic cultures were challenged for 90 minutes at 4°C, and for 8 seconds at 21°C using log reduction, MBEC and Planktonic MIC. Two repetitions of triplicate runs were performed on each strain, and controls of sterility and no treatment were used each time. Biofilm pegs were sonicated to create a suspension. 20 μL from each replicate was serially diluted and spread-plated onto TSA for post-treatment quantification. All plates were incubated overnight at 37°C, and colonies were scored to generate CFUs/mL values the following day. Bacteriostatic concentrations were based on 24-hr TSA growth, and bactericidal concentrations were based on 24-hr optical density at 650 nm using a spectrometer (Biotek).

RESULTS AND DISCUSSION

The chiller and dipping stations offer extended exposure of poultry carcasses to disinfectants prior to packaging, while bringing the temperature down to 4°C as quickly as possible. PAA was ineffective at producing significant log reduction in all strains of bacteria tested at the concentrations currently used in commercial poultry processing facilities. PAA was also only bacteriostatic against planktonic cultures at concentrations ranging from 0.04% to 0.18%, and appeared to reduce *E. coli* numbers. ACH was ineffective in all conditions. Cecure achieved significant log reductions in all strains of *Salmonella*, as well as in *E. coli*, with bactericidal activity at levels as low as 0.03% at 90 minutes, but it cannot be used at that contact time. Cecure was ineffective against *Pseudomonas* biofilm, and was only bacteriostatic at safe ranges against two of the five SH strains at the shorter contact time. The greatest reductions were seen in planktonic SH Historic 1992. SH biofilm consistently proved to be more resistant than other strains.

CONCLUSION

Cecure was most effective in a concentration- and time-dependent manner, but was not reflective of present poultry processing conditions due to current regulations. The historic SH strain appeared to be more sensitive to disinfection than the more recently isolated strains of SH, which also resisted the bactericidal activity of Cecure. Based on our results, at current contact times and concentrations used in practice by poultry processors, the application of disinfectants is not an effective bactericidal or -static step. It therefore appears that while disinfectants might be important for controlling other pathogens in a processing environment, mitigation of *Salmonella*

must essentially focus on preventing contamination in pre-harvest live production systems.

Endemic prevalence of *S. enterica* in the environment provides a large dynamic reservoir for the infection of poultry, a vehicle for contamination of human food products, the rise in incidence of multidrug resistant strains, and an increase in disease severity (11). There appears to be an emergence of acquired reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials. The difficulty of eradication of established biofilm serves to emphasize the priority of unraveling more efficient control strategies. Because they are now seen as more ubiquitous than planktonic bacteria in many environments, including food processing, we propose that the AOAC expand their bacterial sensitivity testing to include biofilms. For companies that are interested in a complete and rapid characterization of zoonotic bacteria, we recommend using MBEC/MIC assays along with log reduction experiments in order to more fully characterize disinfectant efficacy, and provide a context-specific suite of information about a bacterium. Upon introduction of new disinfectants or isolation of new foodborne bacterial strains, the identification of biofilm formation abilities coupled with the experiments described here would offer poultry companies a novel and more comprehensive approach for evaluating and quantifying the effectiveness of antimicrobial strategies in food production systems.

(The full-length article will be published in the *Journal of Food Protection*.)

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Table 1.

Bacterial Strain		90 minutes @ 4°C			8 seconds @ 21°C
		PAA	ACH	Cecure™	
SH field strain 10	P	Static*	Ineffective	Cidal	
	B	Ineffective	Ineffective	Cidal*	Static*
SH field strain 18	P	Static*	Ineffective	Cidal	
	B	Ineffective	Ineffective	Cidal*	Static*
SH field strain 29	P	Static*	Ineffective	Cidal	
	B	Ineffective	Ineffective	Cidal*	Static**
SH field strain 30	P	Static*	Ineffective	Cidal	
	B	Ineffective	Ineffective	Cidal*	Static**
SH historic 1992	P	Static*	Ineffective	Cidal	
	B	Ineffective	Ineffective	Cidal	Static**
S. Ohio field strain 11	P	Static*	Ineffective	Cidal	
	B	Ineffective	Ineffective	Cidal	Static**
S. Senftenberg field strain 65	P	Static**	Ineffective	Cidal	
	B	Ineffective	Ineffective	Cidal	Static**
P. aeruginosa	P	Static*	Ineffective	Static	
	B	Static**	Ineffective	Ineffective	Ineffective
E. coli	P	Cidal*	Ineffective	Cidal	
	B	Ineffective	Ineffective	Cidal	Static*

*Effective at levels above poultry processing facility but still below GRAS
**Effective only at levels above GRAS

PAIRED HOUSE BROILER TRIALS COMPARING BURSAPLEX® TO VAXXITEK® ON BURSAL IMAGING, PCR, AND SEROLOGICAL RESPONSE (ELISA) TO INACTIVATED ND/IB VACCINATION

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INTRODUCTION

The chief concern of infectious bursal disease virus (IBDV) infections in the United States is its potential to cause immune suppression. A field trial was conducted to compare two hatchery applied vaccines for the dynamics of IBDV field infections in the bursa of Fabricius as well as their relative potential to allow for immune suppression. Onset, load and duration of infection were measured as well as bursal lymphoid tissue recovery after infection. Because of this complex's consistently low Newcastle disease virus (NDV) field challenge, a notable depression in titer response to ND vaccination—long considered a gold standard (1,2)—would be used to gauge potential differences in immune suppression caused by IBDV.

MATERIALS AND METHODS

Three broiler farms were selected to house respective flocks vaccinated *in ovo* with either Vaxxitek® or Bursaplex®. Six bursas were collected at three, four, and five weeks of age and submitted for computer imaging analysis (histology) and PCR analysis (sequencing and real time). At five and a half weeks 24 birds per flock were transported to Southern Poultry Research Group facilities where they were tagged, vaccinated via intramuscular injection with a full dose of inactivated ND/IB vaccine and co-mingled into two large floor pens. Birds were bled at four weeks post injection and serology (ProFLOK®) for NDV, IBV and IBDV was performed. Differences were not statistically analyzed and are only descriptive.

RESULTS

Computer imaging analysis. The onset of field infections ranged between 18-22 days in both vaccine treatments. Good lymphoid regeneration was seen in all flocks by five weeks of age except for one Vaxxitek flock that was positive for a second (Classic) IBDV in the five week sampling interval.

PCR analysis. All flocks were IBDV+ at four weeks of age. However, at five weeks of age none of the Bursaplex flocks were IBDV positive while all of the Vaxxitek flocks were still infected—see Figure. Three different IBDV types were isolated:

1) a Del-E/Group-6 hybrid
2) AL2
3) Classic

All three Vaxxitek flocks were strongly positive for IBDV (Ct value ≤ 35 cycles), including one farm that was strongly positive in all three sampling windows. Two of three Bursaplex flocks were strongly positive but only in one testing window (28 days).

Serology. Overall, NDV geometric mean titers (GMT) tended to be similar between Bursaplex flocks and Vaxxitek flocks. However, there was less flock-to-flock variation in Bursaplex flocks (6,085 to 6,652) compared to Vaxxitek flocks (4,228 to 7,530), with the greatest difference coming on Farm C (Bursaplex—6,652 vs. Vaxxitek—4,228). IBDV GMTs tended to be about 10% lower in Bursaplex groups while IBV GMTs were very similar (about a 5% difference).

DISCUSSION

Computer imaging and PCR analysis documented that all six flocks, regardless of vaccine type, were challenged between 18-22 days of age. While this window of challenge is safely beyond the "red zone" of 14-16 days of age, the consistency of the infection window also indicates that the field challenge is quite high. That said, all but one flock showed good lymphoid regeneration by five weeks of age—an indication that both vaccines were at least having a sparing effect on the three-week challenge.

Because the circulating NDV challenge and resulting seroconversion in this complex has historically been very low, the NDV titer responses in this study were attributed primarily to the inactivated

ND/IB vaccination. Having an IBDV-negative control or even a non-vaccinated control group in this study would have been a good comparison, but it was beyond the scope of this trial—which was to compare two IBD vaccination strategies for their potential differences in immune response to inactivated NDV antigen. As such, Bursaplex NDV GMTs were more consistent between flocks but overall there was only a modest 5% advantage to Bursaplex, suggesting no difference in potential immune suppression. The 10% higher IBD GMTs in Vaxxitek flocks may have been due to a better IgG priming effect and/or to the higher, more persistent levels of field virus replication that were documented in Vaxxitek flocks.

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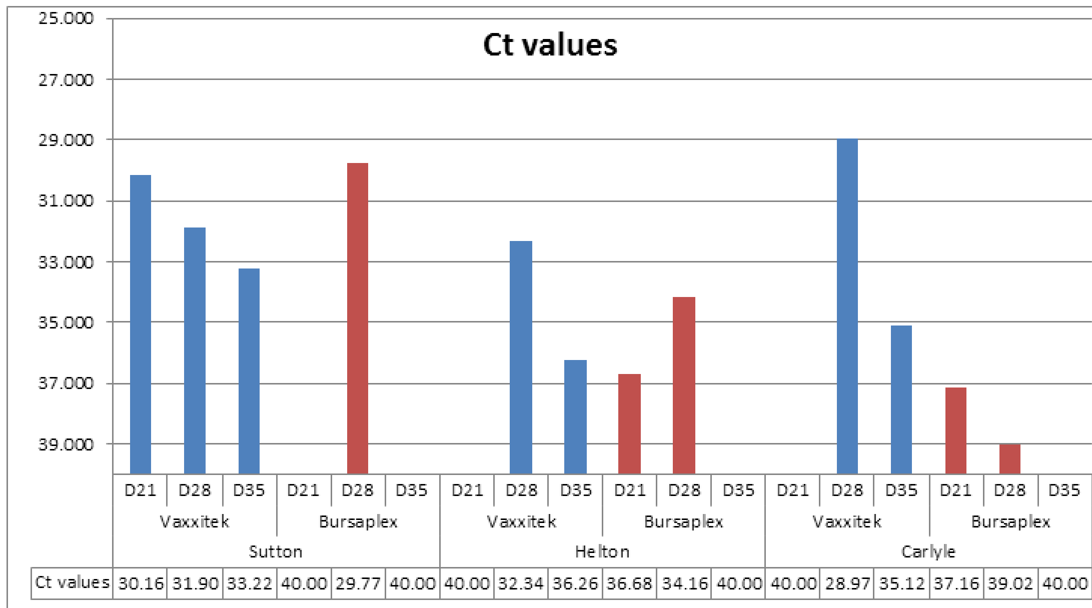
Figure 1. Relative IBD viral load in pooled bursas at each field sampling interval (days of age).



	Sutton						Helton						Carlyle					
	Vaxxitek			Bursaplex			Vaxxitek			Bursaplex			Vaxxitek			Bursaplex		
	D21	D28	D35	D21	D28	D35	D21	D28	D35	D21	D28	D35	D21	D28	D35	D21	D28	D35
Ct values	30.16	31.90	33.22	40.00	29.77	40.00	40.00	32.34	36.26	36.68	34.16	40.00	40.00	28.97	35.12	37.16	39.02	40.00

Note: Samples with strong PCR bands had Ct values ≤ 35. Negative samples had a Ct value of 40.0.

E. COLI VACCINATION TRIAL IN COMMERCIAL BROILERS FACING UNUSUALLY HIGH *E. COLI* MORTALITY SECONDARY TO VARIANT IBV CHALLENGE (DMV/1639)

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INTRODUCTION

Newly emerging variant infectious bronchitis viruses (IBV) are a challenge to the broiler industry because today's vaccine serotypes often do not offer sufficient cross-protection against them. The resulting damage and inflammation of the respiratory tract leaves broilers more susceptible to secondary bacterial infections, most notably *E. coli* related airsacculitis and septicemia/mortality. In contrast, a live *E. coli* vaccine, Poulvac® E. coli (PVEC), has the proven ability to help protect birds against a variety of *E. coli* serotypes in both controlled studies (1,2) and field trials (3). A broiler integrator on the Delmarva Peninsula suffering from high secondary *E. coli* mortality last winter/spring following a high DMV/1639 challenge decided to trial PVEC in the spring/summer months. The goal was to see if *E. coli* vaccination could at least help reduce the high secondary *E. coli* losses while the IBV challenge was still problematic.

MATERIALS AND METHODS

PVEC was given at a half dose to day of age broiler chicks along with the Newcastle disease (ND)/IB vaccine via coarse spray and again at 16 days in about half of the day of age vaccinates. Weeks of control flocks alternated with PVEC weeks for nine total weeks. Complex A raises both six and a half and four pound birds. Complex B raises eight and a half pound birds which also received a chick embryo origin (CEO) infectious laryngotracheitis virus (ILTV) field boost. Treatments were compared based on field performance and condemnations. Differences were not statistically analyzed and are only descriptive.

RESULTS

Grow livability (>2 weeks) improved linearly over non vaccinated controls in Complex A small birds (0.73% to 1.42%) and Complex B flocks (0.68% to 1.65%) when going from one to two doses of PVEC; Complex A large birds improved ~1% using either one or two doses. Adjusted feed conversion improved linearly from one to two doses in Complex A large birds by 0.9 pts and 2.3 pts, respectively. Airsacculitis condemnations decreased linearly from one to two doses in Complex A small birds from 0.57% to 0.41% to 0.27%, respectively. Cost per pound decreased linearly from one to two doses in all three bird sizes. Improvements on two doses PVEC were 0.42 cents (Complex A large birds) and 0.56 cents (Complex A small birds) and 0.34 cents (Complex B). PVEC flocks were 5.8 times less likely to suffer excessive grow mortality (>8%) in Complex B large birds (13.53% vs. 2.35%) and 3.5 times less likely in Complex A large birds (19.28% vs. 5.56%). None of the PVEC small birds in Complex A suffered excessive mortality compared to 6.67% of non-vaccinated small birds. There was indeed a seasonal effect on the incidence of excessive mortality during the course of this trial—it waned as placements got closer to summer—but the order of reductions from controls to PVEC treatments remained consistent (see Figure).

DISCUSSION

While overall livability and performance improved with PVEC vaccination, arguably the most striking benefit of PVEC can be seen in the incidence of flocks suffering from excessive mortality. This was the company's biggest complaint coming out of last winter's IBV challenge and the main reason for this trial. Total live cost is arguably the truest index of bird performance and the PVEC cost savings alone paid for one or two full doses used across the board. Other costs such as running processing plants below full capacity or at reduced line speeds were not factored in but are often even more significant—especially once a novel IBV, for which there is insufficient vaccine cross-protection, becomes well established. Condemnation differences were seen in small birds (five and a half weeks) but were not as apparent in large birds, perhaps because of the timing of the DMV/1639 challenge (usually around four

weeks of age) followed by secondary *E. coli* infections.

These trial results demonstrate the value of immunizing against secondary *E. coli* losses that often occur following a primary respiratory infection—in this case caused by a variant IBV. While the sparing effects of Poulvac E. coli were clear in this trial, it would have been interesting to measure the vaccine effect during the peak of the IBV challenge when even more flocks had suffered high mortality.

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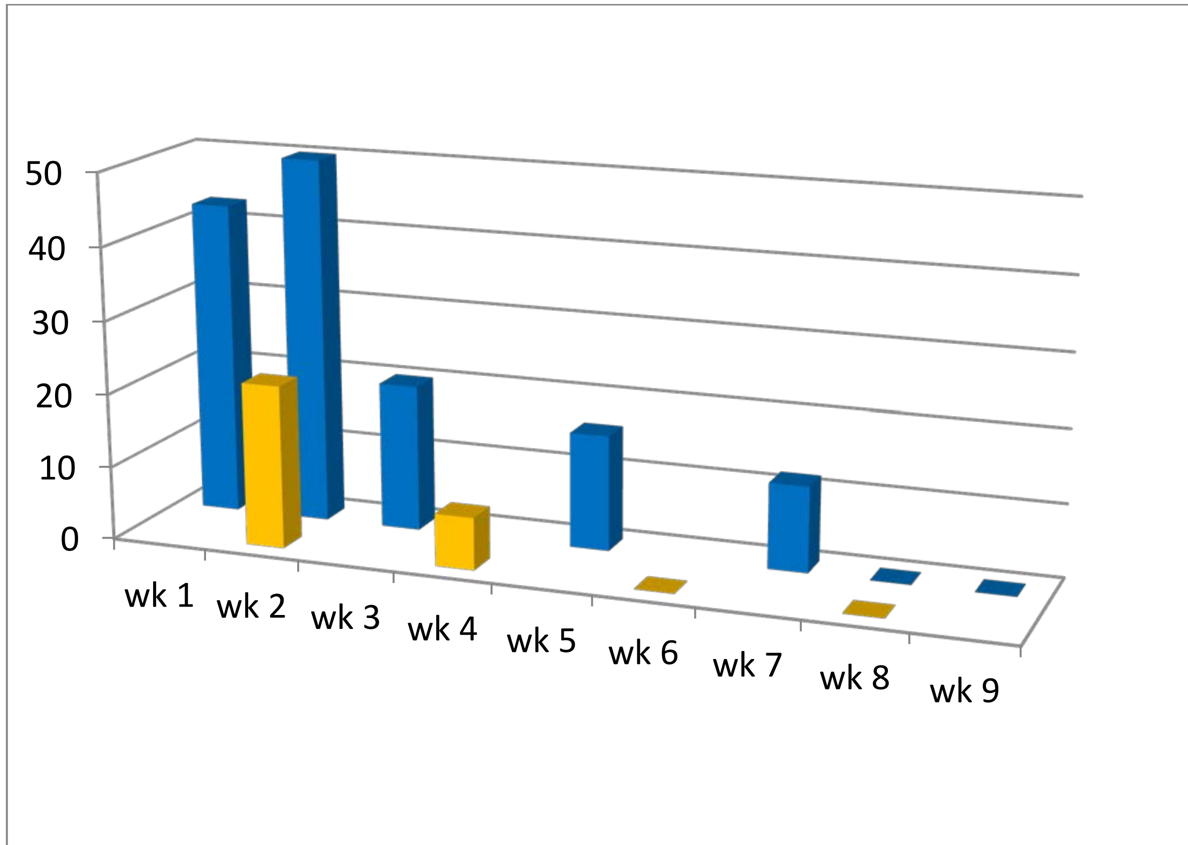
Figure 1. Weekly percentage of flocks with excessive (>8%) grow mortality by treatment group in Complex A (where trial design and treatment balance was maintained).



Note: Lack of a bar indicates an off week for one of the treatments (controls are in blue).

PATHOLOGY OF HIGHLY PATHOGENIC AVIAN INFLUENZA H5N8 IN GYRFALCONS

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After the November 2014 report of H5N2 HPAI outbreaks among poultry in British Columbia, the US Department of Agriculture and the Washington State Department of Agriculture (WSDA) increased surveillance for domestic and wild birds along the US–Canada border. On December 8, 2014, WSDA learned of a captive reared gyrfalcon (*Falco rusticolus*) that had been presented to a private practitioner with clinical signs of depression and lack of appetite for two days. The bird had started having seizures the morning of the presentation and died soon after. Three other gyrfalcons from the same premise, started showing signs of depression and died one to three days later. The birds were housed individually, in separate cages. All four birds had consumed parts of a captured wild American wigeon, which was hunted and caught 8-10 km from the Canadian border by one of the affected gyrfalcons. Heavy metal poisoning was suspected.

Three of these falcons were submitted to the Washington Animal Disease Diagnostic Laboratory for laboratory evaluation. Gross lesions were minimal and nonspecific. The most remarkable changes were pulmonary congestion and enlarged, pale livers. One bird had a large granuloma on the right posterior thoracic air sac.

All birds were examined histologically and a subset of tissues was stained for influenza A antigen by immunohistochemistry. Lesions observed in hematoxylin and eosin stained tissues were most prominent in the brain and pancreas. Two falcons had histopathological changes of acute, multifocal necrotizing encephalitis and gliosis on the cerebral cortex. The third falcon had multifocal to coalescing areas of coagulative necrosis in the pancreas, but no lesions in the brain. Lymphoplasmacytic perivascular cuffing was not a consistent finding. Nonspecific hepatocellular vacuolation was seen in all three birds and interpreted as result of not eating. Immunohistochemical stains on brain and pancreas sections revealed influenza A specific antibody immunoreactivity associated with the necrotic foci. Immunoreactivity for influenza antigen not associated with tissue lesions was present in the brain and various visceral organs. Similar histological changes have been described in previous reports of H5N8 infections (3) and in falcons infected with H5N1 (2).

Molecular assay results for oral and cloacal swab samples from all three falcons were positive for influenza A and H5 viruses. These samples were then forwarded to the National Veterinary Service Laboratory (Aimes, IA) where the virus was classified as influenza A Eurasian lineage H5 clade 2.3.4.4 AIV, A/gyrfalcon/Washington/41088-6/2014 (H5N8) (GenBank taxon no. 1589663) (1).

Blood collected from the first falcon was negative for heavy metals.

The affected premise had 25 raptors and 40 pigeons. No further deaths or illnesses were reported among other birds at the facility. Samples from raptors, pigeons and environment from this premise were tested for avian influenza by reverse transcriptase polymerase chain reaction (RT-PCR) one and two months after the initial diagnosis of highly pathogenic avian influenza. All the bird and environmental samples were negative for AI.

The virus was not detected from any other bird or from the environment. Transmission of avian influenza between individual birds is usually by ingestion or inhalation (4). All four affected gyrfalcons were reported to have consumed the same wild American wigeon a few days prior the onset of clinical signs. Airborne dissemination has been suggested as a potential transmission route over limited distances. Although raptors and pigeons are known to be susceptible to avian influenza, none of the other birds on the premise showed clinical signs. This suggests low lateral transmissibility of this influenza A. The recent epidemiologic report on HPAI (5), suggests that airborne transmission may have a minimal contribution to the spread of HPAI. It is possible that infection of other birds on this farm did not occur because raptors are housed in individual cages, sometimes separated by vegetation

between cages, with little chance of airborne droplet transmission or environmental cross contamination.

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DEVELOPMENT OF A TRIVALENT *BORDETELLA AVIUM* VACCINE

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SUMMARY

In 1979 we developed a successful vaccine against the then prevalent strain of *Bordetella avium* (then known as *Alcaligenes faecalis*), that was causing significant problems in the turkey industry (1,2,3). This vaccine has been available commercially over the years (known as ART-VAX). The basis of the original vaccine was a temperature sensitive mutant that would attach to, and grow in, the cooler temperature of the nasal mucosa but would not grow in the warmer temperature of the lower respiratory tract. In recent years this original vaccine has become less effective due to a possible emergence of differing antigenic strains of *B. avium.*

The purpose of this project was to develop temperature sensitive mutants against three prominent strains of *B. avium* that in recent years have been causing major problems in the turkey flocks in Utah. The three strains were designated OC, CS, and BB, and mutants of each were produced by treatment with NTG (N-methyl-N-nitro-N-nitrosoguanidine)(1). Mutants that grew well at 32°C, and poorly at 40°C, were selected. Growth curves were done on each mutant to determine the level of mutation. Each selected mutant was then inoculated intranasally into two- week-old turkey poults, and two weeks later the nasal mucosa and trachea were cultured on MacConkey agar plates. After incubation at 32°C, for 24 and 48 hr, the level of colonization with the *Bordetella* mutants was measured. Serum antibodies of the inoculated poults were determined by a commercially available ELISA test three to four weeks after intranasal challenge.

Multiple mutants were made of each strain. Those mutants that showed good growth at 32°C, minimal growth at 40°C, produced good colonization of the nasal mucosa and poor colonization of the trachea, and induced a serum antibody response were selected as candidates for the trivalent vaccine.

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PIGEON RACING AND BREEDING IN MEXICO

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SUMMARY

Pigeon racing in Mexico is a frequent activity. There are four categories: Velocity, in which they can fly up to 200 km in one day; Medium endurance, in which they fly 400 km; Endurance, in which they fly 700 km; and Deep endurance, in which they fly up to 1000 km. Breeding starts in January and ends in April. Pigeons are monogamous and their offspring require the parent care to survive. The nest size is two squabs and the rearing lasts one month. Training requires discipline and conditioning to specific sounds related to feed to encourage the birds to go inside the dovecote. Diet consists of a mixture of energetic and protein grains. Pigeons consume 30 g of feed daily and require approximately 90 mL of water. Preventive medicine involves vaccination against paramyxovirus and pox and also internal and external deworming every three months. The main diseases are respiratory and parasitic, being *Haemoproteus columbae* a frequent parasite, causing severe anemia. The objective of this poster is to provide information about basic management and medicine of racing pigeons in Mexico.

INTRODUCTION

Pigeon (*Columba livia domestica*) breeding and racing is an important activity worldwide. Historically, this activity had a strong rise in the time of the Crusades, when the pigeons had military use. The armies used them to send encoded messages attached to the leg of the bird. During both world wars, carrier pigeons saved many lives by delivering messages through the English Channel, showing the next bombing targets, allowing people to take shelter someplace safe. Some of this pigeons were even honored for their services by the Royal Pigeon Racing Association.

PIGEON NAVIGATION

Pigeons possess spatial location features that allow them to return to their original loft after being released from a distant set point. There are different theories to explain their sense of direction and their ability to navigate. Some of them involve detection of the Earth's magnetic fields, the use of familiar topographical landmarks and the sun as a compass (1, 2, 3, 4, 7). All of which allows them to navigate back to their loft. Pigeon racing arise from this feature, allowing different types of competitions based on how fast the pigeons can return to their home lofts from a set point.

In México, racing pigeons can be bred for four different types of races:

1) Velocity: The pigeons are released from shorter distances (170-350 km) and the fastest pigeon wins. Pigeons can fly up to 100km/hour.
2) Medium endurance: The release point is 370 - 500 km from origin.
3) Endurance: Release point 600 - 700 km from home.
4) Deep Endurance: Release point 800 - 1000 km.

It is important to note that even in the endurance races, made to prove the pigeon's resistance to long flights, the birds are able to perform them in less than a day. Therefore, racing pigeons are high performing athletes, Velocity pigeons can be compared to "sprinters" and endurance pigeons to "marathon runners".

HOUSING

The pigeon loft must be located in a well ventilated and easy to clean place. Most pigeon lofts are located on rooftops of houses and buildings, but some can be found in open ground. The lofts usually have separated areas for breeders, squabs and flying birds (the racing team). The breeders need to have nests within their cages while the other areas only need perches. All of the areas need enough feeders and drinkers.

REPRODUCTIVE FEATURES

Reproduction is mainly monogamous. A couple can produce squabs all year, but since the racing season is at the end of the year, the rearing season usually comprises from January to April. Based on phenotypical features the owner can either set the

partners by confining the female with the selected male for several days until she accepts him; or, he can allow the males to fight each other for the ownership of a perch, to which each one of them will courtship a selected female.

After 10 to 12 days of mating, the egg laying starts. The incubation period lasts 18 days. The squabs are completely dependent of their parents to feed them. Both male and female participate in the feeding and caring of their offspring during a 25 to 28-day period. The squabs are fed by regurgitating a mix of pre-digested food and crop milk, a secretion of the crop lining induced by prolactin (6). It is common to have a second clutch of eggs after 14 days of rearing of the first squabs, allowing the mix of both squabs and eggs in the same nest.

When the squabs are between seven to nine days old, a marking ring is placed in the leg. The ring has a specific color based on the year of birth, the racing association, the country of origin, and a unique consecutive number associated with the bird for life.

TRAINING

When the squabs turn 28 to 30 days old and are almost the same size as their parents, they are separated to another area; at this point the homing and training starts.

The homing process involves placing the young pigeons in the access ramp every day to teach them how to enter through the trapdoor and how to recognize the loft. This process requires patience and discipline, since repetition is essential. Usually, after one or two weeks the pigeons start searching for the entrance by themselves. Simultaneously, the pigeons are taught to associate a specific sound (it can be a whistle or a metal can being shaken) with imminent feeding.

The training then starts, releasing the pigeons and locking them out of the loft, to force them to explore the surroundings and fly away. Then they are called using the specific sound related to feed, making them return to the loft.

The time between releasing and calling is spaced more and more to allow them to explore further and further away every day. When they have spent over an hour away through several days, it is time to put them in small baskets (cages) and take them even further from the loft to be released. The first flights normally are two to five km from the loft, and follow the flight line set by the competitions. As the pigeons improve their physical condition, the distance is then increased gradually to 10, 20, 50, and 100 km. The overall training process last between three and four months.

The racing competition consists of putting the pigeons in the baskets and taking them to a certain location and releasing them so they will return to their loft. The fastest pigeon wins. To establish the flight time with accuracy, each pigeon carries a special ring with an electronic built-in chip that is detected by an antenna set underneath the access ramp. The antenna sends the data to an electronic watch that registers the exact arriving time.

Due to the different locations of each of the lofts participating in the race, and the resulting differences in distance to the release set point, the judges calculate the weighted mean distance and consider it along with the flight time to select the winner. The difference between first and second place can be of just seconds. Each association establishes the scoring system and the prizes.

FEEDING

One of the most challenging aspects of pigeon breeding and racing is feeding. Pigeons are granivorous and require a proper mixture of energetic and protein grains to fill their needs. The most common energetic grains are sorghum, wheat, barley and oats. The protein sources include lentil, chickpea, soy and green peas. Depending on the level of physical activity grains with high lipid content are included in the diet, for example sesame seeds, safflower and sunflowers. There are different dietary formulations for maintenance, racing, breeding and recovery.

PREVENTIVE MEDICINE

Considering that during competition there is mixing of pigeons from different lofts, preventive medicine is another critical aspect. Vaccination is carried out once a year against avian paramyxovirus (by eye drop) and pox virus (by wing web puncture). Deworming is conducted every three to four months using oral ivermectin. To avoid external parasites like the pigeon fly (*Columbicola columbae*) and the louse (*Pseudolynchia canariensis*) the pigeons are bathed with a cipermetrin solution. This also prevents transmission of *Haemoproteus columbae* (5). Additionally, a bowl big enough to fit a pigeon should be provided and regularly filled with water and a few grams of sea salt and vinegar, to let the pigeons bathe themselves.

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ANTIVIRAL EFFECT OF HYBRID QUATERNARY AMMONIA-GLUTARALDEHYDE DISINFECTANT ON VERY VIRULENT INFECTIOUS BURSAL DISEASE (vvIBDV) AND LOW PATHOGENIC AVIAN INFLUENZA (LPAI) VIRUSES

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Infectious bursal disease (IBD) is a highly infectious immunosuppressive disease of chickens that is endemic in most poultry-producing areas around the world (8). The non-enveloped virus that causes the disease, infectious bursal disease virus (IBDV), is highly stable and resistant to inactivation by common disinfectants, persisting in the environment despite thorough cleaning and disinfection (9). Disinfectants capable of inactivating IBDV include formalin and invert soap, when combined with 0.05% NaOH, while IBDV was resistant against treatment with phenol and merthiolate (1, 10).

Avian influenza viruses (AIV) on the other hand, are highly vulnerable to most disinfectants because of their enveloped nature, making them easier to eliminate (6). Despite the vulnerable nature of the virus, AIV poses a major threat to the poultry industry as the outbreaks in 2014 in the U.S. have shown. AIV can be inactivated completely after 1 min of contact time with potassium peroxymonosulfate, sodium dicholoroisocyanurate or glutaraldehyde (6).

Some of the aforementioned studies, other than being outdated, were conducted in laboratory settings without taking into consideration factors that would affect the disinfectant efficacy once used in the field, such as organic material. Therefore, the aim of this study was to investigate the efficacy of a commercial combination of quaternary ammonium and glutaraldehyde as a disinfectant against IBDV and AIV in the presence of organic material commonly found in the commercial poultry industry: fecal matter alone, feather dust mixed with feces, and bedding material mixed with feces.

Five g of chicken feces were spiked with 1 mL of very virulent IBDV (vvIBDV) S7610 resulting in a final viral titer of $2x10^4$ EID_{50} /g of feces. Ten to twenty g of feather dust and clean wood shavings were placed in separate large petri dishes and a third petri dish was left empty. One g of IBDV-spiked feces was added to each dish, mixed well with feather dust or wood shavings, and left at room temperature for five min.

For testing the efficacy against AIV, 10 g of chicken feces were spiked with low pathogenic avian influenza (LPAI) H6N2 to achieve a titer of $5.6x10^5$ EID_{50}/g of feces. Ten to twenty g of feather dust and clean bedding material were placed in separate dishes, while a third petri dish was empty. Ten g of LPAI-spiked feces were added to each dish, mixed well, and left at room temperature for five minutes.

The combination of quaternary ammonium and glutaraldehyde was diluted in autoclaved deionized water at varying concentrations based on which organic matter it was applied to and following the manufacturer recommendations: 2% on feces alone; 0.5% on feather dust with feces and 1% on shavings/ bedding material contaminated with feces. Five mL of disinfectant solution at the appropriate concentration were then added to each petri dish and left for 10 minutes to allow the disinfectant to act on the virus-infected samples.

After the contact time with the disinfectant, each petri dish was vigorously swabbed with a cotton-tipped applicator; the swabs were placed in tryptose phosphate broth and vortexed thoroughly. After centrifugation, virus re-isolation in embryonated SPF chicken eggs was attempted using routine methods (8, 11). After two passages, the CAM and embryos of the eggs used for re-isolation of IBDV were tested by RT-PCR as described by Jackwood et al. (2, 3, 4, 5), while the allantoic fluid of the eggs used for re-isolation of AIV was tested by hemagglutination assay (7).

Neither IBDV nor AIV were re-isolated from the samples treated with the disinfectant.

In conclusion, the combination of quaternary ammonia and glutaraldehyde was effective in the inactivation of vvIBDV and LPAI at the recommended concentrations on all the spiked

organic materials used: fecal matter alone, feather dust, and wood shavings spiked with feces.

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THE FORGOTTEN *EIMERIA* OF CHICKENS

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SUMMARY

These poorly referenced organisms (*Eimeria hagani, E. mivati and E. praecox*) are now commonly seen in commercial chickens. *E. hagani* and *E. praecox* appeared to be associated with a transient impairment in performance, whereas *E. mivati* is associated with morbidity and mortality. Specific anticoccidial programs appeared to be associated with these unusual *Eimeria; E. hagani* in particular seem to be prevalent in birds younger than 30 days of age.

INTRODUCTION

Tyzzer (1928) suggested eight important criteria for differentiating and identifying new species of *Eimeria*. These criteria have been used in the identification and naming of almost all the known species of chicken *Eimeria* in the USA. Nine species named for the chicken; these include *E. tenella* (Raiellet and Leucet, 1891), *E. maxima, E. mitis, E. acervulina* (Tyzzer, 1929), *E. praecox, E. necatrix,* (Johnson, 1930), *E. brunetti* and *E. hagani* (Levine, 1938) and *E. mivati* (Edgar and Seibold, 1964). After the naming and description of *E. mivati*, researchers described finding this parasite throughout the world. Long and Tanielian (1965), found these parasites in chickens in Lebanon. Reid *et al* (1965) reported *E. mivati* in Great Britain, Germany, Holland, France and Canada.

In the early 1970s, several researchers developed deep reservations about the validity of *E. mivati and E. hagani.* Long and Reid (1982), *E. hagani* and *E. praecox* stated that these species are rare or non-existent. Shirley and Jeffers, (1983) stated that these species should be considered "*nomina dubia*" — in other words, their existence is doubtful. Since then the seven species that have been considered parasites of chickens are *E. acervulina, E. brunetti, E. maxima, E .mitis, E. necatrix, E. praecox and E .tenella.* Some of these species have been used extensively in research (*E. acervulina, E. maxima and E. tenella*); therefore there are an inordinate amount of references as compared to the other species.

Levine's (1938) description of *E. hagani*_and the pathology it causes was very brief but fairly accurate. However, Oluleye (1982) described the lesions of *E. hagani* as diffuse hyperemia and catarrhal inflammation. There was tremendous engorgement of the capillary bed in the lamina propria. This species does not infect the yolk stalk diverticulum or the mid gut region (unpublished data).

During the late 1960 and early 1970s Long published his findings on *E. mitis, E. mivati*, and *E. acervulina,* those papers might have led to the current perceptions about these species. Riley (1980) showed that *E. acervulina* and *E. mivati* were antigenically dissimilar and the *E. mivati* strains maintained at Houghton were contaminated with *E. acervulina* or vice versa. Riley's belief was Long had been working with mixed species rather than pure isolates.

MATERIALS AND METHODS

Fecal, litter and or intestinal samples were submitted to determine the species of coccidia in the samples. From the intestinal samples wet mount smears were taken from the duodenum, jejunum, ileum and ceca and placed on clean microscope slides and examined with a compound microscope. The litter or fecal samples were thoroughly mixed and an aliquot taken from each sample and diluted in twice the volume of water; mixed thoroughly and a droplet from each sample was place on a clean microscope slide and examined. Several selected samples were collected, sporulated and inoculated into coccidia naïve birds for an expansion of the sample.

RESULTS AND DISCUSSION

Eimeria isolates from commercial operations are not easy to work with as the sample could be a composite of several species. However, someone skilled in the arts would have it easier to sort it out. In recent years, several of these forgotten or rare or non-existent species such as *E. mivati, E. hagani* and *E. praecox* have been seen in samples from commercial broiler operations (50% of recent samples). Most often these unusual organisms are seen during the period in which chemical anticoccidial are used. These species are easily missed during routine necropsy sessions, in which biological samples are only taken from the mid gut region.

When these organisms are inoculated into naïve chickens, the birds become positive by 88-93hr post inoculation (pi). By 120 hr the average number of oocysts produced per bird was approximately $144.0x10^6$, therefore indicative of high fecundity. The current belief is that these coccidia are non-existent and or non-pathogenic, but current observations showed otherwise. Growth can be significantly impacted even while the animal might be on an anticoccidial. These agents do not cause mortality but cause transient morbidity and may be easily confused with more pathogenic species such as *E. tenella or E. brunetti*.

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SUMMARY OF RECENT *ORNITHOBACTERIUM RHINOTRACHEALE* INFECTIONS IN UTAH TURKEYS

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CASE REPORT

Introduction. Prior to 2014 presence of *Ornithobacterium rhinotracheale* (ORT) infection in Utah commercial turkeys had not been detected. In October 2014 mortality from a flock of 18-week-old toms was submitted to the Central Utah Branch of the Utah Veterinary Diagnostic Laboratory (CUVDL). Gross lesions suggestive of fowl cholera were present; pure growth of ORT was isolated from the lungs of multiple toms. An increasing number of flocks between 13 and 17 weeks of age began showing similar respiratory signs (with ORT isolation from lungs and pericardial sacs predominantly). Some turkeys from these flocks that previously showed respiratory signs developed severe tenosynovitis in the hocks. Over the period 2014-15 ORT isolation from similarly age flocks exhibiting higher than expected mortality continued to be made; however, severity of lesions, associated mortality, and frequency of the tenosynovitis sequel decreased over time.

The description of ORT in turkeys and interaction with various pathogens have been well characterized, and excellent general reviews of ORT have been previously published (1,2,3,4,5,6), to name just a few. This report revisits ORT by describing the sudden appearance, lesions, and general course of infection and management of an ORT epornitic in an apparently naïve population of domestic turkeys.

History and clinical signs. Dead birds from an 18-week-old flock of toms exhibiting signs suggestive of fowl cholera were submitted to the Utah Veterinary Diagnostic Laboratory, Central Utah Branch (CUVDL) in October 2014. Mortality had steadily increased over the previous few days before submission, with moribund birds hanging along the side of the building and dying. Usual field observations by company personnel and growers were increased mortality with no clinical respiratory signs. During the next few months, mortality from various flocks ranging in age from 13 to 17 weeks was submitted to the CUVDL with similar mortality pattern and history.

Gross and histologic findings. Lesions consisted of unilaterally or bilaterally diffuse fibrinoheterophilic pneumonia, pleuritis, and pericarditis (Figures 1, 2, 3, and 4). Some turkeys also exhibited fibrinoheterophilic airsacculitis of thoracic airsacs, but this was only occasional and not a predominant finding.

In some birds the hocks were markedly swollen and contained an abundance of straw-colored cloudy fluid (Figures 5 and 6). Histology revealed a severe, subacute, diffuse, heterophilic and fibrinonecrotizing tenosynovitis characterized by large amount of fibrin admixed with cell debris, viable and degenerative heterophils, and fewer macrophages infiltrating and expending the tendon sheets. The synovium was infiltrated by numerous inflammatory cells transmigrating towards the lumen and early granulation tissue formation was present multifocally (Figure 7).

Although *O. rhinotracheale* was sporadically isolated from swollen hocks, the isolation rate was much less than from lung and heart lesions.

Isolation and identification. Initial isolation was accomplished using swabs from tissues streaked onto blood and MacConkey agar plates. No growth was detected on MacConkey agar. After 48 hr incubation at 37°C, small circular gray colonies were visible on blood agar. Gram staining revealed gram-negative pleomorphic rods (Figure 8). Biochemical characterization and identification were performed using API® 20NE and MALDI-TOF. No serotyping was pursued.

Management considerations. From a management standpoint, an effort was made to reduce the stress in affected flocks. This was accomplished by optimizing air exchange rate and minimizing temperature fluctuations within the building.

Beginning May 2015 all toms were vaccinated with a live ORT vaccine at 28 days of age. Within three months all birds in the company were being vaccinated at 21 days. Serology data using ELISA

test indicated vaccinated birds seroconverted to ORT exposure. Since the vaccination program was begun, the number and severity of ORT cases have been reduced by 70 to 80%.

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Figure 1. Typical gross appearance of the lungs showing diffuse fibrinonecrotizing pneumonia and pleuritis.

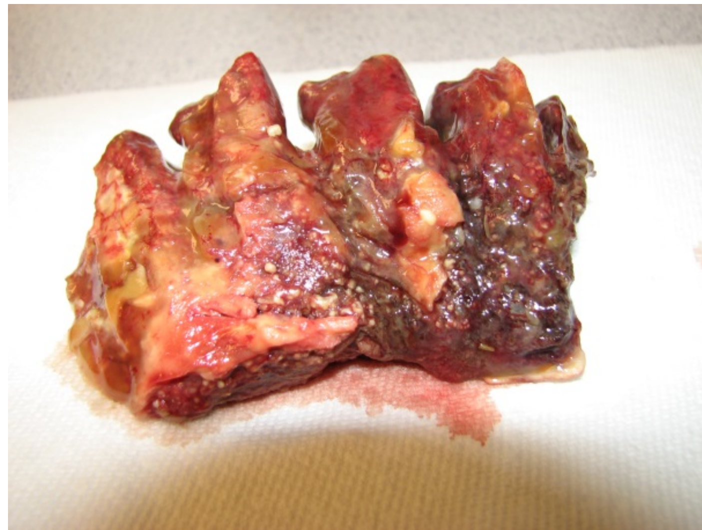


Figure 2. Fibrinoheterophilic pericarditis. The pericardial sac is filled with fibrinoheterophilic exudate.

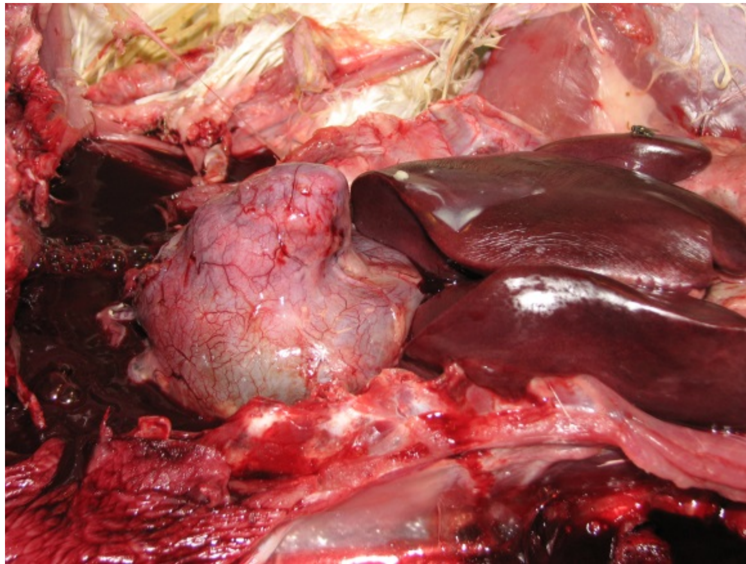


Figure 3. Lung histology depicting fibrinoheterophilic pneumonia. (10X, bar = 200 µm, H&E staining).

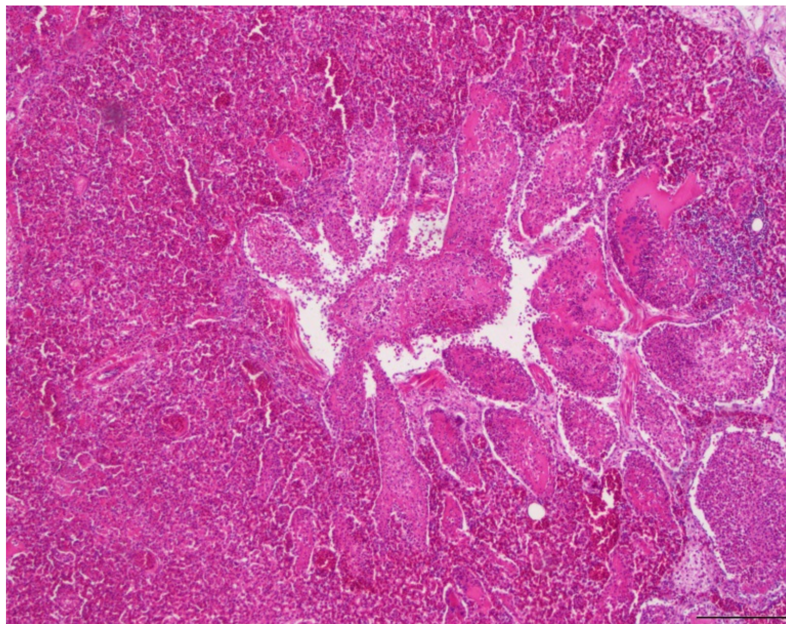
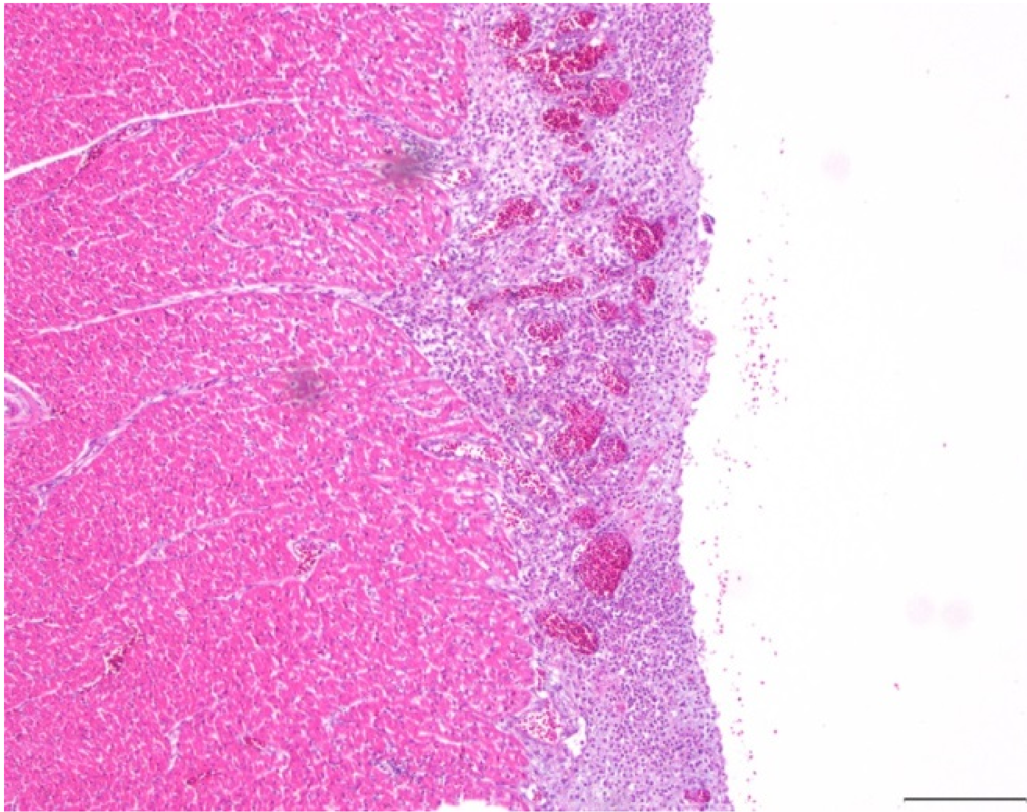


Figure 4. Histology of the heart with fibrinoheterophilic epicarditis. (10X, bar = 200 µm, H&E staining).



Figures 5 and 6. Swollen hock joint containing yellow cloudy exudate.

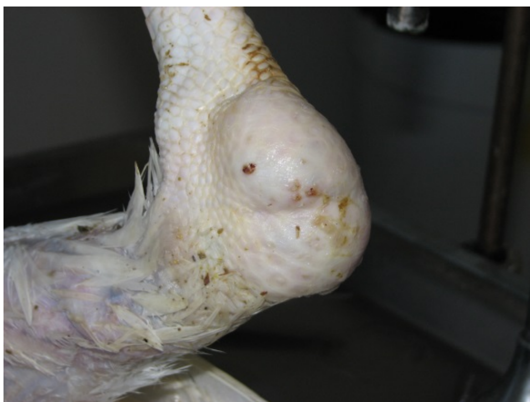


Figure 5

Figure 6

Figure 7. Cross section of tendon sheath from the hock area showing severe fibrinoheterophilic tenosynovitis (2X, bar = 1 mm H&E staining).

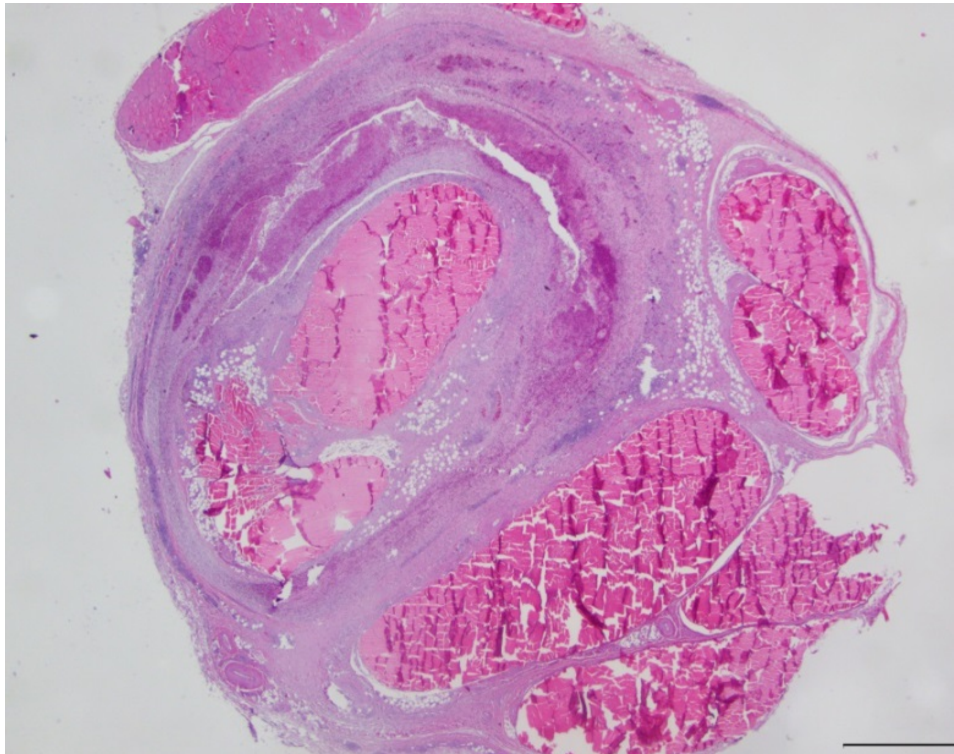
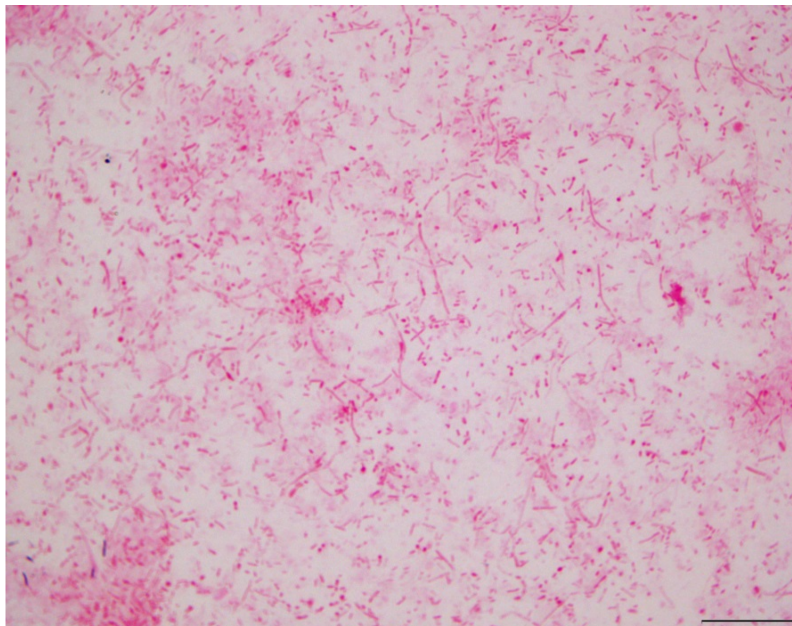


Figure 8 Gram stain of *Ornithobacterium rhinotracheale* (1000x; bar = 20µm).



ANALYSES OF *BORDETELLA* ISOLATES COLLECTED FROM TURKEYS WITH RESPIRATORY DISEASE USING MALDI-TOF MASS SPECTROSCOPY AND COMPARISON TO A *BORDETELLA AVIUM* VACCINE

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SUMMARY

Bordetella avium has been isolated from turkeys showing clinical signs of respiratory disease and increased mortality in Sanpete County Utah, despite receiving the *B. avium* vaccine. To determine if recent *B. avium* isolates are related or unrelated to the vaccine strain, 25 isolates from different time periods and different locations in the U.S. were collected for comparison by MALDI-TOF mass spectroscopy. Spectra were evaluated by MALDI Biotyper software (Bruker Co.) to determine relationships among the clinical isolates. Cluster analysis of the spectra showed four major clusters using the principle component scores for the three spectral peaks in highest abundance. These clusters also accounted for >70% of the variability in the data based on identification score values. Four of five Utah isolates were in the same cluster as the vaccine strain. However, one isolate from Utah and isolates from other locations did not cluster with the vaccine strain.

INTRODUCTION

Tracheal swab cultures were used to isolate *Bordetella avium* from turkeys showing clinical signs of respiratory disease and increased mortality in Sanpete County Utah, despite receiving the *B. avium* vaccine. To determine if recent *B. avium* isolates are related or unrelated to the vaccine strain, 25 isolates from different time periods and different locations in the U.S. were collected for comparison by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectroscopy.

MALDI-TOF mass spectroscopy is a powerful new tool for identification of pathogens in clinical specimens. Compared with other methods, including 16S rRNA and *rpo*B gene sequencing, reported accuracy of identification of bacteria, fungi or parasites has been 95.4% to 99.5% (3).

Bacterial colonies are mixed with a matrix solution and placed and dried onto a target plate. The matrix solution (cinnamic acid) cocrystallizes with the bacterial sample on the target plate (96 sample plate). Samples are then exposed to short laser pulses under high vacuum, vaporizing the bacteria and the matrix. Bacterial proteins are ionized, and an electromagnetic field accelerates the ions as they enter the flight tube. The time of flight (TOF) required for analytes to reach the detector at the end of the flight tube is measured and recorded. A "characteristic spectrum" based on the TOF provides a specific sample fingerprint, considered unique for each bacterial species resulting in precise identification (1). Software compares the collected spectra with a databank of reference spectra of bacterial isolates. A numerical score value of similarity to known isolates' spectra is calculated. A score value above 2.0 is defined as a valid species level identification, while values between 2.0 and 1.7 represent reliable genus level identifications.

MATERIALS AND METHODS

Spectra were obtained from clinical isolates collected from turkeys, grown overnight on blood agar plates, and evaluated by MALDI-TOF mass spectroscopy. The MALDI-TOF spectra from the clinical isolates were evaluated using the Bruker MALDI Biotyper software (IVD MALDI Biotyper 2.3) to determine relationships among the clinical isolates. This was done by comparing the MALDI-TOF results to the database of microorganisms using the Biotyper software for peak-matching and by multivariate analyses using the three principal component scores (2). After completion of the peak-matching algorithm, the score value was evaluated for the suggested matches. A score between 2.3 and 3.0 had a high degree of confidence that the correct species was identified. A score between 2.0 and 2.29 was also reliable, although indicates a lower level of

confidence in the species identification. We manually selected samples with high scores and processed the data sets into both dendrograms and cluster charts. Types of analyses included: presumptive species, location of collection, and year of collection. In the cluster charts, the relative distance between points is indicative of bacterial similarity.

RESULTS

Spectra were evaluated by MALDI Biotyper software to determine relationships among clinical isolates. Cluster analysis of the spectra showed four major clusters using the principle component scores for the three spectral peaks in highest abundance. These clusters also accounted for >70% of the variability (considered significant) in the data based on identification score values (Figure 1). Four of five Utah isolates were in the same cluster as the vaccine strain. However, one isolate from Utah and isolates from other locations did not cluster with the vaccine strain.

DISCUSSION

In recent years, clinical manifestations similar to bordetellosis have been observed in turkeys that have previously been vaccinated against *B. avium*. From these observations, we conclude that the infectious agents associated with bordetellosis in these turkeys may include *B. avium, B. hinzii*, or other closely related species. Future studies will involve additional biological characterization, and species-specific PCR, to determine if the clusters observed by MALDI-TOF have additional biological relevance.

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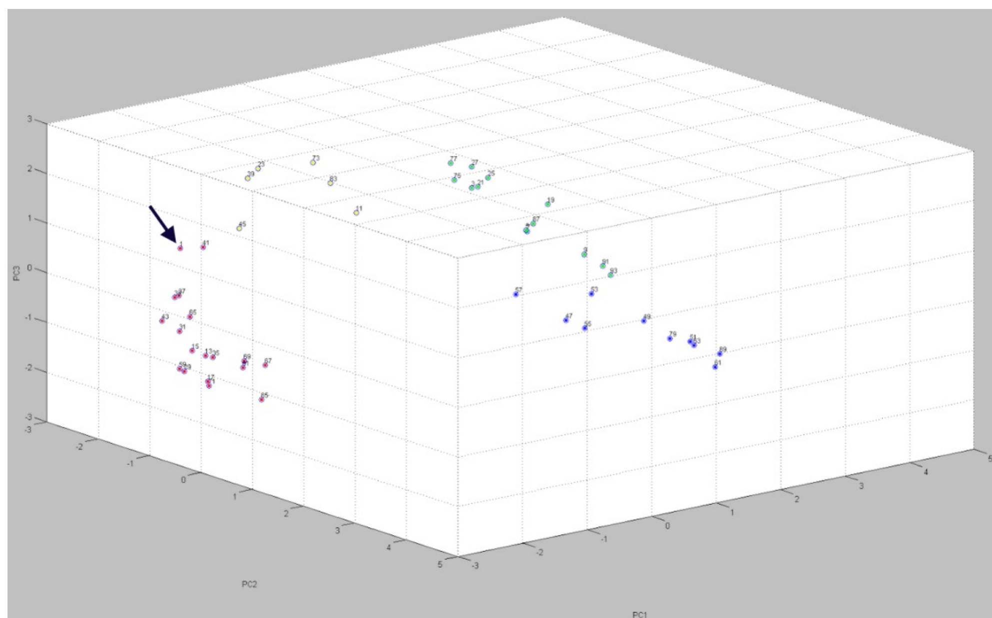
Figure 1: Cluster analysis for three spectral peaks in highest abundance from clinical isolates. Sample No. 1 (arrow) is the *B. avium* vaccine strain. Axis units indicate variance, with distances farthest from 0 being most different. Four major clusters, representing diverse samples, account for >70% of the variability in the data.



IMMUNE RESPONSE ENHANCEMENT OF LIVE ATTENUATED VACCINES AGAINST POULTRY RESPIRATORY VIRUSES

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INTRODUCTION

Live attenuated vaccination against poultry respiratory viral diseases such as infectious bronchitis (IB) and Newcastle disease (ND) is widely used in the commercial poultry industry in the U.S. The common practice is to apply them massively, through drinking water or spray, or otherwise individually using the eye drop route. The main objective is the generation of a protective mucosal immunity via stimulation of innate responses subsequently triggering the adaptive immune response on site and systemically.

Adjuvants have been used for decades in commercial poultry in inactivated vaccines. They are capable of recruiting immune cells activating innate immune responses. Their use in commercial live vaccines is limited. Experimentally, chitosan has been used in live NDV vaccines with success (13). In addition, polymers and nanoparticles have been used as adjuvants in live IBV, as well as in Eimeria vaccines (4, 7, 8). Our laboratory has been working on the use of diatoms and diatomaceous earth (DE) as novel vaccine adjuvants with promising results. Diatoms are single-cell eukaryotic microalgae present in all water habitats (5). DE is a siliceous sediment of fossilized diatoms. Because of its structural, mechanical and chemical properties DE can be sonicated and used as fine particles to further increase vaccine distribution and antigen absorption (1-3, 5, 10, 12). In addition, silica particles have shown adjuvant properties with even better performance than aluminum hydroxide (alum), which is commonly used in inactivated vaccines in humans and less in animals due to its high cost (9). The goal of this research project is to improve the efficacy of live attenuated IBV vaccines by mixing them with diatomaceous earth (DE) as a cheap and readily available vaccine adjuvant and antigen carrier.

MATERIALS AND METHODS

Specific-pathogen-free (SPF) chickens were divided into 6 groups (n=10). Groups 1, 2 and 3 were vaccinated ocularly (DE+live attenuated IBV; live attenuated IBV and DE alone). Groups 4, 5 and 6 (n=10) were vaccinated using a spray cabinet (DE+live attenuated IBV; live attenuated IBV and DE alone). Vaccinations with an IBV Ark DPI strain were performed at three days of age.

At four and 11 days post vaccination (DPV) four chicks of each group that received the virus with or without DE by either route were necropsied and pools of spleen and blood were collected and processed for flow cytometry to detect monocyte/macrophages, $CD4^+$ and $CD8^+$ T cells. At seven, 14 and 21 DPV blood and tears were collected for IgY and IgA ELISAs respectively.

At 21 DPV the remaining birds were challenged with an IBV homologous Ark field strain. Clinical respiratory signs were assessed at two, four, and six days post-challenge (DPC) as described in (6). Flow cytometry was performed from blood and spleen at six DPC. Viral load is being assessed in tears at two and six DPC. Histopathology and histomorphometry of tracheas, Harderian glands, and eyes will be performed.

RESULTS AND DISCUSSION

A general poor response was observed after the vaccination with the Ark DPI vaccine. This can be related with the vaccine titer used, which after back titration showed to be 1 x $10^{3.5}EID_{50}$/mL. What surprised us was the response on the spray groups, which was lower than in the ocular groups for IgG measured in serum and IgA measured in tears. These results correlate with observations made by Roh in which she demonstrated that chickens vaccinated using Ark DPI vaccines via spray cabinet in the hatchery showed poor protection against homologous challenge (11). Six days after a homologous challenge, performed at 21 days of age, we saw a

stronger IgG and IgA antibody response in groups previously vaccinated ocularly with IBV and IBV+DE. These responses were not seen in the groups previously vaccinated via spray cabinet with IBV alone or IBV+DE. We attribute these results to an effective prime vaccination and the challenge that acts as a second boost generating a stronger and faster secondary response. No clear adjuvant effect of DE was detected when we compared the IBV and the IBV+DE vaccinated groups in which vaccines were applied ocularly or spray. The respiratory signs evaluation at 4, 5 and 6 DPC showed no respiratory signs in the ocular IBV vaccines at all times demonstrating the efficacy of the vaccine and the route. Interestingly when the vaccine was applied via spray and mixed with DE there was a reduction of the respiratory signs at five and lack of respiratory signs at six DPC compared with the spray IBV vaccinated group not using DE. Viral load after vaccination and challenge, macrophages, CD4 and CD8 cell populations from spleen and blood are being analyzed and will provide more insights in terms of the effect of DE in live attenuated IBV vaccination.

In conclusion, no clear effects on immune response enhancement, have been detected after the incorporation of DE in live IBV vaccination.

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SERUM METABOLOMICS PROFILE OF NEONATAL BROILER CHICKENS FOLLOWING TOLL-LIKE RECEPTOR AGONIST CPG-ODN ADMINISTRATION

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ABSTRACT

Despite the tremendous advances seen in the field of immunology over the past few years, the immune system's regulation of its metabolism and the influence of the metabolic pathways on phenotypes and functions of immune cells remains poorly understood. We have previously shown that oligodeoxynucleotides containing cytosine phosphodiester guanine (CpG) motifs (CpG-ODN) protect neonatal broiler chickens against *Escherichia coli* septicemia. Whether the administration of CpG-ODN in chickens affects the metabolome (small-molecule chemicals found within a biological sample) is not known. The objective of this study was to identify the metabolomic profile of neonatal broiler chickens following CpG-ODN administration. Birds were divided into two groups; one group was administered intramuscularly CpG-ODN (50 μg/50μL/bird) and the control group was administered saline (50 □l). Blood was collected from five birds per group at three and 24 h post-administration of CpG-ODN or saline. Serum was separated and metabolomic analysis was performed using Nuclear Magnetic Resonance (NMR) spectroscopy and Direct Injection Liquid Chromatography Mass Spectrometry (DI/LC-MS/MS) techniques. Analysis of metabolomics data was conducted using MetaboAnalyst 2.5 software. Serum metabolome differed between CpG-ODN and saline treated groups for a variety of metabolites demonstrating crucial link between metabolism and immunity. We believe that the metabolomics profile analysis will further help to enhance our understanding of biochemical pathways associated with CpG-ODN mediated immunomodulation.

INTRODUCTION

During the first week of chicken life, microbial infections cause massive losses to poultry industry. Therefore, chicken industry is constantly looking for novel methods to prevent infection in young chicks. Several studies have demonstrated that CpG motifs within specific DNA sequences in bacteria as well as their synthetic counterparts, CpG oligonucleotides (CpG-ODN), carry immune stimulatory properties (1). Recent work revealed that in chickens, CpG-ODN binds with an intracellular Toll like Receptor 21 (TLR 21), orthologous to TLR 9 in mammals and stimulate macrophages (2, 3). Studies confirmed that CpG-ODN imparts a significant immune protective function in chickens against bacterial infections such as *Escherichia coli* and *Salmonella* Typhimurium when administered as an intramuscular injections or as an *in ovo* injection (4, 5). However, there is a lack of studies regarding the mechanisms behind the CpG-ODN-mediated immune protection in chickens.

Metabolomic analysis is a newly emerging field of "omics" research. In this analysis, the complete set of small molecule metabolites (<1500kDa) found in a specific cell, organ or organism known as the "metabolome" is identified and quantified using high throughput techniques.(6). Metabolomic analysis is currently used in drug discovery, toxicology, nutritional, medical and biochemical studies in humans, animals and plants(7). Since metabolomics analysis technique correlates the quantified metabolite data with biology and metabolism (6), thus it could be a valuable tool to discover immune modulatory pathways initiated by CpG-ODNs. The objective of this study was to identify the metabolomic profile of neonatal broiler chickens following CpG-ODN administration.

MATERIALS AND METHODS

Broiler chickens (11 days old) were divided into two groups: one group was administered intramuscularly CpG-ODN (50 µg/50µL/bird) and the control group was administered saline (50 µL). Blood was collected from five birds per group at three and 24 h post-administration of CpG-ODN or saline. Serum was separated and metabolomic analysis was performed using Nuclear Magnetic Resonance (NMR) spectroscopy and Direct Injection Liquid Chromatography Mass Spectrometry (DI/LC-MS/MS) techniques on the serum. Analysis of metabolomics data was conducted using MetaboAnalyst 2.5 software.

RESULTS AND DISCUSSION

Serum metabolome analysis revealed that there was no difference in the serum metabolome in the saline group between the 3 h and 24 h time points. Interestingly, serum metabolome differed between CpG-ODN and saline treated groups for a variety of metabolites including acetone, myoinositol, 3hydroxybutyrate, glutamine, phenylalanine, malonate, alpha.aminoadipic acid, several glycerophospholipids, and sphingolipids. Of note, we found that serum metabolome in CpG-ODN group differed between the serum collected at 3 h and 24 h post CpG-ODN administration. These results clearly suggest that CpG-ODN administration caused the serum metabolome to change over time. Given the recent studies demonstrating crucial link between metabolism and immunity, we believe that the metabolomics profile analysis will further help to enhance our understanding of biochemical pathways associated with CpG-ODN mediated immuno-modulation.

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ADVANCES IN LAYER FORMULATION TO ENHANCE HEALTH AND PERFORMANCE FOR THE MODERN COMMERCIAL LAYER HEN

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SUMMARY

Formulating diets for the modern laying hen has changed as genetics have dramatically improved the number of eggs per hen per year. Consequently, diets have to be able to at least maintain, if not improve, health, performance, egg shell quality and size for the high producing layer hen. Production from the North American layer farms were collected to access the difference between conventional and reformulated diets with new technologies to provide better biological availability of minerals, enzymatic action to assist with digestibility and enhance energy uptake, and improve gut health stability. Performance measurements (e.g., egg production, salable eggs) improved with reformulated diets. Ensuring that a diet is formulated for the health and performance of the hen, in addition to maximizing return on investment for feed cost, will help to provide eggs to meet consumer demand while enhancing layer production, minimizing unsalable eggs and effects on the environment.

INTRODUCTION

The carbon footprint of the laying hen in 2010 was 50% less than in 1960 (1). This is through a combination production of 27% more eggs, with 26% less feed, for 42% better feed efficiency (1). This change has been brought about by enhanced genetics and management leading to improved egg production, lower discarded eggs, and lower mortality. Consequently, formulating for the modern layer hen requires a transformation in how the birds are fed for optimum performance. Inclusion of several novel technologies to improve the biological availability of minerals, enzymatic action to assist with digestibility and enhance gut health stability has been explored.

Over the last half century much has changed with regard to trace mineral requirements. Originally, these requirements were defined in terms of inorganic trace minerals (2); however, modern research has demonstrated the benefit of proteinated transition trace minerals (3). Egg shell quality has been reported to improve when specific proteinated minerals replaced inorganic trace minerals (4). In addition to egg shell quality improvements (4), the lower inclusion levels of these proteinated trace minerals were also shown to reduce the environmental impact of the layer hen through reducing the amount of minerals in the manure (5). The full impact of replacing inorganic minerals with proteinated trace minerals in commercial production was subsequently tested in a case study that was conducted over time (6). This case study demonstrated that with the addition of proteinated trace minerals the over the life of a layer flock the total number of under-grade eggs was reduced from 4.93% to 1.52% (6). Transitional trace minerals are not the only trace minerals that benefit poultry when supplemented in the proteinated form. Selenium, when supplemented as selenium enriched yeast, has been shown to influence shell quality (7) and increase the selenium concentration in the egg (8-9) which can be translated to the consumer for an added health benefit.

Providing exogenous enzymes has been repeatedly shown to assist digestibility and energy uptake both with conventional and non-conventional ingredients. Beta-glucanases were among the first exogenous enzymes to be adapted by the feed industry (10); however, phytases have been the most widely adapted exogenous enzymes (11). Many recommended use rates are based on the enzyme units which were developed by the quality control program for that enzyme manufacturer. Consequently, there has been no industry standardization and variable systems have resulted. Liquid fermentation is a commonly used fermentation method for enzyme production (12); however, this process often predominates in one enzyme activity (13). Solid state fermentation is an ancient but less commonly used technique to produce a synergistic combination of enzymes grown together on a specific substrate (12-13). Solid state fermentation (12) has been the production method of choice for Alltech. Consequently, the quality control procedure for most of the enzymes produced in solid state fermentation would be different than that of

liquid fermentation due to the difference in the fermentation systems (14). Formulating to the recommended nutrient matrix with the synergistic exogenous enzymes provided through solid state fermentation has been demonstrated to be a successful means to effectively using the product (15).

Optimum returns for layer producers are when egg production meets a specific size demanded by the market. While many markets exist for egg production, most focus on the weight of the egg. Providing a large (53 to 62 g egg as per Canadian regulations [16]) egg is desirable in some markets while in other markets large and extra large eggs are more sought after. The amount of shell material does not increase with egg size; thus, controlling egg size and not allowing the size to increase beyond the target weight will control cracked eggs. As a result, there are advantages to supplying digestible nutrients to produce the desired size of egg. If enzymes are used in this process they should be formulated in the ration following the supplier's matrix so the nutrient profile is met to optimize egg size for the production market.

DISCUSSION

Providing novel feed technologies through modification of diet formulation for the modern laying hen has the potential to provide a diet that is devised to support hen health and performance as well as maximizing eggs to meet consumer demand. Beginning in 1989, Coburn Farms began working with Alltech to improve their production efficiency. Over time, many of the technologies listed above were adapted. For easy application these were combined into what became known as Alltech Poultry Pak® (17). The benefits of this program have been tracked at this farm and the continued use of the program has shown consistent improvement. Using a corn-soy based ration the most recent flock has peaked at over 97% production per hen-housed at 25 weeks of age and at 37 weeks of age they have just dropped under 97 % (D. Coburn, personal obs.). Under-graded eggs remained low at 1.97% at 37 weeks, of these 0.8% were cracked eggs (D. Coburn, personal obs.). The previous flock that was marketed was at 86.7% production per hen-housed at 70 weeks of age (335.8 eggs per hen housed) with under-grade eggs at a total of 1.79% for the flock and of these 0.74% were cracked eggs (D. Coburn, personal obs.). Improved shell quality, as determined by reduced cracked egg percentage, help to explain the increase in eggs per hen-housed and saleable eggs as less eggs are being broken during production.

The Alltech Poultry Pak has also been used commercially with wheat-canola based rations. A commercial layer producer on this program for at least four years had 91% average production per hen housed at 72 weeks of age (334 eggs per hen housed). When assessing saleable egg production and total percent under-grade eggs (grade C cracked eggs and leaking eggs) between 42 and 48 weeks of age those layers on Alltech Poultry Pak performed numerically better than comparable layer hens not on the program. The percentage of large eggs during this period was 54% (average 61 g egg) for the Alltech Poultry Pak fed layers compared to 48% (average 62g egg) with comparable layer hens not on the program. The percentage of under-grade eggs during this period was 2.29% for the Alltech Poultry Pak fed layers compared to 2.42% with comparable layer hens not on the program. The hens on Alltech Poultry Pak were able to achieve a high level of production while maintaining the egg size and quality needed for the local market.

A recent field trial with a mid-west United States egg integrator was conducted using Alltech Poultry Pak. The objective of this trial was to maximize the amount of 61 g eggs, while minimizing the number of eggs exceeding that size. Thus, through feed formulation the desired egg size had to be reached as fast as possible and limit further increase in egg size. To do this rations were formulated with Alltech Poultry Pak with the recommended matrix values so the available nutrients would follow the desired nutrient matrix for the bird based on current and target production. For comparison purposes there was a separate control ration that was formulated without the added supplemented package and fed to the layers in the control barn. Weekly production reports where monitored to review egg production, body weight, and egg size (Figure 1). During early production energy and amino acid intake in addition to key elements determining egg size (18) where maximized. However, as egg size approached 61 g per egg the layer hen ration was restricted via increasing fiber intake while holding dietary energy levels relatively constant. Control rations without the added supplemented packaged were formulated and fed to compare the two programs and their impact on egg production and egg size (Figure 1). Generally, the layers on the Alltech Poultry Pak program had numerically higher percent production and consistent egg size throughout production.

Despite each set of the above mentioned commercial production information originating from different flocks similar trends were noted. Those laying hens fed Alltech Poultry Pak reformulated into the diet with the company specified nutrient matrix

showed numerically increased egg production and saleable eggs with reduced under-graded (non-saleable) eggs. Consequently, the novel technologies within the packaged and reformulated solution provided a benefit to both the hen and the producer.

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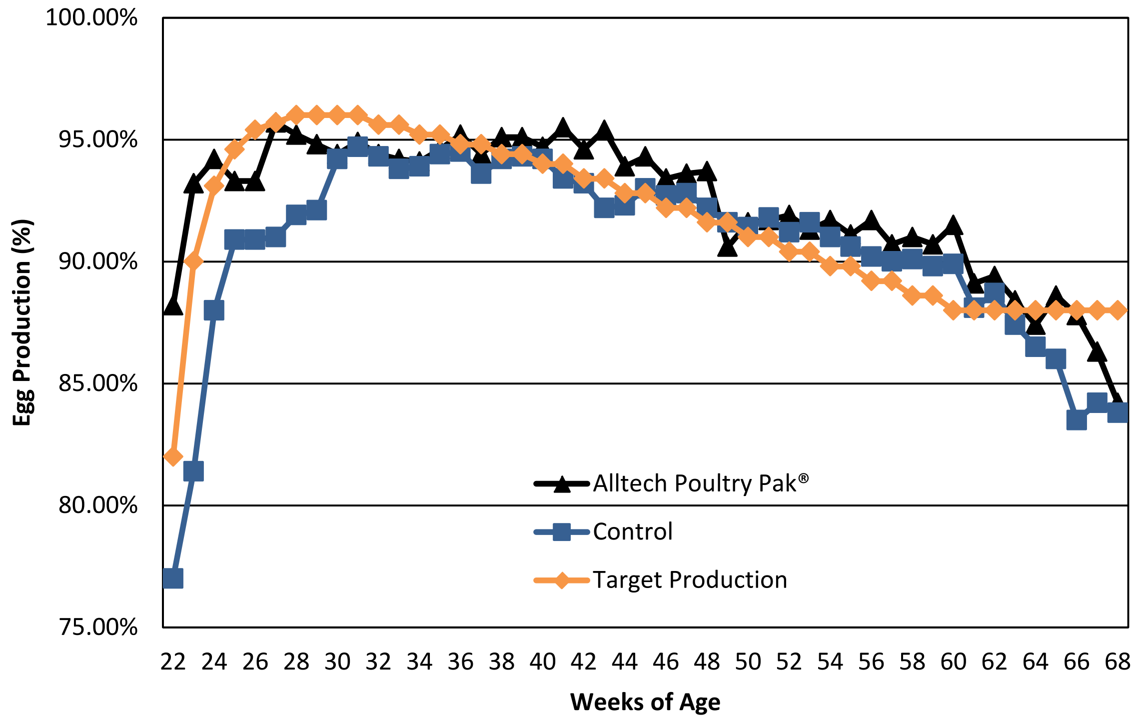
Figure 1. Percent egg production by week to 68 weeks for the control, Alltech Poultry Pak and standard curve flocks. The egg production has been higher for the Alltech Poultry Pak flock. Peak egg production for the Alltech Poultry Pak layer hens was 95.7% at 27 weeks of age compared to 94.7% at 31 weeks of age. This production has translated into an average of 1,114 more cumulative dozen eggs produced compared to the control flock. Generally, both the control and Alltech Poultry Pak flock achieved an average egg size of 61g throughout the entire production. These flocks will be marketed at 85 weeks of age.



IMMUNOPROTECTIVE EFFECTS OF CPG-ODN AGAINST INCLUSION BODY HEPATITIS IN CHICKENS

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ABSTRACT

The immune system in young chicken is not fully developed, thus protection of chicks from infectious diseases has been a great challenge in poultry industry worldwide. Successful protection against infectious diseases largely depends upon the proper activation of innate and adaptive immunity. Cytosine phosphodiester guanine (CpG) motifs containing synthetic oligodeoxynucleotides (ODN) (CpG-ODN) has been widely used to activate host's immune system against range of bacterial, viral and parasitic infections. We have previously shown that *in ovo* delivery of CpG-ODN was able to significantly protect neonatal broiler chickens against *Escherichia coli* or *Salmonella* Typhimurium infections. In this study, we investigated whether the CpG-ODN-mediated immune activation can induce protective immunity against fowl adenovirus (FAdV), which causes inclusion body hepatitis, an economically important viral disease in broiler chickens. Fifty broiler birds, with no maternal antibodies against FAdV, were intramuscularly injected with either multiple doses (three or four) of CpG-ODN (50µg per bird) or saline at days 12, 14, 16 and 18 of age. All birds were challenged with FAdV 11 serotype at 14 days of age. We observed statistically significant protection against FAdV 11 following four but not three doses of CpG-ODN. Further study is needed to investigate the underlying cellular and molecular mechanisms for the antiviral effects of CpG ODN.

INTRODUCTION

Infectious diseases have been a great threat in poultry industry worldwide. Preventing disease occurrences in newly hatched chicks is a very challenging task, as chick's innate and adaptive immune systems are not fully developed. Unmethylated, non-vertebral CpG dinucleotides (CpG motifs) are recognized as molecular patterns that can activate and stimulate the vertebral immune system (1). Synthetic preparations of CpG-ODNs, simulating pathogenic DNA, have shown similar immunostimulatory activity (11). These synthetic CpG-ODN's have been widely used to activate host's immune system against range of bacterial, viral and parasitic infections in many vertebral species including chickens (2, 3, 5-7, 9, 10). We have previously shown that *in ovo* delivery of CpG-ODN was able to significantly protect neonatal broiler chickens against *Escherichia coli* or *Salmonella* Typhimurium infections (6). In this study, we investigated whether the CpG-ODN-mediated immune activation can induce protective immunity against FAdV, which causes inclusion body hepatitis (IBH), an economically important viral disease in broiler chickens.

MATERIALS AND METHODS

Synthetic CpG-ODN. The sequence of CpG-ODN (CpG 2007) used in this study was 5'-TCGTCGTTGTCGTTTTGTCGTT- 3'. ODNs were synthesized with a phosphorothioate backbone (Operon Biotechnologies, Inc. Huntsville, AL). Synthetic CpG-ODNs were diluted in sterile, pyrogen-free saline (50µg/100µL) and administered in a 100µL volume per bird by the intramuscular route into thigh muscle of chicks using 22-gauge-1-inch, hypodermic needles.

Virus. In this study, FAdV 11 serotype was used as a challenge strain. Challenge virus was prepared from liver tissue homogenate collected from birds infected with FAdV 11 serotype. At 14 days of

age, birds were challenged with FAdV11 (10^6 TCID/ 100µL/bird) by administering virus through intramuscular route into the thigh muscle.

Experimental animal and their management. Experimental birds were obtained from vaccine free broiler breeders maintained for research, at the Animal Care Unit at the Western College of Veterinary Medicine, University of Saskatchewan, Canada. Chicks were allocated randomly into groups and placed in an animal isolation room at the Animal Care Unit, University of Saskatchewan. Water and commercial broiler ration were provided *ad libitum*. Each room was ventilated with filtered, non-recirculated air at a rate of 10–12 changes/h. Air pressure differentials and strict sanitation was maintained in this isolation facility. This work was approved by the University of Saskatchewan's Animal Research Ethics Board and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

EXPERIMENTAL DESIGN

Fifty broiler birds, with no maternal antibodies against FAdV, were intramuscularly injected with either three doses: n = 10 (at day 12, 14, and 16 of age) or four doses: n =10 (at day 12, 14, 16, and 18 of age) of CpG-ODN (50µg/100µL/ bird) or saline: n = 30. All birds were challenged with FAdV 11 serotype at 14 days of age. The body conditions of birds were evaluated three times daily for 10 days post challenge. Birds were observed for clinical signs and everyday each individual was assigned a clinical score. Clinical scores were based on a 3-point scale:
0 = normal; 0.5 = slightly abnormal-inactive appearance, slow to move; 1 = depressed, reluctant to move; 1.5 = reluctant to move, may take a drink and peck at feed occasionally; 2 = unable to stand or reach food or water; and 3 = found dead. Birds that received a clinical score of 2 were euthanatized by cervical dislocation. Chicks that were found dead or euthanatized were necropsied immediately and their liver was examined for gross pathological lesions typical to IBH. Livers were further processed for histology to confirm diagnosis of IBH.

Statistical analysis. Survival data were analyzed with the use of Prism (Prism 5.0, GraphPad Software Inc., San Diego, CA) and Statistix7 (Analytical Software, Tallahassee, FL) with a significance level of $P < 0.05$. The survival patterns and median survival times were compared using the log-rank test and chi-square statistic.

RESULTS AND DISCUSSION

Previous studies have reported CpG-ODN as an adjuvant for vaccines against viral diseases in chickens (8). However, there is a lack of study investigating the role for CpG-ODN alone in the orchestration of immunoprotection against viral diseases in chickens. In this study, we observed that four but not three doses of CpG-ODN alone administration significantly (p=0.0166 compared to saline control) enhanced the survival in birds challenged with IBH causing fowl adenovirus serotype 11. Our results suggest that CpG-ODN administration enabled chicken innate immune system to effectively counter the invading virus, which is also corroborated by a previous study that showed that CpG-ODN given through *in ovo* route activated innate immune responses that suppressed infectious bronchitis virus replication in chicken embryos (4). Our study showed that multiple doses of CpG-ODN induced protective effects against FAdv11. We hypothesize that significant protection may be due to delayed virus replication, stimulation and preparation of the immune system against the disease. However, further studies are needed to investigate the reason behind the CpG-ODN-mediated protection against IBH and identify the cellular mechanisms of the immune system which leads to protection against such diseases in chicken.

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ENVIRONMENTAL DETECTION OF H5N2 HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS IN THE ENVIRONMENT OF INFECTED TURKEY FARMS IN MINNESOTA

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ABSTRACT

In 2015, the novel Eurasian H5N2 highly pathogenic avian influenza (HPAI) virus substantially impacted domestic poultry in Minnesota (MN) (4). Domestic turkeys infected with HPAI virus shed viruses in large amounts (3), leading to contamination of the environment. The extent and the distribution of HPAI viral contamination on infected turkey farms are largely unknown. The objective of the study is to evaluate contamination of various areas inside and outside of infected turkey barns. A total of 141 environmental samples were collected from commercial turkey growers and breeder farms in MN. The sampling areas included the inside of the barn, the entries of the barn and the surrounding area. Viral RNA in the samples was detected by influenza matrix rRT-PCR. Samples collected inside the barns and at barn entries had higher detection rates (50% and 30.8%) than samples collected further from the barn (<20%). The HPAI viral RNA was widely distributed in the environment of infected turkey barns but diminished with the distance away from the infected barn. The study provides insights into the spreading pattern of HPAI viruses in poultry operations, as well as the guidance for the cleaning and disinfection practices.

INTRODUCTION

In 2015, the novel Eurasian H5N2 HPAI virus substantially impacted domestic poultry in MN (4). Turkeys infected with HPAI shed large amounts of the virus (>5 $\log_{10}$ EID_{50}/mL) through oropharyngeal and cloacal routes (3). Previous studies have proved that HPAI viruses survive for long periods of time in various environments (1,2). To date, little is known about the role that environmental contamination played in the perpetuation of the HPAI outbreak in 2015. To investigate the extent of HPAI viral contamination on infected turkey farms, we collected 141 environmental samples from nine farms within 20 days of the initial diagnosis.

MATERIALS AND METHODS

Sample collection and viral RNA detection. Environmental samples were collected from nine commercial turkey farms identified as HPAI positive at the National Veterinary Service Laboratory (NVSL). Various locations were sampled, including the drinkers inside the infected barns, floors, fields surrounding the barns, etc. Viral RNA was extracted from environmental samples by Kingfisher Model 700 Magnetic Particle Processor with MagMAX™ 96 Viral RNA Isolation Kit (Ambion) and detected by IAV matrix gene rRT-PCR with AgPath-ID™ One-Step RT-PCR Kit (Ambion). Samples that were positive for matrix rRT-PCR were tested by HPAI H5 HA gene PCR to confirm the subtype. We used Ct value of 38 as the cutoff to determine positive/negative status of the samples.

Data analysis. PCR results were categorized by the location of samples. Average Ct value, the positive rate, and the standard deviation of Ct values were calculated. Analysis of variance (ANOVA) and pairwise t-tests were used for multiple group comparisons. To analyze the persistence of viral RNA in the environment, the time between the initial diagnosis and the sampling were calculated. Linear regression was applied to characterize the viral RNA survival time and the amount of viral RNA detected (Ct values).

RESULTS

Table 1 shows the PCR results of environmental samples categorized by collection location. Of the

141 samples, those collected inside the barn had the most viral RNA (average Ct value: 35.2) and the highest percentage of positive samples (50%). In addition, 30.8% of samples collected at the barn entries were positive. With the exception of samples collected 10-15 ft from the barns, the percentages of positive samples decreased as the distance from the infected barn increased. The lowest average Ct value of positive samples was from inside the infected barns. ANOVA analysis shows that the means of Ct values differ significantly between groups ($p\text{-value} < 0.001$). Pairwise t-test indicates that the differences of Ct values between the inside the infected barns group and other groups are significant ($p\text{-value} < 0.001$).

The time between the initial diagnosis and the sampling ranged from 0–20 days. By applying a linear regression model, we discovered no linear relationship between Ct values and the time of survival ($R^2 = 0.04$).

DISCUSSION

In this sampling effort during the HPAI outbreak in 2015, we estimated the level of HPAI viral contamination in the environment of infected turkey farms prior to clean up. We found that detectable level of viral RNA was spread widely on the infected farms. The highest amount of RNA was detected from samples collected inside the infected barns. It is worthwhile to point out that viral RNA was detected in samples collected beyond 15 ft radius of infected barns. The results clearly indicate that the viral RNA was spread from the center of the infection to the surrounding region, as would be expected. The analysis on viral RNA survival time and Ct values revealed that IAV viral RNA might persist for a long time in the environment.

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Table 1. Ct values summarized by the location of sample collection.

Location	Total number of samples	% of positive samples	Average Ct value of positive samples	Sample type of positive samples
Inside the infected barns *	44	50	30.6	A, B, C, D, E
Barn entries	13	30.8	33.5	C
<10 ft from the barns	30	16.7	33.5	A, C, E
10-15 ft from the barns (inclusive)	12	8.3	37.3	D
>15 ft from the barns	42	11.9	33.8	C, E
All	141	26.2	31.9	

Sample type: A: boot socks; B: drinker swab; C: gauze swab; D: air sample; E: turkey feathers

FOWL ADENOVIRUS (FADV) SHEDDING IN BROILER BREEDERS FOLLOWING INFECTION WITH FADV 8AB

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ABSTRACT

Experimental infections of specific-pathogen free (SPF) chickens with fowl adenoviruses (FAdV) have been successful in demonstrating fecal shedding of FAdV. The role of neutralizing antibodies (NAb) against FAdV preventing FAdV shedding is not yet known. Infection of naïve broiler breeders with FAdV8ab was conducted to investigate fecal shedding and the effect of serum NAb on FAdV shedding. Naïve broiler breeders (n=48), were randomly divided into two groups (n=24/group). At 17 weeks of age, half of the birds in group one were orally infected with FAdV8ab with a dose of 1 x 10^4 $TCID_{50}$/bird, while all birds in group two remained as an uninfected control. To study fecal shedding and NAbs, cloacal swabs from both groups were collected weekly from 16 to 23 weeks of age as well as at 31, 33, 36, 40, and 43 weeks of age. Sera was also collected at 16, 23, 33, and 48 weeks of age. Viral DNA was detected in cloacal swabs by real-time QPCR while NAb were measured by a serum neutralization assay. At 16 weeks of age, prior to infection, all birds were confirmed to be naïve. However after infection, fecal shedding of FAdV8ab was detected and peaked at day seven post-infection in the infected group, which then declined as NAb increased. Birds in group one that were not infected, but rather in contact with FAdV infected birds, shed FAdV comparably to intentionally infected birds. Similarly, all broiler breeders in the FAdV exposed group seroconverted post-FAdV exposure. Maximum NAbs was reached at 16 weeks post-infection and remained constant until the termination of the experiment. It was determined that NAbs were negatively correlated with FAdV shedding.

INTRODUCTION

Upon natural exposure to the pathogenic strains of the FAdVs, severe diseases, for instance, inclusion body hepatitis (IBH) (1, 5), hydropericardium hepatitis syndrome (HHS) (2) and gizzard erosions (9) occur in young broiler chickens. Short term studies in leghorn chickens and SPF chicks have shown that FAdVs are shed intermittently in the feces (4, 6, 8). Fecal shedding of FAdVs results in horizontal transmission of the virus (1). Despite the economic importance of FAdV infection in broiler chicken, there is a lack of study on fecal shedding pattern in adult broiler birds. Also, whether serum NAbs produced following seroconversion affect viral shedding is not known. Therefore the goal of the present study was to investigate the long-term fecal shedding pattern in adult broiler breeders from 16 through 48 weeks of age. This study provides information on the pattern of FadV 8ab fecal shedding in broiler chicken and its association with the serum neutralizing antibodies.

MATERIALS AND METHODS

Preparation of virus. The virus was grown in Leghorn male hepatoma (LMH) cells (ATCC). Briefly, the cells were infected at one MOI, incubated for one h at 37°C in 5% CO_2. After one h, the inoculum was supplemented with DMEM-12 media with FBS, glutamine and HEPES and gentamicin to a final concentration of 5%, 2 mm and 20 mm and 1:1000, respectively. The cells were harvested 72 h post-infection, freeze thawed five times, homogenized, and spun at 3000 rpm at 4°C for 30 min. The supernatant was collected and titrated by Reed and Munch method.

Virus quantification. Virus quantification was performed by real-time QPCR or virus isolation method. TaqMan real-time QPCR assay

(PRIMETIME) with FAdV 8 specific primer- probe combination was used for viral DNA quantification. Briefly, a 25 uL reaction was comprised of 12.5 µL of 2X RT PCR buffer, 1 uL of primer-probe mix (2:1), 1 µL of 2X RT-enzymes mix (Ambion), 2 uL of DNA and nucleus free water to make volume 25 uL. QPCR was run at cycling conditions of 95°C for 10 min, 94°C initial denaturation and 60°C for 45 sec final extension. The standard curve is generated in QPCR by 10-fold serial dilution of plasmid containing hexon gene. The copy number of hexon gene was calculated by using the formula; plasmid DNA amount (g/µL)/ (size of plasmid including insert x 660). The number so obtained multiplied by Avogadro number.

Neutralization test. Sera samples were heat inactivated at 56°C for 30 min and 2-fold serial dilutions were made in 96 well plate. A constant amount of virus (10 $TCID_{50}$) was used per well, the mixture was incubated for one h and transferred to LMH cells (1 x 10^4 cells per well), which were prepared in DMEM-12 with 2.5 % FBS. Positive and negative controls included each time. Serum NAbs were determined against FAdV serotypes; FAdV 8a-Stanford, FAdV 8a- TR59, FAdV 8ab, FAdV 7-x11 Like, FAdV 2-P7A, FAdV 2-685 and FAdV 11-1047.

Animal trials. Adult broiler breeders were randomly divided in two groups of 24 birds each (n = 24/group). Half of the birds in the treatment group was infected orally with FAdV8 ab (1 x 10^4 $TCID_{50}$/mL/bird) at 17 weeks of age and the other half were housed together with the infected birds. The second group was kept as uninfected control. The birds were sampled for sera (n = >15) at time points; 16 (before infection), 23, 33 and 48 weeks of age for both the groups. The cloacal swabs (n = 6) were collected at various time points, treatment group; 16 (before infection), 17 -through- 23 and at 31, 33, 36, 40, 48 weeks of age (three from infected birds and three from non-infected pan-mates). The birds from uninfected control group were sampled at: 16, 23, 33, 48 weeks of age, n = 5.

Viral detection in fecal shedding. Fecal samples were collected in cotton swabs and placed in 0.5 mL tryptose broth (Sigma) with antibiotic-antimycotic solution (1:100). Mean weight of the swabs was calculated and was 0.25 g. The swabs were vortexed and 140 uL of broth was used for DNA extraction by Viral RNA mini kit (Qiagen). The rest of the broth was used for virus isolation after filtration through 0.22µm filter.

RESULTS AND DISCUSSION

All the birds were seronegative for FAdV 8ab, FAdV 8a TR-59, FAdV 8a Stanford, FAdV 7 x 11like, FAdV 11-1047, FAdV2 P7A, FAdV2 685, and no fecal shedding was detected in the birds before the commencement of experiment. Neither FAdV 8ab DNA nor infectious virus was isolated form uninfected control group through-out the experiment. Fecal shedding of virus (FAdV DNA) or infectious virus peaked at 7 days post-infection (DPI)(100% birds). Infectious virus was last detected at 21 DPI (33% birds) and no shedding was detected afterwards. Serum NAbs were first detected at 23 weeks of age (first time point) and remained consistent through-out the experiment. Uninfected pen-mates had contact with FAdV infected birds also excreted the FAdV-DNA as did experimentally infected birds. Cross-neutralizing antibodies were present for the serotypes of species E, but not for species D.

This study found that fecal shedding of the pathogenic FAdV 8ab was a brief event. Broiler breeders shed infectious virus in feces that peaked at 7 DPI and last detected at 21 DPI. Though, infectious shedding ceased at 21 DPI, viral DNA was detected in feces for longer time. This might be possible as viral DNA could be present in sloughed off cells from the gut. Alternatively, although, anti-FAdV 8ab IgA was not determined in this study, based on previous studies showing anti-viral IgA exists in gut mucous (3) or at other mucosal surfaces (7), it is quite possible that IgA might have neutralized the infectious virus at the gut mucosal surface. Our finding that shedding of infectious virus led to the horizontal spread of virus to the unchallenged pen-mates which is also supported by previous studies (1). Following seroconversion, no infectious virus shedding was detected, thus it could be concluded from this study that broiler breeders shed infectious virus for brief time and NAbs helps preventing replication of the virus in enteric mucosal surfaces. This information could be used to design strategies to use live FAdV to vaccinate breeders at 17 weeks of age providing enough time before laying to seroconvert.

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PROTECTIVE IMMUNITY AGAINST LARYNGOTRACHEITIS (LT) IS ENHANCED BY COMBINED *IN OVO* VACCINATION WITH HERPESVIRUS OF TURKEYS-VECTORED LT VACCINE *AND* FOWL POXVIRUS-VECTORED LT VACCINE (HVT-LT + FPV-LT)

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ABSTRACT

Two commercially-available, virus-vectored vaccines (CEVA Biomune) were evaluated for prevention of laryngotracheitis (LT) in commercial broiler chickens. Protective immunity induced by combined, *in ovo* vaccination with herpesvirus of turkeys-vectored (HVT-LT) and fowl poxvirus-vectored (FPV-LT) vaccines (HVT-LT + FPV-LT) was evaluated using both a 14 day and 28 day challenge exposure, and compared with conventional vaccination using chicken embryo-origin (CEO) vaccine.

In ovo vaccination of chickens with both virus vectored vaccines (HVT-LT + FPV-LT) induced highly effective immunity against LT virus challenge. Compared with unvaccinated chickens, *in ovo* HVT-LT + FPV-LT prevented development of severe clinical disease as indicated by low clinical scores and prevention of mortality, significantly reduced microscopic tracheal lesion scores ($p < 0.05$), significantly improved weight gain ($p < 0.05$), and significantly ($p < 0.05$) reduced concentrations of both infectious virus and viral DNA in tracheas after LTV challenge. Protective immunity induced by *in ovo* vaccination with HVT-LT + FPV-LT was comparable to that induced by CEO vaccine administered in drinking water at 14 days of age. These findings suggest utility of combined, *in ovo* vaccination with both HVT-LT and FPV-LT (HVT-LT + FPV-LT).

MATERIALS AND METHODS

Experimental design. Embryonated commercial broiler chicken eggs were *in ovo*-inoculated at 18 days of embryonation with HVT-LT + FPV-LT using one full dose of each vaccine. CEO vaccination was done at 14 days of age via drinking water.

Vaccine effectiveness was evaluated after LTV challenge at 14 and 28 days of age; challenge consisted of 2000 PFU Illinois-N71851 administered intratracheally (1). Effectiveness was evaluated based on clinical signs and mortality, microscopic lesions in trachea at 4 days PE, weight gain during the seven day postchallenge period, and concentrations of LTV (DNA and infectious virus) in trachea at four days PE.

Six chickens were randomly selected on day four postchallenge and necropsied. Tracheas were removed; a segment of trachea approximately one centimeter caudal to the larynx was collected and immediately placed in 10% buffered neutral formalin for histopathology. Tracheal exudates were collected from tracheas by vigorously scraping with a polyester-tipped swab, and immediately placed in one ml PBS containing 0.1 mg/mL gentamicin and 5 ug/mL amphotericin B. Tracheal exudate samples were stored at -80°C; they were homogenized by vigorous vortex mixing prior to DNA extraction and plaque-assay.

Clinical evaluation and histopathology. Clinical scores (intratracheal pathogenicity indices [ITPI]) and microscopic lesion scores were determined as described (1).

Virus infectivity assays. Infectious LTV titers were determined by plaque assays. Tracheal exudates were prepared as serial ten-fold dilutions, inoculated onto confluent monolayers of LMH cells in six well tissue culture plates, and incubated one h at 37°C; the inoculum then was replaced with an agar overlay. Plates were returned to incubator and incubated six days. Plaques were visualized using a neutral red agar overlay.

Real-time LTV PCR assay. A quantitative real-time PCR procedure was used for detection and quantitation of LT virus DNA. This procedure was modified from a real-time Taqman PCR assay previously described (2).

Statistics. Microscopic lesion scores, weight gain, infectious LTV titers, and viral DNA concentrations (genome copies) were evaluated by one-way analysis of variance (ANOVA) using analytical software (Statistix 8.0, Analytical Software, Tallahassee, FL).

RESULTS AND DISCUSSION

Effectiveness of HVT-LT + FPV-LT. At 14 challenge, unvaccinated, LTV-challenged chickens experienced severe clinical signs and one mortality (clinical score [ITPI] = 0.45), severe microscopic lesions in tracheas examined on day four postchallenge (lesion score = 4.5), and a significant ($p < 0.05$) depression in weight gain during the seven day postchallenge period.

Chickens *in ovo*-vaccinated with HVT-LT + FPV-LT had low clinical scores (0.11), no mortality, significantly ($P<0.05$) reduced microscopic tracheal lesion scores (lesion score = 1.3), and significantly improved weight gain during the seven day post challenge period compared with unvaccinated, LTV challenged chickens (Fig. 1).

Mean infectious LTV titers (plaque-forming units) and mean DNA concentrations (genome copies) detected in tracheal exudates from chickens vaccinated with HVT-LT + FPV-LT were significantly lower ($p < 0.05$) than those in unvaccinated/challenged chickens. These findings indicate that *in ovo* vaccination of chickens with HVT-LT + FPV-LT induced effective protective immunity against challenge as early as 14 days of age. This combined vaccine regimen prevented clinical disease and induced protective immune responses within tracheal tissues of vaccinated chickens that impeded virus replication when challenged at 14 days of age.

Effectiveness of HVT-LT + FPV-LT and CEO vaccination. At 28 day challenge, unvaccinated, LTV-challenged chickens experienced severe clinical disease (ITPI = 0.40), and one mortality, severe microscopic lesions in tracheas examined on day four postchallenge (lesion score = 4.0), and a significant ($p < 0.05$) depression in weight gain.

Chickens vaccinated with HVT-LT + FPV-LT and CEO had low clinical scores (0.04, 0.05), no mortality, significant reduction ($p < 0.05$) in tracheal lesion scores (lesion score = 1.8, 2.0), and significantly improved ($p < 0.05$) weight gain during the seven day postchallenge period compared with unvaccinated, LT virus-challenged chickens (Fig. 1).

Mean infectious LTV titers (plaque-forming units) and mean LTV DNA concentrations (genome copies) detected in tracheal exudates from chickens vaccinated with HVT-LT + FPV-LT were significantly lower ($p < 0.05$) than those in unvaccinated/challenged chickens, and not significantly different from unvaccinated/ nonchallenged and CEO vaccinated chickens.

These findings indicate that *in ovo* vaccination of chickens with HVT-LT + FPV-LT induced effective protective immunity against LTV challenge at 28 days of age. *In ovo* vaccination with HVT-LT + FPV-LT induced protective immune responses in chickens comparable with conventional vaccination using CEO vaccine. *In ovo* vaccination with HVT-LT + FPV-LT, like conventional CEO vaccination, induced immunity in vaccinates that prevented clinical disease, and induced local immunity within tracheal tissues that impeded tracheal virus replication.

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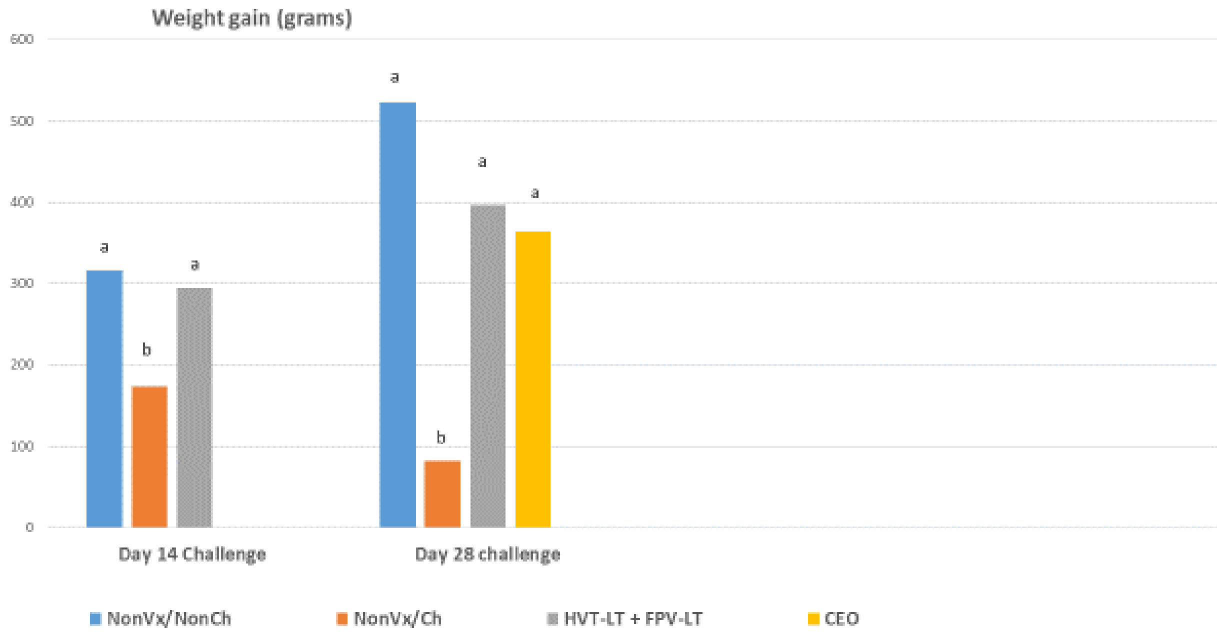
Figure 1. Weight gain during 7-day post challenge periods (day 14 and 28 challenges): unvaccinated/unchallenged (NonVx/Nonch), unvaccinated/ LTV challenged (NonVx/Ch), *in ovo* HVT-LT + FPV-LT vaccinated/ LTV challenged, and CEO vaccinated/LTV challenged chickens. Values with same lowercase letter are not statistically different (P>0.05).



OVERVIEW OF THE 2015 HPAI OUTBREAK IN THE MIDWESTERN REGION OF THE USA

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OVERVIEW

Highly pathogenic Eurasian origin H5 influenza A viruses were first detected in North America in November 2014 on the West coast. In late February, 2015, an unprecedented multi-state, multi-introduction of highly pathogenic avian influenza (HPAI) H5N2 in the Midwestern USA began in central Minnesota. The first commercial cases were in turkeys and from Mar 4–April 11, the outbreak was restricted to turkeys. On April 11, the first egg layer flock was infected. From April 11–June 5, both turkey and egg laying hens were infected. The final cases from June 5–18 were in egg laying chicken flocks.

The outbreak happened across the Midwestern region of the USA, an area of more than 1.5 million km^2. The region is covered by Eastern forest and grasslands biomes and is largely defined by the Mississippi river and its tributaries, the Ohio and Missouri rivers. The climate of the area varies from subtropical at the southern extent of the range to a humid continental climate at the northern extent. The region is covered by two migratory bird flyways, the Central and Mississippi flyways, and nine states. In this region, there are nearly 47 million turkeys, more than 100 million egg laying hens and approximately 236 million broiler chickens. The combination of climate, migratory flyways and large number of susceptible hosts in the region likely contributed to the magnitude of the outbreak which affected more than 48 million birds on 198 commercial farms in the Midwestern USA.

It was apparent during and after the outbreak that, in addition to these regional risks, the barriers to cooperative efforts in infection control also contributed to the outbreak. The barriers to effective cooperative control that may go unrecognized in an emergency event include the activities and communication channels that exist to make everyday activities of business and government go smoothly. Component parts of infection control include state animal health agencies; USDA APHIS VS; the layer, turkey and broiler industries; the scientific community; and the media, customer and public. In the absence of an outbreak, these groups interact very differently than how they need to interact during an outbreak. The speed, efficiency and success of infection control depends on the ability of these groups to work cooperatively to address a goal.

The need to share information. At the onset of a disease outbreak all the information needed to control the outbreak is held by the broiler, layer and turkey industries, but the information is fragmented between them; this changes as the disease and control efforts progress. The regulatory community gains information during the outbreak, and hoarding of information may leave the industries, the scientific communities, and the public without needed knowledge. The eradication team may not have time to access information held by the scientific community.

Infection control strategies vary. Perspectives on poultry production and health as well as on what constitutes effective disease control vary within the broiler, layer and turkeys industries. The poultry industries are structured to reduce the risk of common diseases but have little experience in the control of diseases that go beyond the boundaries of a specific commodity or a state. Regulatory officials have specific training and expertise in the control of transboundary diseases but are often unfamiliar with standard operating procedures in the poultry industries.

Containment requires resources. The eradication team is faced with a great challenge in an emergency disease situation - they must mobilize equipment, vehicles, and scores or hundreds of people with a goal of depopulating and disposing of sick or dead poultry. The affected farmer may have little to do, while the industries must figure out how to keep operating safely. This may result in inattention to details surrounding virus containment on the infected farm while these scores or hundreds of contaminated people leave the farm each day.

History is written by one's perspective. As the outbreak unfolds, great confusion exists about the source of the virus and the way it is moving about. The source of the virus in the 2015 outbreak is still not known, but it was clear very early in the outbreak that the industries had experienced multiple primary introductions presumably from the wild bird reservoir. Whether these primary introductions arose from dust borne virus carried into barns or from

being tracked in has not been established. Lateral transmission from farm to farm appeared to occur via familiar means: movement of live or dead birds or contaminated people and equipment. Silos tend to keep fragmentary phylogenetic analysis, survey results, and industry information from coalescing into an enlightening epidemiology report of what happened (1).

The combined efforts of industry, state and federal workers brought the outbreak to an end, eliminating infected commercial poultry in Arkansas, Missouri, Iowa, Minnesota, South Dakota, North Dakota, Nebraska and Wisconsin. The successful and timely control of this outbreak is testimony to the preparation steps that had been taken in these same states. In spite of the barriers of information holding, varied control strategies, limited resources, and perspective, the outbreak was stopped in three months; and continuity of business efforts successfully allowed low-risk movement of poultry industry products and day-old poultry.

Lessons learned include the continuing need to break down communication silos involving industries, agencies and academics; to explore regional biosecurity practices; to change industry cross talk and continuity of business planning; and to practice realistic scenarios.

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RELATIONSHIP OF ENTERIC INFLAMMATION AND LEAKY GUT ON BACTERIAL TRANSLOCATION AND LAMENESS IN BROILERS

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SUMMARY

Previous reports indicate that enteric inflammation and associated permeability may play a crucial role in pathogenesis of multiple diseases associated with lameness in poultry including bacterial chondronecrosis with osteomyelitis, turkey osteomyelitis complex, and spondylolisthesis. Additionally, dexamethasone has been shown to increase mucosal permeability, measured by increased fluorescein isothiocyanate-dextran (FITC-D) in serum and bacterial levels in liver. In Experiment 1, treatments consisted of negative control, eight h water withdrawal immediately prior to sampling, dexamethasone in feed (d4-11), rye-based diet (d7-11), and 15% dried distillers grain with solubles (DDGS). FITC-D and liver bacteria were measured on day 11. Dexamethasone, rye-based diet, and water withdrawal resulted in the highest levels of serum FITC-d, and percent incidence of positive bacterial translocation to liver were 80%, 73.3%, and 60%, respectively, compared to only 26.7% for DDGS group and 33.3% for control. In Experiment 2, all chicks were exposed to a mild cold stress, (30°C for six h) at three days of age plus an inflammation-inducing treatment, followed by *Enterococcus cecorum* (EC) challenge on day 11, except for control which received only cold stress treatment. On day 11 serum FITC-D levels were measured, and EC was recovered from the free thoracic vertebrae region on day 15. Birds were monitored for lameness through day 70. Serum FITC-D and EC recovery were highest ($P<0.05$) in dexamethasone treated birds, plus rye-based diet and DDGS diet also increased. However, by day 70, there were no differences in total lameness or occurrence of spondylolisthesis, and incidence of ascites compared to control groups. This suggests that while dexamethasone and dietary treatments increase mucosal permeability, low level, and perhaps undetectable, stressors such as temperature change and short-term water withdrawal may be sufficient for inducing meaningful amounts of decreased enteric integrity. These experiments stress the importance of early management on development and maintenance of enteric integrity and long-term health of flocks.

As the use of low level antibiotics as growth promoters becomes less acceptable worldwide, alternative methods for growth promotion and performance improvement must be found. One hypothesis for the action of growth promotion by antibiotics is a decrease in gastrointestinal inflammation, which leads to improved nutrient absorption and utilization. When the mucosal barrier in an animal is compromised, translocation of microorganisms can occur, the innate immune system is initiated, and nutrient absorption can become impaired. We have recently developed mucosal permeability models in chickens for the study of enteric inflammation (2, 3, 6). These representations of inflammation can cause a decrease in nutrient absorption efficiency and growth. Though morphological changes are perhaps the best method for describing the inflammatory effects of gastrointestinal insults, it is a long and tedious process not conducive to high-throughput screening models. Additionally, morphological repairs are slow to develop and may not be fully indicative of enteric function once treatment has begun. Liver recovery of bacteria may be an indicator of tight junction function and leaky gut, and FITC-D has become a common indicator of this pathology.

Bacterial translocation from the GIT into circulation has previously been associated with bone pathologies such turkey osteomyelitis complex (1) and bacterial chondronecrosis with osteomyelitis (7). The studies presented here investigated the role of mucosal permeability and *Enterococcus cecorum* in the development of kinky back, as well as other types of lameness in broilers.

MATERIAL AND METHODS

Experimental animals and diets. Two experiments were conducted to evaluate the effect of stress on intestinal permeability induced by administration of dexamethasone in feed, feed

restriction, or modified diets previously shown to induce enteric inflammation and leaky gut (4, 5). Broiler chickens were obtained from Cobb-Vantress (Siloam Springs, AR, USA), randomly assigned to treatment groups, and placed in a controlled age-appropriate environment with unrestricted access to feed and water (with the exception of 24 h feed restriction prior to test; FR). In each experiment, chickens received an antibiotic free diet meeting the nutritional requirements of poultry recommended by National Research Council (1994). All animal handling procedures were in compliance with Institutional Animal Care and Use Committee at the University of Arkansas.

Serum determination of FITC-D leakage. Fluorescein isothiocyanate dextran (FITC-D; MW 3-5 KDa; Sigma Aldrich Co., St. Louis, MO) levels were detected in serum. After collection from chickens, blood was kept at room temperature for three h, centrifuged (500 X *g* for 15 min) to separate serum from red blood cells, and diluted 1:1 in PBS. Levels of FITC-D in serum were measured at excitation wavelength of 485nm and emission wavelength of 528nm (Synergy HT, Multi-mode microplate reader, BioTek Instruments, Inc., Vermont, USA). Fluorescence measured was then compared to a standard curve with known FITC-D concentrations. Gut leakage for each bird was reported as µg of FITC-D/mL of serum.

***Enterococcus cecorum* challenge.** *Enterococcus* cultures were obtained from field cases and identified as *E. cecorum* in a veterinary service laboratory. For challenge, cultures were individually grown in tryptic soy broth under microaerophilic conditions in a candle jar at 37°C overnight and washed by centrifugation three times. After washing, each of the four challenge strains was re-suspended in equal volumes of saline and combined into a single test tube, CFU were determined after combining all cultures by plating serial dilutions. *Enterococcus* was administered by oral gavage at a dose of ~$1x10^8$ per chicken.

Recovery. Free thoracic vertebrae region was aseptically removed from each bird, generally cleaned of extraneous tissue, and crushed with Carmalt forceps before placement in a sterile sample bag. Sterile saline was added at 4X w:v and samples were massaged by hand for 10s. Serial dilutions were plated on BD CHROMagar Orientation medium, which is selective for *Enterococcus*. Selected colonies were confirmed by Gram staining and microscopic observation of Gram positive chaining cocci.

Hepatic bacterial translocation. To measure bacterial translocation (BT) from the intestinal tract to blood circulation, portions of the liver were aseptically removed from each chicken, collected in sterile bags, homogenized, weighed and 1:4 w:v dilutions were made with sterile 0.9% saline. Serial dilutions of each sample were made and plated on tryptic soy agar for determination of total aerobic bacteria translocation levels (TSA, catalog no. 211822, Becton Dickinson, Sparks, MD) or media selective for *E. cecorum* (described above).

Statistical analysis. All numerical data were subjected to Analysis of Variance as a completely randomized design using the General Linear Models procedure of SAS (SAS Institute, 2002). Incidence data were compared using the Chi Square Test of Independence, testing all possible combinations. Data are expressed as mean ± standard error or percent recovery ($P \leq 0.05$).

RESULTS AND DISCUSSION

As observed in previously observed studies from our laboratories, inclusion of dexamethasone, high levels of rye, or 15% DDGS in the diet caused leaky gut. Each of these treatments resulted in numerically or significantly increased *Enterococcus cecorum* from liver samples at day 15 (challenge on day 11). This increased mucosal translocation and liver recovery was mirrored by the induction of enterocyte tight junction breakdown as indication of absorption of FITC-D (~4000Da), which was largely excluded by the enteric epithelium of control chicks (Figure 2A). Moreover, the pattern of recovery of EC from the free thoracic vertebrae on day 15, suggests that this organism has the ability to bypass the liver and directly seed bone tissue (Figure 2B).

These data suggest that leaky-gut, cause by a variety of enteric inflammation insults, allows EC to translocate from its normal enteric habitat and, possibly in conjunction with high stress loads on the FTV in heavy broilers, allows expression of the lesions and resulting signs of Kinky Back.

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

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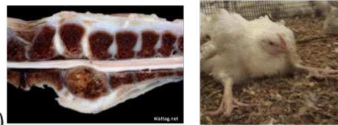
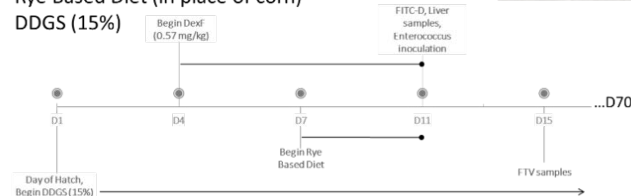
Figure 1. Spondylolisthesis (Kinky Back) model.

Spondylolisthesis (Kinky Back) Model

Treatment Groups:
Control
Enterococcus cecorum only
Feed Dex (0.57 mg/kg feed)
Rye Based Diet (in place of corn)
DDGS (15%)





Serum FITC-D levels (µg/mL) at 11 Days of Age after Enteric Inflammation Treatment

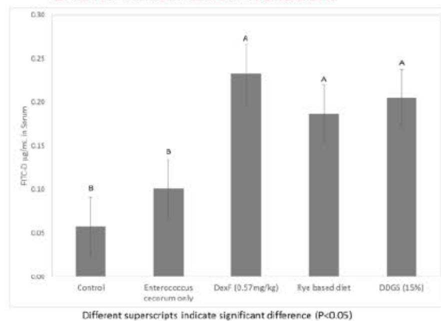


Figure 2a

Incidence of *Enterococcus cecorum* in FTV Region at 15 Days of Age after Enteric Inflammation Treatment

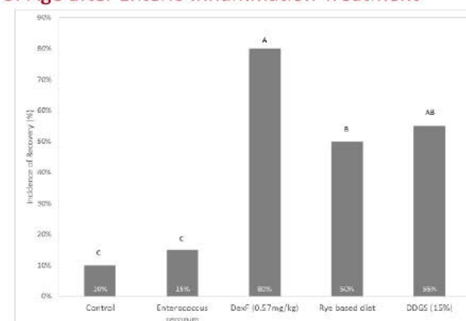


Figure 2b

THE ROLE OF FEED ADDITIVES IN THE MANAGEMENT OF PROTOZOAL DISEASES IN POULTRY

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SUMMARY

Coccidiosis and histomoniasis are economically significant diseases in chickens and turkeys. Management, vaccination and medication are key tools in the control of these diseases but these tools are not without challenges. Work undertaken in both the laboratory and the field has looked at the impact of oregano-based (Orego-Stim) and carrier-based organic acid products on the prevalence of *Eimeria* infections or histomoniasis. Data indicate that Orego-Stim and/or organic acid presented in feed are able to positively influence bird performance during periods of exposure to *Eimeria* and, to a lesser extent, *Histomonas*. Early indications suggest these two types of additives might have an integral role in protozoal management programmes.

INTRODUCTION

Eimeria and *Histomonas* are responsible for two of the main protozoal diseases that affect poultry: coccidiosis and Blackhead (histomoniasis). The economic impact of these diseases can be severe. For example, coccidiosis is estimated to cost the US poultry industry $127 million annually (1). Turkeys appear to be more susceptible to *Histomonas meleagridis* than chickens, where if left untreated, it can cause mortality approaching 100%. There is also some suggestion that the virulence of *Histomonas* is affected by the presence of bacteria, notably *Escherichia coli*, *Clostridium* spp. and *Bacillus subtilis* (2), indicating, perhaps, a relationship with the gut, and particularly cecal, microbiota.

Antiprotozoal drugs and vaccines have long been used in commercial poultry production but there are no new compounds coming to market. With the withdrawal of some existing products as well, the tools to help manage protozoal poultry infections are becoming limited. Feed additives, such as those based on organic acids or essential oils are increasingly used in poultry production as alternatives for antibiotics and to help manage gut microbiota. Can such products also help in the management of protozoal diseases, where gut health is known to be a significant factor?

OREGANO, ORGANIC ACIDS, AND COCCIDIOSIS

The use of oregano oil in laboratory infections with *Eimeria* has been well documented with promising results, although typically the studies are run in laboratory settings on clean litter systems. Recent work at Southern Poultry Research (Georgia, USA) demonstrated synergy between a commercial oregano-based product (Orego-Stim (OS)) in feed and a commercial anticoccidial vaccine (Coccivac B52, Merck, USA). Birds were reared on re-used litter (to ensure a high natural bacterial and protozoal challenge) for 42 days and given a mixed *Eimeria* spp. (*Eimeria acervulina*, *E. maxima*, and *E. tenella*) challenge at 21 days of age. Where hatchery vaccination with a anticoccidial vaccine significantly adversely affected performance relative to a salinomycin (60 ppm) treatment (FCR: 1.87 and 1.64, weight gain: 1.917 kg and 2.375 kg, respectively), the inclusion of OS at 150 g/T in feed was able to significantly ameliorate the vaccines negative performance impact (FCR: 1.73, weight gain: 2.194 kg) without adversely impacting immune status to coccidial challenge (Figure 1). Indeed, OS (450 g/T) on its own delivered similar performance to salinomycin (FCR: 1.67, weight gain: 2.412 kg).

In a further study in Brazil on reused litter, the combination of a propionic/formic acid mixture on a silica carrier and OS was compared to birds on a standard antibiotic growth promotor/anticoccidial program given both an *Eimeria* and bacterial challenge at 10 days. Preliminary data indicate a synergy between the acids and OS such that the acid/OS group performed numerically better than birds on the standard program (FCR: 1.63, 1.57, weight gain: 2.652 kg and 2.712 kg, respectively), and indeed better than either the OS or acid group

separately, demonstrating a synergy between the acid and OS.

OREGANO AND HISTOMONIASIS: FIELD OBSERVATIONS

Reports on the efficacy of natural feed additives for *Histomonas* control have been mixed at best. In a laboratory study, four out of 43 plant substance extracts demonstrated *in vitro* efficacy against *Histomonas meleagridis* but all failed to be protective when tested *in-vivo* (3). Similarly, several commercial products including a mixture of cinnamon and garlic, an aromabiotic, and an essential oil mixture were effective against *H. meleagridis in vitro* but again, not *in vivo* (4). Where natural feed additives have appeared to demonstrate some improvement in turkey performance following challenge with *Histomonas*, the challenge appeared to be subclinical in nature and did not cause mortality (5). Anecdotal evidence indicates that in field infections involving *Histomonas* that can only be resolved by drug treatment, OS in feed can help ameliorate the impact of the parasite. Typically, the concurrent supplementation of OS in the feed reduced mortality to single digits where mortality was 50-60% with nitarsone-only medication. Further laboratory work is underway to understand the interaction of oregano and *Histomonas*.

CONCLUSION

Feed additives have an important role in managing gut health in poultry production. Laboratory and field experience support the potential of a commercial oregano-based product, Orego-Stim, alone or in combination with a carrier-based organic acid to have a significant role in protozoal (*Eimeria* and *Histomonas*) management programs. More work is necessary to explore and understand their potential further.

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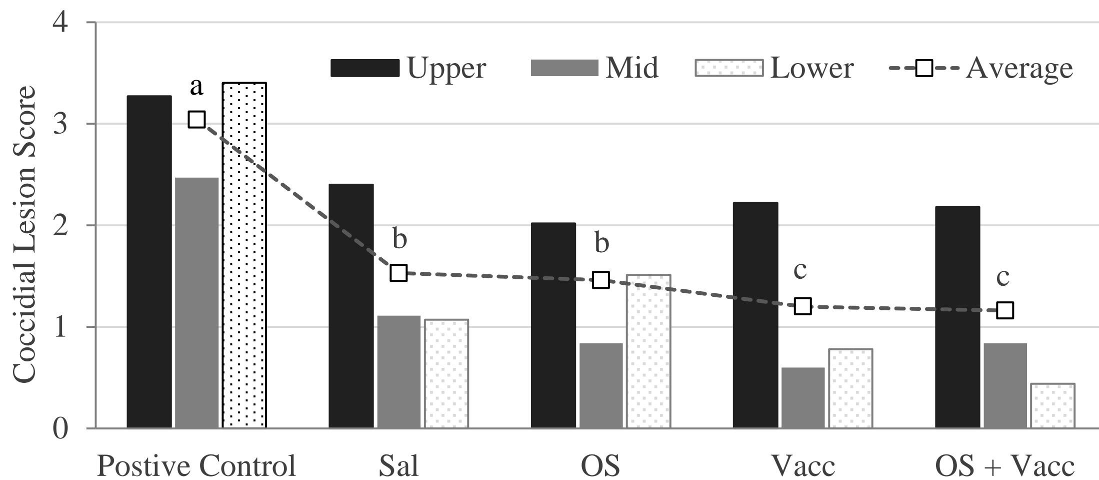
Figure 1. Coccidial lesion scores following *Eimeria* challenge at 21 days in birds administered salinomoycin (Sal), Orego-Stim (OS), Anticoccidial vaccine (Vacc) or Orego-Stim + Anticoccidial vaccine (OS + Vacc). Different superscript denotes significant difference at $p \leq 0.05$.



RETROSPECTIVE STUDY OF TRANSMISSIBLE VIRAL PROVENTRICULITIS IN BROILER CHICKENS IN CENTRAL CALIFORNIA: 2000-2014

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Transmissible viral proventriculitis (TVP) is a disease of chickens, mostly broilers, of two to eight weeks of age. Characteristic gross lesions are enlargement, atony and pallor of the proventriculus and weakness of the gastric isthmus (9). Microscopic lesions consist of glandular epithelial necrosis, ductal epithelial degeneration and hyperplasia, infiltration of the glandular interstitium by lymphocytes and replacement of glandular epithelium by hyperplastic ductal epithelium (1, 7). By impaired digestion, it causes poor feed conversion and slow growth (6, 8). Due to the fragility of the proventriculus, they tend to rupture during evisceration at processing, causing spillage of the proventricular contents, resulting in condemnation of affected carcasses because of contamination (10).

Historically, a number of different infectious and non-infectious causes have been attributed to TVP (2, 3, 10). The most likely etiology for TVP so far identified is a birnavirus, referred to as chicken proventricular necrosis virus (CPNV) (4, 5).

In order to gain more insight in the occurrence of the disease and the lesions caused by it, the electronic database of the California Animal Health and Food Safety (CAHFS) Laboratory System at the at the CAHFS branches in Fresno, Tulare and Turlock was searched for cases in which TVP had been diagnosed for the period 2000-2014. Only submissions from commercial broiler flocks were included. Cases, in which Marek's Disease or Avian Encephalomyelitis were diagnosed, were excluded.

Altogether, 330 submissions meeting the criteria were found. When multiple submissions from the same day and from the same ranch but from different houses were regarded as one case, 294 different cases were identified. The fact that 28 cases were from two to four different submissions showed that TVP frequently affects several houses on one ranch.

Submitted birds originated from at least 79 different ranches, mostly affiliated with one of three different companies. The yearly number of cases varied widely between three and 56 showing no clear trend over the years. Monthly numbers were more evenly distributed and varied between 13 and 33 without a clear peak season. The flocks were between six and 60 days old. Their average age was 35 days and the median age 36 days.

Clinical history indicated increased mortality in 166 cases and normal mortality in 54. In 74 cases the submitters gave no indication regarding mortality. In 76 cases, no specific clinical signs were reported and in 43 cases birds were submitted for monitoring purposes only. In the remaining 177 cases, most commonly reported clinical signs were respiratory symptoms (100 cases), runting/stunting (50 cases) and leg problems (36 cases). Other reported clinical signs included diarrhea, dermatitis and nervous signs.

Between two and 50 birds were investigated in each case. While in 124 cases no bird showed gross lesions, in eight cases all birds did. In average, 24 % of the birds in a case showed gross lesions. The most common finding, in 115 cases, was enlarged or dilated proventriculi followed by thickened walls in 71 cases and pale or mottled appearance in 54 cases. Other described lesions of the proventriculus included prominent papillae, petechiae, hemorrhages or reddened appearance. Histopathologically, inflammation was observed in 290 cases, mostly of the glands (227 cases) and more rarely of the mucosa or submucosa (78 cases). The inflammation was mostly characterized by infiltration with lymphocytes, plasma cells or other mononuclear inflammatory cells (252 cases) and only occasional infiltration with heterophils (37 cases). Other histopathological lesions included necrosis of the glandular region, dilated or hyperplastic glands, fibrin deposits and hemorrhages.

The results show that TVP is a frequent disease in California broiler flocks and can affect the productivity in a negative way. Further investigations

are needed in order to ascertain the etiology of the disease in those cases.

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SALMONELLA VACCINE PERSISTENCE STUDY

K. Hayes, B. Lungu, S. Peppers, and P. Eidson

SUMMARY

A study was conducted in a primary broiler breeder operation to determine the persistence of a live *Salmonella* vaccine in breeder flocks and their progeny. Samples reviewed included poultry, poultry house environmental, and chick and meconium. The objectives of the study were also to develop and validate methods for the isolation, identification, and confirmation of a particular live *Salmonella* vaccine strain. No evidence was found that the commercial live *Salmonella* vaccine persisted in poultry, their environments, or their progeny following vaccination of breeder flocks during the course of the study.

INTRODUCTION

As part of a comprehensive biosecurity program, vaccination can play a role in protecting breeding flocks and their progeny from *Salmonella*. Historically both live and dead *Salmonella* vaccines have been used as part of the *Salmonella* control plan and research supports the reduction in incidence or lowered prevalence and a decrease in egg contamination when incorporating vaccination programs (3, 4). The goals of utilizing vaccination in a *Salmonella* control program are to prevent intestinal colonization thus reducing fecal shedding and horizontal transfer by egg shell contamination. Preventing systemic infection and minimizing or eliminating vertical transfer is critical to preventing the transfer and proliferation of *Salmonella*.

Various commercially available vaccines have been administered to breeder flocks in the industry within the United States and worldwide. The live vaccines are chiefly derived from *S*. Enteritidis (SE) or *S*. Typhimurium (ST). Inactivated vaccines also center on SE and ST control but autogenous products, composed of various serotypes, are utilized as well depending on local challenges. There has been considerable interest in the use of live attenuated vaccines for many years. The use of modified ST strains as immunization agents has gained remarkable popularity, as these attenuated strains have no ill effects and may provide some protection against other related serovars as well (1, 2, 5, 6, 7, 8, 9). However, there has been some concern that the attenuated vaccine strains might regain the ability to persist in birds or in the poultry environment and be subsequently transmitted to the progeny of birds. In addition, the question of reversion back to the virulent wild type has also been suggested. This investigation was prompted from a historical concern that a live *Salmonella* vaccine might persist in breeders and be capable of vertical transmission to progeny.

Therefore, the objective of this study was to develop and validate methods for the isolation, identification, and confirmation of a live PVST vaccine strain. The tests would then be used to monitor breeder flocks and their progeny as well as the poultry environment following vaccination to determine persistence. In addition, a longitudinal study was initiated to monitor results and try and ascertain persistence or determine if a reversion incident may have occurred.

MATERIALS AND METHODS

Poulvac ST vaccine was obtained from Zoetis and used in field vaccinations of breeder flocks. Pure cultures of the PVST vaccine strain were used as controls for all culture and PCR testing. All other bacterial cultures used in this study were obtained from the company's S*almonella* bank.

The *Salmonella* vaccination program for flocks represented in this study consisted of administering the commercial live *Salmonella* PVST vaccine to birds via spray application at day of age. The birds received a second booster vaccination applied via spray application at 14 days of age. Liver/spleen and ceca samples were collected from 20 birds in each trial group (A, B, & C) at 3, 8, 14, 17, 21, 28, and 35 days of age and processed for both *Salmonella* culture and PCR testing. Trial group A consisted of birds that received no ceftiofur at hatch and no probiotics at hatch or day of placement. Trial Group B birds received no ceftiofur at hatch but did receive probiotics via drinking water day of placement. Trial Group C birds received ceftiofur at hatch and probiotics via drinking water day of placement.

GGP & GP poultry house environmental samples were evaluated from July 2014 through February 2015. Samples included dust and shoe cover samples and were collected from all breeder flocks every four weeks and processed via approved culture methods. In addition, pooled liver and spleen samples from 10 birds were collected every four weeks from GGP breeder flocks from onset of production through flock depletion.

The hatchery monitoring samples collected consisted of pooled meconium samples and chick organ cultures.

RESULTS

For the GGP results, all samples tested *Salmonella* negative by the real-time PCR and/or approved culture methods with the exception of one test which was identified to the serotype level as S. Newport. For the GP results, two samples were PCR positive, but culture negative. The PCR positive samples were group B and D negative. All other samples were negative by the real-time PCR and/or culture tests. The results from the investigation to determine whether the vaccine strain was persisting in flocks are depicted in Table 1 which shows results from liver/spleen and ceca samples collected from Trial A, B, and C vaccinated birds.

DISCUSSION

These data indicate that PVST did not persist in the poultry environment, the flock or their progeny during the time frame it was used in either generation. In evaluating the results obtained from testing performed on chicks following vaccination, the detection of PVST from liver/spleen and ceca from vaccinated chicks yielded lower results than we expected. It is possible that other factors may play a role in limiting the colonization or detection of Poulvac ST. Additional trials need to be conducted to further evaluate these concerns.

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Table 1. Detection of Poulvac ST from liver/spleen & ceca samples of Trial Group A, B, and C vaccinated birds.

Day of Age	Sample #	Oric #/% Positive			STPV #/% Positive			Culture #/% Positive		
TRIAL GROUP		A	B	C	A	B	C	A	B	C
3	40	7 /17.5	3 / 7.5	1/ 2.5	6 /15	3 / 7.5	1 / 2.5	0	0	0
8	40	2 /5	5 /12.5	0	2 / 5	4 /10	0	0	0	0
14	40	1 / 2.5	1 /2.5	0	1 /2.5	1 / 2.5	0	0	0	0
17	40	2 / 5	1 / 2.5	1 / 2.5	1 / 2.5	1 /2.5	0	0	0	0
21	40	0	0	1 / 2.5	0	0	1 / 2.5	0	0	0
28	40	0	0	0	0	0	0	0	0	0
35	40	0	0	1 / 2.5	0	0	1 / 2.5	0	0	0

Note: “The full-length article “Persistence of a Commercial Live Salmonella Vaccine in Broiler Breeder Operation” has not yet been submitted for publication.

A NEW MOLECULAR AND GENOMICS-BASED APPROACH FOR AVIAN INFLUENZA SURVEILLANCE IN WILD WATERFOWL USING ENVIRONMENTAL SAMPLES

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INTRODUCTION

Avian influenza (AI) is a viral disease of chickens and turkeys that has significant negative impacts on the poultry industry, the local and national economy, and food security. There have been repeated outbreaks of AI in the Fraser Valley (FV), the most recent of which occurred in 2014/2015. The source of AI is known to be migratory waterfowl, who shed the virus in their feces and who bring the virus into new geographic areas during their annual migrations (6). Although waterfowl always have the potential to be carrying AI, it is not feasible for poultry producers to institute prolonged periods of heightened biosecurity every year. Indeed, this 'blanket' approach is known to cause 'biosecurity fatigue' (5) and lead to critical protocol breakdowns that can result in a transmission event. Similarly, it would be prohibitively expensive to engage in intensive annual AI surveillance of domestic poultry. What is needed, therefore, is a type of early warning system that notifies producers and government of the presence/absence of AI viruses in waterfowl each fall so that biosecurity and surveillance measures appropriate for the level of risk can be implemented.

The problem is that current surveillance techniques focusing on testing individual wild waterfowl for the presence of AI have major drawbacks in terms of efficient and efficacy (4). It is of note that these techniques were in place in 2014 and failed to detect the presence of AI in waterfowl in advance of the domestic poultry outbreak.

To address this problem, we aimed to develop a new surveillance approach based on molecular and genomic analysis of wetland sediments. Given that waterfowl congregate on wetlands, by testing wetland sediments, we may be able to efficiently screen a large number of waterfowl encompassing a wide range of potential reservoir species.

SAMPLE AND DATA COLLECTION

During the 2014/2015 FV AI outbreak (December 2014 – January 2015), there were five farms that were believed to have been infected by waterfowl (vs. contact with other infected farms). Using data from eBird (ebird.org), we identified the closest major wetlands where dabbling waterfowl were likely to congregate and roost (n = 15). A total of 300 superficial sediment samples were obtained from these wetlands (20/wetland). For each sampling site, a water sample was also collected in order to analyze the amount of *E. coli* in the water (termed 'coliform count'). Given that AI is shed in waterfowl feces, which also contains *E. coli*, we hypothesized that high *E. coli* levels in the water would be linked to an increased likelihood of having an AI-positive sediment sample. At time of sampling, data pertaining to 19 different environmental variables were collected. These variables included a range of different wetland characteristics, as well as wetland use by waterfowl. Concurrently, 41 sediment samples were obtained on infected farms from areas of standing water where waterfowl were observed to frequent. All samples were collected during the outbreak period.

PCR-BASED SCREENING FOR AI PRESENCE

Samples were subjected to total RNA extraction using a modified commercial protocol in order to maximize the amount of RNA obtained and minimize PCR inhibition. Specifically, the RNA was extracted from ~2 g of sediment sample using the RNA PowerSoil® Total RNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) with additional chloroform purification (1). RNA extracts were screened for the presence of influenza virus matrix

gene by real time reverse-transcription PCR as previously described (3) using Applied Biosystems™ 7500 Fast Real-Time PCR thermocycler (Applied Biosystems, California, USA).

For the 300 wetland sediment samples, a total of 23 (7.7%) were matrix-gene positive and 49 (16.3%) were suspect-positive. For the 41 on farm sediment samples, 15 (36.6%) were matrix-gene positive and six (14.6%) were suspect positive. All PCR products showed 100% similarity to previously described AI sequences. Farms believed to have been infected by waterfowl were more likely to have an AI positive sediment sample compared to farms that were infected through contact with other farms ($p < 0.05$).

The 19 environmental variables, as well as water coliform counts, were used in a multi-level model (controlling for clustering by wetland and sampling site) to identify which combination of variables best predicts an AI positive sediment sample. Coliform count and the visible presence of feces on the shore were the only variables significantly associated with AI positivity ($p < 0.05$).

In order for sediment analysis to be an effective surveillance tool, the information it provides must be contemporaneous. This means that, for each year, AI in the sediment must reflect that being brought in by that season's migratory waterfowl and not AI 'left over' from previous years. To begin to address this question, we collected 20 sediment samples from sampling sites where AI was detected in August 2015. Samples were subjected to the aforementioned RNA extraction and PCR protocol and all were negative, suggesting that AI contamination does not last into the following year.

GENOMICS ANALYSIS OF AI-POSITIVE SAMPLES

Our current work focuses on characterization of the influenza strain types present in matrix gene-positive sediment samples using a genomics approach. RNA from these samples is being reversed-transcribed and enriched using two different platforms. The first method is based on PCR amplification of target genes. Briefly, in collaboration with the BC Genome Science Centre, we developed primers targeting various AI genes and subtypes for use in a Wafergen Biosystems (California, USA) microfluidic-based RNA amplification platform. The second method is based on RNA capture technology. Briefly, proprietary capture probes specific to AI genes have been designed by Fusion Genomics to 'filter out' AI-specific sequences. Next Generation Sequencing using an Illumina platform will be performed on the enriched samples followed by bioinformatics analysis to identify the AI subtypes present in each sample and their relatedness to previously described strains.

CONCLUSION

In conclusion, since we were able to detect AI virus in up to 37% of sediment samples, as opposed to a 1.0% rate of AI detection in individual waterfowl included in the current Canadian National surveillance program (2), our study suggests that, in the future, molecular and genomics-based screening of wetland sediment will be an effective AI surveillance tool. Interestingly, our preliminary study also suggested that this new approach could be used to better ascertain how AI is transmitted from wild waterfowl to domestic poultry, since on farm sediment testing revealed heavy waterfowl related AI environmental contamination on farms believed to have been infected directly by wild waterfowl, but not farms that were infected through indirect contact with other infected farms. Ultimately, our goal is to use sediment surveillance as the cornerstone for developing an effective provincial AI early warning system.

(The full-length article will be submitted for publication. Journal to be determined.)

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CULTURE, HISTOLOGIC, AND MICROFLORA EVALUATION OF THE INFLUENCE OF COCCIDIA VACCINATION AT ONE-DAY-OF-AGE ON THE SHED OF *SALMONELLA* HEIDELBERG

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SUMMARY

The objective of this study was to demonstrate that the method of coccidia control, either coccidia vaccine or by an ionophore, has an effect on the ability of *Salmonella* to colonize the ceca and translocate to the liver and spleen in broiler chickens. Natural coccidia challenge from used litter from a previous study and a *Salmonella* Heidelberg challenge of one-half the chicks gavaged in each pen was the model used in this controlled study to reproduce an industry field study (7) which found a significant correlation in lower *Salmonella* over 66 flocks of broilers in flocks having coccidia vaccine as the control for coccidia.

In the presence of a low coccidia challenge to all pens, the Coccivac-B52® resulted in significantly lower *Salmonella* Heidelberg translocation to the spleen and liver vs. the ionophore (5% vs. 18.3%) and numerically lower *Salmonella* Heidelberg colonization of the ceca (30% positive Coccivac B52® vs. 35% positive salinomycin). In this study it was demonstrated that the earlier *E. tenella* cycling with the Coccivac-B52 resulted in increased thickness of the ceca lamina propria and less total gut inflammation in birds colonized with *Salmonella*. There was not a significant difference in the diversity of the ceca flora between treatments but there was a consistent trend to a less diverse flora with the Coccivac B52 treatments group.

This study has demonstrated that the method of coccidia control may have a significant impact on the amount of *Salmonella* translocating from a bird's intestines to be harbored in internal organs such as the spleen and liver. It appears to be more important for *Salmonella* programs to obtain coccidia immunity early in a bird's life and to prevent any *E. tenella* cycling near processing age.

INTRODUCTION

As USDA-FSIS has lowered the *Salmonella* performance standards for *Salmonella* in broiler, whole bird, and ground chicken, it has become more important for broiler companies to find ways to keep the level of *Salmonella* lower prior to the processing plants. *Eimeria tenella* and *E. necatrix* are two species of coccidia that have been associated with increased *Salmonella* colonization and shed (1, 5). Alternatively, it has been demonstrated that a low dose of *E. tenella* could result in less ability for *Salmonella* to invade (6). In this controlled study with a natural coccidian challenge and natural spread of the *S.* Heidelberg, it will be determined if a subclinical coccidia infection from a commercial coccidian vaccine vs. control with an ionophore in the feed will have an effect on *S.* Heidelberg colonization and shed from the ceca and also affect the ability of *Salmonella* to translocate to internal organs like the liver and spleen.

MATERIALS AND METHODS

One thousand and eight hundred male broiler chickens, Cobb x Cobb were randomly divided into three treatments; 50 chicks per pen with 12 pen-level replicates/treatment. The control broilers received no coccidia prevention, the coccidia vaccinated broilers received a full dose of Coccivac-52 by coarse spray at one-day-of-age and the salinomycin treated broilers were fed 60 g/ton of salinomycin from day of age to 35 days. Half (25) of the broiler chicks upon placement (Day 1) were orally gavaged with 3.9 X 10^5 colony forming units (CFU) per chick of a nalidixic acid resistant *S.* Heidelberg. These seeders were also identified. The remaining birds (25) in each pen served as contact control or horizontal exposure. All had bedding of soft wood shavings that had been used one time before for a broiler grow out. This provided a natural/more typical coccidia challenge.

At 42 and 43 days-of-age 10 horizontal/contact exposed broilers from each pen were humanely euthanized, ceca and liver/spleens were removed aseptically and placed into sterile whirl pack bags onto ice for transport. Liver/spleens from each bird were pooled in one bag and ceca in another bag. *Salmonella* culture was performed for prevalence of *Salmonella* using tetrathionate enrichment and XLT-4 agar containing 25 mg of nalidixic acid. Also, the *Salmonella* in the ceca were enumerated by the most probably number method (MPN) using tetrathionate and XLT-4 (2).

Histology samples. Five mid body pieces of ceca from horizontal exposed broilers were collected at 42-days-of-age and placed into 10% buffered formalin. The mucosa was evaluated for infiltration of inflammatory cells and thickness of the lamina propria.

Ceca flora evaluation. Four ceca contents were obtained at 46-days-of-age from four birds and pooled into one sterile whirlpak bag.

16S r/RNA bacterial microbiome profiling. DNA was extracted using the MO BIO PowerSoil DNA Isolation Kit according to the manufacturer's instructions (MO BIO Laboratories, Carlsbad, CA). The V5-V6 hypervariable regions of the 16S rRNA gene was amplified by the University of Minnesota Genomics Center using the following two-step amplification protocol: samples were first amplified using V5-V6 Nextera primers and KAPA HiFi hot start taq polymerase; thermocycler parameters used were an initial denaturation at 95°C for 5 min, followed by 20 cycles of 98°C for 20 sec, 55°C for 15 sec, and 72°C for 1 min. The resulting PCR products were diluted 1:100 and 5µl was used as template for a second amplification using Illumina indexing primers and thermocycler parameters of 95°C for 5 min followed by 10 cycles of 98°C for 20 sec, 55°C for 15 sec, and 72°C for 1 min. Samples were then size-selected and pooled, denatured with NaOH, diluted to 8 ppM in HT1 buffer (Illumina), spiked with 15% PhiX and denatured with heat treatment at 96°C for 2 min. Sequencing was performed using Illumina MiSeq 2x300 paired-end technology.

Statistical analysis. Descriptive statistics for continuous variables were reported using the mean and standard deviation. Because the variances within treatment groups were not homogeneous with respect to pen-level fecal oocyst counts, separate nonparametric Kruskal-Wallis tests were used to compare groups at each time point. Pairwise multiple comparisons were performed using a nonparametric Bonferroni procedure to limit the Type I error rate to five percent over all comparisons. Cecal *Salmonella* MPNs and prevalences were compared between treatment groups and challenge status categories using generalized estimating equations linear and logistic models, respectively, to account for the correlation between responses of birds from the same pens. Generalized estimating equations models were performed using robust standard errors and an exchangeable working correlation structure. All statistical testing assumed a two-sided alternative hypothesis, and $P < 0.05$ was considered significant. Analyses were performed using commercially available statistical software (Stata version 12.1, StataCorp LP, College Station, TX).

RESULTS AND DISCUSSION

Coccidia results. Despite the use of re-used litter, the early coccidia challenge was not initially very high for any of the three major *Eimeria* species of broilers (*E. tenella*, *E. maxima*, and *E. acervulina*) (Figure 1). As would be expected, the no anticoccidia control group had overall higher average oocyst counts throughout the study. The coccidia vaccine, Coccivac-B52, had the highest total oocysts counts early and then rapidly declined as the birds approached study end at 46 days-of-age. In Figure 1, the *E. tenella* oocysts counts in the salinomycin treated group became quite elevated between day 28 and 46, while the Coccivac-B52 was declining. This shift would be expected since the salinomycin was withdrawn from the feed at day 35, as would normally occur in a commercial flock, and the Coccivac-B52 birds had developed immunity to the *E. tenella* at this time. This shift may be significant in regard to *Salmonella* as will be discussed.

The negative effect of coccidia cycling on the intestine is further supported by the histology results (Table 1), where the mean total coccidia index was 0.08 salinomycin treated pens versus a mean of 0.03 for Coccivac-B52 at 46 days-of-age.

***Salmonella* results**. The *Salmonella* Heidelberg seeder bird challenge method was successful in colonizing all pens as evidenced by 92% of pens positive at 14 days and 100% positive at 46 days. This model is a realistic, but strong challenge with 50% of the chicks in each pen receiving *Salmonella* Heidelberg at one day-of-age. These horizontal exposed birds are more naturally exposed by their pen mates. The horizontal or contact exposed are the ceca sampled at 45 and 46 days-of-age.

The Coccivac-B52 significantly prevented *Salmonella* translocation to the liver and spleen (Table 2) versus the salinomycin treated chicks. There was no significant difference between the no anticoccidia treated and the Coccivac-B52. This may be explained by a lower *Eimeria* challenge in all pens, thus the nontreated controls exposure to

coccidia was early, similar to the coccidia vaccine exposure. This may have resulted in immunity similar to the Coccivac-B52 where at withdrawal of the salinomycin, immunity was incomplete and the *E. tenella* damage occurred, allowing the *Salmonella* colonized in the ceca to translocate to the liver and spleen. This would be in agreement with a previous study (6).

The ceca *Salmonella* colonization trends followed the spleen and liver, but the differences were not significant. This can be seen in Table 2, where 30% of the Coccivac-B52 treated chicks were *Salmonella* Heidelberg positive versus 35% positive for the salinomycin treated chicks. There also were more salinomycin treated pens having 90% or more of the birds sampled positive for *Salmonella* Heidelberg.

Numbers of *Salmonella* in positive ceca are also important to note (Table 3) and in the *Salmonella* positive ceca of the Coccivac-B52 treated birds the amount of *Salmonella* Heidelberg was numerically lower for the $\log_{10}$ MPN, 0.17 versus the positive ceca for the salinomycin treated at 0.49 estimated marginal means $\log_{10}$ MPN/g of ceca.

Histology Results

A 1992 study found that at a challenge dose of *E. tenella* there were increased colonization of *Salmonella* (1). However a different study theorized that lower doses of *E. tenella* could result in mild inflammation and an increase in intestinal thickness resulting in less ability of *Salmonella* to invade (6).

This study, despite the lower level of coccidia challenge in all pens, had a numerical (not statistically significant, Table 1) increase in the mucosal thickness of the Coccivac-B52 chicks versus salinomycin treated chicks. When this increased ceca mucosa thickness is coupled with the enteritis index (Coccivac-B52 2.22 versus salinomycin 2.28) and total gut index (Coccivac-B52 2.25 versus salinomycin 2.37) (Table 1) it is a clear trend to support the Tellez, et al. theory of increased mucosa thickness coupled with less inflammation as an explanation for the lower *Salmonella* Heidelberg colonization of the ceca and especially less translocation to the liver and spleen in the Coccivac-B52 pens (6).

Cecal flora evaluation. Since we are unable to observe phenotypic differences in bacteria as can be done in larger organisms, such as animals; DNA sequences are used in an attempt to understand differences in bacterial diversity. Sequence clusters delineated in this way are called operational taxonomic units or OTU's.

As expected in the ceca the most abundant phyla of bacteria were Firmitcutes and Bacteroidetes. There was no statistically significant difference in either the Phyla or the relative number of each between treatment groups. Although there was not a significant difference in the diversity of ceca flora between treatment groups, there was a consistent trend to less diversity of flora with the Coccivac B52 treatment group.

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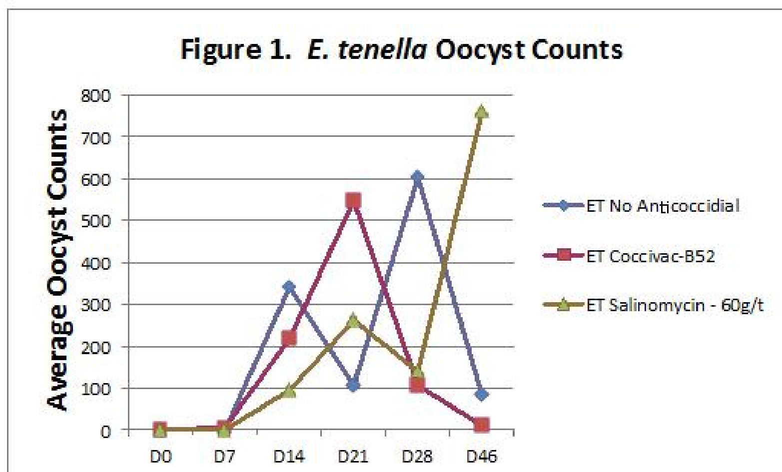

Table 1. Mean of histopathology lesion scores.

	No Anticoccidial	Coccivac-B52	Salinomycin
Cocci Index	0.06	0.03	0.08
Enteritis Index	2.37	2.22	2.28
Total Gut Index	2.43	2.25	2.37
Russell Bodies	1.75	1.59	1.83
Marginal means Mucosal Thickness* (micrometers)	276.6 (7.0)[a]	266.5 (6.9)[a]	259.9 (6.8)[a]

*P = 0.226

Table 2. *Salmonella* prevalences in liver/spleen and ceca samples from 10 birds/pen in each of 12 pens per treatment group.

Treatment	n	Liver/spleen No. positive (%)	†*P*	Ceca No. positive (%)	†*P*
Untreated Control	120	8 (6.7)[a,b]	0.020	33 (27.5)[a]	0.839
Coccivac B	120	6 (5.0)[b]		36 (30.0)[a]	
Salinomycin (60g/ton)	120	22 (18.3)[a]		42 (35.0)[a]	

†Generalized estimating equations logistic model adjusted for clustering by pen. Percentages with a superscript in common do not differ with a level of significance of 5% over all comparisons.

Table 3. Estimated marginal means (95% CI) for $\log_{10}$ *Salmonella* MPN per gram in culture-positive ceca samples from each treatment group.

Treatment	n	Mean (95% CI)	†*P*
Untreated Control	33	0.17 (-0.09, 0.43)[a]	0.210
Coccivac B	36	0.17 (-0.27, 0.61)[a]	
Salinomycin (60 g/ton)	42	0.49 (0.22, 0.77)[a]	

†Generalized estimating equations linear model adjusted for clustering by pen. Means with a superscript in common do not differ with a level of significance of 5% over all comparisons.

OPPORTUNITIES AND CHALLENGES FOR THE CANADIAN POULTRY INDUSTRY

K. Robin Horel

SUMMARY

The challenges for the Canadian poultry industry are diverse and, it could be argued, have never been greater. Many of the current challenges are common to the poultry industry throughout North America and around the world. Some of the challenges are unique to Canada due to the supply management system that is in place for chicken, eggs, turkey and broiler hatching eggs. Most of these challenges present opportunities, opportunities that the Canadian industry should be well positioned to take advantage of.

Robin Horel, President and CEO of the Canadian Poultry & Egg Processors Council will address this subject to attendees at the 65th Western Poultry Disease Conference. Mr. Horel's presentation will deal with challenges and opportunities within three overarching areas: sustainability, Canadian customer and consumer demands, and supply management.

Sustainability includes, but is not limited to: environmental impact, food safety, animal welfare, animal health and financial considerations. These challenges are common to the international industry, but Robin will highlight the Canadian perspective and note where our industry is well positioned to turn these into opportunities.

Canadian consumers, like consumers in most of the developed world, are multiple generations removed from the farm. Nevertheless, they are demanding more information on how their food is produced. Taste, nutrition, convenience, food safety, and cost continue to be important, but animal welfare, locally sourced and environmentally friendly are becoming more important considerations as well. Consolidation of retail customers and attention to brand by both retail and food service customers are additional factors that must be considered.

The Canadian system of supply management for poultry production offers its own set of challenges but also results in some interesting opportunities for the industry. The member companies of the Canadian Poultry & Egg Processors Council process, grade &/or hatch over 90% of Canada's chicken, turkey, eggs, and broiler hatching eggs. They buy their raw material from supply managed farmers in these supply chains.

Against a backdrop of erosion of the Canadian dollar compared to the US greenback, avian influenza outbreaks in North America, a Trans Pacific Partnership (TPP) agreement and lagging consumer confidence, the Canadian poultry industry had a successful 2015. Dealing successfully with the upcoming challenges to our industry will ensure that this success continues.

IMPACT OF IMMUNOSUPPRESSION ON POULTRY PRODUCTION: THE ROLE OF INFECTIOUS BURSAL DISEASE VIRUS

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ABSTRACT

Immune suppression can be the underlying cause of clinical respiratory and enteric disease in layer and broiler flocks. It can also cause vaccination failure and reactions including vaccine induced disease. The sub-clinical disease that accompanies immune suppression caused by infectious bursal disease virus (IBDV) can also have a significant economic impact on poultry production. The flock may appear healthy but feed efficiency is reduced, growth rate is slowed and time to market is lengthened. Although mortality is usually not an issue, the cost of production in an IBDV infected flock can be prohibitive. Gumboro disease continues to be a problem because vaccines have not kept pace with the antigenic changes in the viruses. We now know that even minor amino acid changes can have profound effects on the antigenicity and pathogenicity of IBDV. Evolutionary forces at work for over half a century have selected for phenotypic changes among IBDV strains. As a result, the viruses we are trying to control today are much different than the viruses that were infecting chicken flocks 50, 25 or even five years ago. The inactivated vaccines used to produce maternal immunity and the live-attenuated and recombinant HVT-IBD vaccines used to produce active immunity in chicks all seem to provide better protection when their antigenic structure matches that of the field viruses.

INTRODUCTION

Gumboro disease is caused by IBDV. This virus is a member of the family *Birnaviridae*, genus *Avibirnavirus*. The antigenic and pathogenic types of IBDV have evolved considerably since it was first identified approximately 50 years ago (4). These viruses no longer fit into the two antigenic classes (classic and variant) identified in the 1980s. Numerous antigenic strains of IBDV have been identified. In addition, a wide range of pathogenicity exists from naturally attenuated to very virulent (vvIBDV) strains (25).

PRODUCTION LOSSES ASSOCIATED WITH GUMBORO DISEASE

IBDV have been generally classified into sub-clinical (scIBDV), classic virulent (cvIBDV) and very virulent (vvIBDV) pathotypes (25). Although there are some countries that do not have vvIBDV, phylogenetic studies indicate these highly pathogenic viruses have spread to nearly all poultry producing countries in the world including the United States (7, 23, 24). If these vvIBDV are in North America, why are we not seeing the high mortality and devastating clinical disease reported in Europe, Asia and Latin America? Our studies indicate that in regions where scIBDV predominate, viral competition and maternal immunity may play a role in reducing the severity of the disease typically observed following a vvIBDV infection (11).

In North America, production losses from IBDV induced immune suppression are usually due to scIBDV strains of the virus. These viruses are ubiquitous and typically cause a sub-clinical disease with depletion of lymphocytes without inflammation in the bursa of Fabricius. Alone, these viruses can cause slower growth rates, poor feed efficiency and uneven flock weights. In well managed flocks, secondary infections can be innocuous but in poorly managed flocks, these secondary infections exacerbate economic losses.

ANTIGENICITY

To understand why IBDV infections are still causing immune suppression, it is necessary to review what is known about their antigenic structure. Two serotypes of IBDV have been identified. The serotype 1 viruses typically infect chickens and cause immune suppression (4). Serotype 2 viruses infect numerous avian species including turkeys but thus far no disease has been attributed to them (3, 19, 20).

The serotype 1 viruses have been divided into two antigenic groups known as classic and variant. This is an oversimplification of the antigenic variability observed among these viruses however

and several antigenic subtypes have been described within each group. Antigenic drift is largely responsible for the continued emergence of new antigenic strains of IBDV.

The antigenic phenotype of IBDV is determined by the hypervariable sequence region of VP2 (hvVP2) (1, 2, 8, 10). Four loop structures in the hypervariable sequence region were identified and designated P_{BC} P_{DE}, P_{FG}, and P_{HI} (6). The antigenic characteristics of IBDV are controlled by specific amino acids at the apex of these loops (5, 17).

Phylogenetic analysis of the VP2 loop structures of IBDV has been used to delineate antigenically diverse groups of the virus. Compared to a whole genome analysis, a phylogenic analysis of the hvVP2 genetic region that is subject to frequent mutations allows greater discrimination between viruses (18). Figure 1 shows the Neighbor Joining phylogenetic tree of hvVP2 from IBDV strains around the world. Although three major groups (vvIBDV, Classic and Variant) are identified, there are numerous sub-branches within each group that were shown to be antigenically diverse (9, 13, 17, 21).

CONTROL OF GUMBORO DISEASE AND IMMUNE SUPPRESSION

To consistently produce healthy poultry flocks, IBDV infections and the resulting immune suppression must be controlled. Current vaccination and control strategies used by the poultry industry rely heavily on maternal immunity being passed to progeny chicks. Inactivated commercial vaccines are available for the production of maternal immunity in breeder flocks. These vaccines are decades old however and because of antigenic drift they often do not fully protect progeny chicks against IBDV infection and immune suppression. Live-attenuated commercial vaccines have been used in young chicks in an attempt to improve protection but they too are only marginally effective against new antigenic types of IBDV and most are not effective against the vvIBDV (16, 22). Recombinant HVT-IBD vaccines have improved protection to some IBDV strains but these vaccines express only one IBDV VP2 protein and may not offer full protection against evolving IBDV strains.

The increased use and success of autogenous vaccines and recent publications on the molecular basis for antigenic drift (13) indicate the evolution of IBDV has made some commercial IBD vaccines obsolete. The spread of vvIBDV and genome reassorted vvIBDV throughout the world, including the US, suggests the vaccines used for this acute strain of the virus could also perform better (14, 15). Multivalent vaccines for IBDV exist and they do a good job protecting against some strains of the virus. Using one multivalent vaccine product to protect against all possible strains of IBDV however may not be practical because antigenic change will continue as immunologic selection pressures drive the evolution of these viruses. We have observed geographically restricted antigenic drift among IBDV (16). Controlling these viruses may only be possible using custom made vaccines that match their antigenicity (12). Regardless of the method or vaccine used, reducing the impact of immune suppression on poultry production will require the control of IBDV infections.

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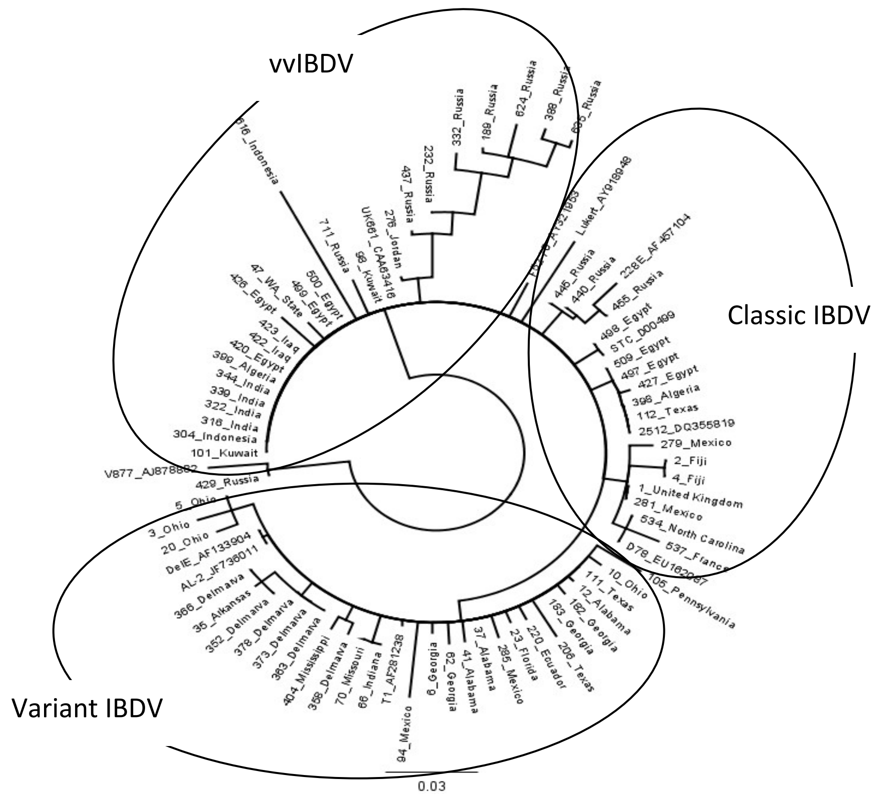
Figure 1. Neighbor Joining Phylogenic Tree. Loop structure amino acid sequences of the IBDV VP2 protein were analyzed and generally grouped into antigenic type strains. Sub-branches within each type strain represent antigenic diversity among the viruses.



IMPACT OF RESPIRATORY DISEASES WITH SPECIAL EMPHASIS TO EMERGING INFECTIOUS BRONCHITIS VIRUS

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SUMMARY

Respiratory diseases in commercial poultry are likely the costliest diseases encountered in a modern poultry operation. Management of diseases like avian influenza, Newcastle disease, infectious laryngotracheitis, avian mycoplasma and infectious bronchitis are a constant and frustrating challenge. Infectious bronchitis virus (IBV) is a gamma coronavirus that causes a highly contagious upper-respiratory disease in chickens. The virus can also affect the reproductive tract and some strains can cause nephritis. Different serotypes and genetic types of the virus have been identified worldwide and for the most part do not cross-protect. In addition, new types of the virus continue to arise due to mutations and recombination events in the viral genome making this virus difficult to identify and extremely difficult to control. Proactive surveillance and identification of IBV types, particularly emerging new virus types is necessary for effective control of the disease.

INTRODUCTION

Avian respiratory diseases have an enormous economic impact on commercial poultry. Some of the most difficult diseases to manage are avian influenza, Newcastle disease, infectious laryngotracheitis, avian mycoplasma and infectious bronchitis. The pathogens that cause these diseases exist as different serotypes (avian influenza virus and IBV) different pathotypes (infectious laryngotracheitis virus and Newcastle disease virus) and different strains (avian mycoplasmas) making them extremely difficult to control. In addition, they can lead to secondary bacterial infections resulting in even more severe disease. Losses from respiratory disease include production losses (weight gain and egg production) condemnations in the processing plant and in some cases death.

Although not the most pathogenic respiratory disease agent in commercial poultry, IBV is perhaps the most difficult to control because it exists as multiple different types that do not cross-protect and it is constantly changing to emerge as new types capable of causing disease. This presentation will focus on the ongoing challenges associated with control of infectious bronchitis in commercial poultry.

HISTORY

The Massachusetts (Mass) type of IBV was the first virus isolated (circa 1930), and for many years the Mass serotype was the only known virus type until Jungherr (2) reported a different IBV type in 1956 from Connecticut. Using the virus neutralization test, he found that antisera against the Mass type virus did not neutralize this new virus designated Conn. This was the first indication that different types of the virus exist. Since that finding many different serotypes (defined by neutralizing antibodies), and genetic types (based on the sequence of the spike gene), have been described worldwide.

EMERGENCE OF NEW VIRUS TYPES

IBV is a gamma coronavirus with single stranded-positive sense RNA genome surrounded by a lipid envelope. The viral genome encodes for the viral RNA-dependent RNA-polymerase (RdRp), and structural proteins spike (S), envelope, membrane, and nucleocapsid. The S glycoprotein contains epitopes for neutralizing and serotype specific antibodies mediates cell attachment, virus-cell membrane fusion, and plays an important role in host cell specificity. Spike is the most variable protein in the virus. This variability is what contributes to the emergence of new virus types and allows the virus to continue to be maintained in poultry.

Genetic diversity in IBV is due to rapid replication, a high mutation rate and genome recombination events. Although the mechanisms contributing to genetic diversity among IBV types are not completely understood, it is well known that the viral polymerase has low fidelity and limited ability to correct mistakes when replicating the viral genome. When the resulting mutations give the virus a selective advantage, that 'new' IBV type will emerge to be the predominant virus population in the host. Thus, IBV exists as a population of genetically different viruses in the host, and there is sound

evidence that these genetically different subpopulations of the virus exist even in vaccine strains (3, 5). It has further been shown that certain subpopulations, which may be minor components of the overall virus population, can be more adept at infecting and replicating in embryonated eggs whereas other subpopulations are more fit to infect and replicate in chickens. This was specifically shown for the ArkDPI type IBV vaccine (3, 4, 5). Although recombination events have been documented for IBV, recombination is typically associated with shifts in pathogenicity or host specificity and likely play a minor role in the emergence of variant strains of IBV (1). Thus, host selection acting on existing virus subpopulations as well as variant viruses that result from a high mutation rate contribute to the emergence of new IBV types in commercial poultry.

CONTROL

Attenuated live vaccines are used in broilers and pullets and killed vaccines are typically used in layers and breeders. Because IBV exists as multiple different types with limited cross-protection, effective control involves identification of the virus type causing disease followed by vaccination with an appropriate vaccine against that type. However, control is challenging because there are approximately six different types of IBV vaccines commercially available in the USA, whereas, countless different types and variants of the virus capable of causing disease can be found throughout the USA and the world.

Proactive surveillance to identify IBV types circulating in the field is extremely important for control of this disease. Identification is typically done by reverse transcriptase-polymerase chain reaction (RT-PCR) amplification of the spike glycoprotein gene or the hypervariable 5´ end of the spike gene followed by nucleic acid sequencing and analysis with other similar spike sequences. Genetic typing of IBV is rapid and relatively inexpensive making it possible to type a large number of virus isolates in a short period of time. In addition, we have the ability to compare the relatedness of different virus types from all over the world allowing for a more informed control strategy. Currently circulating strains of IBV in the USA are Ark, CA99, Mass, DE072, GA98, GA08, DMV/1639 and GA13. Vaccines exist for Ark, Mass, DE072, GA98 and GA08, and indeed a proportion of those types identified in the field are likely vaccine origin viruses.

A successful control strategy for IBV involves a sound vaccination program that includes vaccines for currently circulating strains as well as proactive surveillance for the identification of new IBV types so the vaccination program can be adjusted accordingly. Of course this assumes that vaccines for all of the different IBV types circulating in commercial poultry are available, which is often not the case. The most significant challenge for the future control of IBV is the rapid development (and licensing) of safe and efficacious vaccines for newly emerging IBV types.

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UNDERSTANDING INFECTIOUS BRONCHITIS VIRUS SUBPOPULATIONS AND SPRAY VACCINATION FAILURE OF THE ARKANSAS TYPE VACCINES

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SUMMARY

Virus subpopulations (defined by the sequence of the S1 gene) are naturally generated in infectious bronchitis virus (IBV) as a result of virus replication. Herein, we identify the implications of those subpopulations on vaccine performance for the Arkansas-DPI (Ark-DPI) type vaccines. When Ark-DPI type vaccines are given using a hatchery spray cabinet, they largely fail to infect the chick whereas after eye drop administration vaccine virus can readily be demonstrated in the chicks and sound protection following homologous challenge is observed. We have conducted a number of studies examining the characteristics of the Ark-DPI vaccines including subpopulations of the virus, in an attempt to elucidate the causes of the poor infectivity and immunity following spray vaccination in broilers. This data is important given that almost all broiler companies in the USA vaccinate with the Ark-DPI strain and that over 80% of all IBV isolates from broilers in the USA are Arkansas type viruses.

INTRODUCTION

One of the goals of our laboratory is to understand why IBV Ark-DPI type vaccines are not efficacious when delivered using a hatchery spray cabinet. It appears that that an immunizing dose of Ark-DPI vaccine is not reaching the chicks. This assumption is based on our findings that the Ark-DPI vaccine virus when delivered by eye-drop is efficacious but the same amount of virus delivered by spray cabinet is not. Since Mass type IBV vaccines are efficacious when delivered by a hatchery spray cabinet, we were interested in determining if physical parameters of the hatchery spray cabinet were possibly affecting infection and/or replication of the Ark-DPI vaccine, and if we could obtain a minimum infectious dose of Ark-DPI vaccine by increasing the amount of vaccine virus delivered using a hatchery spray cabinet.

MATERIALS AND METHODS

Viruses and virus titrations. The IBV vaccines used in this study were commercially available Ark-DPI vaccines from three different manufacturers. We also included a commercially available Mass type IBV vaccine as a control. Virus titrations were conducted in 9 to 11 day-old embryonated eggs as described (7).

RNA extraction and qRRT-PCR. Viral RNA extraction and detection by quantitative real time RRT-PCR was conducted as previously described (1).

Transmission electron microscopy (EM). Vaccine samples were examined using a JEM-1210 transmission electron microscope (JEOL, Inc., Tokyo, Japan) to identify any virus particle morphological differences as previously described (4).

Sequencing and analysis. The S1 spike genes were amplified using the 5' oligo and 3' degenerate primers as previously described (2). Samples were sent to the Georgia Genomics Facility (Athens, GA) for standard Sanger sequencing. The spike gene sequence was assembled and analyzed using DNAStar Lasergene 12, Vir 12.3.1.

RESULTS AND DISCUSSION

We examined if shearing forces associated with spray vaccination damaged Ark-DPI vaccine virus particles. The structure of the virus particles and the average number of spikes associated with each virion was examined by EM before and after spray and compared to a Mass vaccine control. The number of spikes on the Ark-DPI type vaccines from three different manufacturers varied slightly but there was no substantial difference in the number of spikes before and after spray which was similar for the Mass vaccine. In addition, we measured the titer of the vaccines before and after spray and there was a half log drop in titer after spray but this was similar for both Ark-DPI and Mass type vaccines. This data indicates that the spraying process apparently does

not damage Ark-DPI virus particles any more than Mass vaccine particles suggesting that failure of the Ark-DPI vaccine is not due to mechanical damage from hatchery spray cabinet administration.

Since an infectious dose of Ark-DPI vaccine is apparently not reaching the chicks, we examined a 1x, 10x and 100x dose of vaccine delivered in a hatchery spray cabinet to determine if we could obtain a level of infection similar to eye drop vaccination. We found that using a 100x dose, approximately 88% (Ct= 32.16) and 90% (Ct= 31.96) of the chicks were positive for vaccine virus at seven and 10 days post-vaccination respectively. This was similar to infection and virus replication levels achieved by eye drop vaccination using a 1x dose of the same Ark-DPI vaccine.

Sequence analysis of re-isolated Ark-DPI vaccine virus showed sequence polymorphisms when compared to the original vaccine given to the birds. The polymorphisms were similar to previous reports (3, 5, 6) and were consistent regardless of vaccine dose or time of re-isolation. Rapid emergence of a vaccine virus subpopulation in vaccinated birds with specific changes in the S1 gene is evidence of a minor subpopulation present in the original vaccine that is more proficient at infecting and replicating in birds than the major virus population in the vaccine. This data indicates that a critical amount of the minor virus subpopulation in the Ark-DPI vaccine that is capable of infecting and replicating in the chicks (ie. the amount found in a 100x dose) is needed to establish a minimum infectious dose of the Ark-DPI vaccine when given by a hatchery spray cabinet. Obviously giving a 100x dose of Ark-DPI vaccine by hatchery spray cabinet is not economically feasible. Thus, a new Ark vaccine or alternative vaccine strategy is needed.

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EFFECT OF *BACILLUS SUBTILIS* AND *BACILLUS LICHENIFORMIS* ON PERFORMANCE OF LATE FIRST CYCLE LAYING HENS

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INTRODUCTION

The objective of this trial was to evaluate GalliPro® Tect (*B. licheniformis*) and GalliPro® Max (*B. subtilis*) on the performance of late first cycle laying hens.

MATERIALS AND METHODS

Animals and experimental design. The study was conducted at the Iowa State University research farm during the first half of 2014. A total of 234 Hy-Line W-36 laying hens, with an initial age of 69 weeks and a 1.63 kg initial average body weight, were evaluated for an experimental period of 20 weeks. Birds were kept in a windowless house with full control of the environment with temperature maintained between 20°C to 27°C. Birds were kept in cage batteries at a density of 438 cm^2/bird. Feed, in mash form, was manually filled in the feeders with controlled feeding and offered 100-105 g/bird/day. Nipple automatic drinkers were used to provide *ad libitum* access of water. Lighting schedule was 15.5 h of light and 8.5 h of dark. Eggs were collected manually. Birds were randomly assigned to each experimental unit. Each experimental unit consisted of a cage with six birds. There were three treatments with 13 replicates per treatment.

Feeds and test article. There were three treatments:

1) Control diet
2) Control diet + GalliPro Tect (*B. licheniformis* 1.60E+06 CFU/g)
3) Control diet + GalliPro Max (*B. subtilis* 8.00E+05 CFU/g)

There were 12 replications (cages) per treatment and six hens per cage at a density of 438 cm^2.

Data collection and statistical analysis. Live body weight- individual weights were measured. Feed consumption was measured weekly, and feed efficiency was calculated (egg mas/kg feed). Egg production was recorded every day. Eggs were collected manually at the same time every day. Eggs of different treatments were collected separately. Hen day egg production (HDEP) was calculated. Egg quality measurements: egg weight, shell weight, shell thickness, yolk weight, albumen weight, albumen height, and specific gravity were measured. Haugh unit = 100*log (albumem height – (1.7 egg weight)^0.37 + 7.6). Energy/calorie conversion was calculated.

The data were analyzed with SAS 9.4 using PROC MIXED (SAS Institute Inc., Cary, NC) for Randomized complete block design. Performance measurements analyzed with repeated measures to determine treatment, week, and treatment x week effects. If significant, Tukeys test was used to separate means of repeated measures. Performance values averaged over two week periods. Significance was accepted at $P \leq 0.10$.

RESULTS AND DISCUSSION

Laying performance was summarized in Table 2. Birds fed a diet with GalliPro Max had the highest HDEP (77%, $P<0.05$) among three the treatment groups. Both GalliPro Max and GalliPro Tect resulted in heavier egg weight than Control ($P<0.10$). GalliPro Max also significantly improved feed efficiency ($P<0.10$) over Control, with GalliPro Tect being intermediate.

Egg quality parameters are shown in Table 3. Both GalliPro Tect and GalliPro Max increased albumen height over the Control ($P=0.10$). GalliPro Tect resulted in higher shell weight ($P<0.10$) than Control, with GalliPro Max intermediate. There were no differences observed on egg weight, shell thickness, yolk weight, albumen weight, Haugh unit and specific gravity among the three treatments.

Energy conversion and related parameters are shown in Table 4. Feed conversion ratio calculated as amount of feed consumed per unit of egg produced. Both GalliPro Max and GalliPro Tect resulted in better FCR than Control ($P<0.10$). Energy or calorie conversion is expressed as amount of kilo calorie required to produce per kg eggs, GalliPro Max and GalliPro Tect had 232 Kcal and 49 Kcal less ME vs Control for each kg eggs produced, which were equivalent to 114 and 23 Kcal ME per kg feed, respectively.

Feed efficiency was improved with GalliPro Max in comparison to the control fed birds, with the GalliPro Tect intermediate (P = 0.08). GalliPro Max and GalliPro Tect treatments improved dietary energy conversion with calorie value of 114 and 23 Kcal/kg feed, respectively.

CONCLUSION

During the late first cycle laying period, GalliPro Max significantly increase hen day egg production by 2.7% over Control. Both GalliPro Max and GalliPro Tect resulted in heavier eggs (1.2%), higher egg mass (4.1%) and improved feed efficiency (5.5%) vs. Control. Hence, GalliPro Max and GalliPro Tect improved energy or Calorie conversion, with ME equivalent of 114 and 23 Kcal/kg feed, respectively.

The mechanism is in part due to the enzymes produced by *Bacillus* in the GI tract, resulting improved nutrient digestion, absorption and utilization in the metabolic processing (1, 2) in addition to other well established functions, such as enhanced immune function (3) and reducing pathogenic bacteria in the GI tract (4), especially under relatively higher physiological stress condition during the late laying cycle period.

This research finding has significant implications to the feed and layer industry, with potential feed cost savings opportunity.

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Table 1. Experimental feed diet formulation.

Ingredients	Treatment		
	Control	GalliPro® Tect	GalliPro® Max
	------------------------- % ----------------------		
Corn	60.72	60.67	60.67
SBM 48	19.22	19.22	19.22
DDGS	5.00	5.00	5.00
MBM	3.89	3.89	3.89
Soy oil	1.14	1.14	1.14
Other*	10.03	10.03	10.03
GalliPro® Tect		0.05	-
GalliPro® Max		-	0.05
Total	*100.00*	*100.00*	*100.00*

*Limestone (50/50 mixture small & large particle), vitamin & mineral premix, salt, DL methionine, choline chloride, Biolys®(Evonik), xylanase, and phytase.

Ingredients	Treatment		
	Control	GalliPro® Tect	GalliPro® Max
	------------------------Analyzed -------------------		
Crude protein, %	17.27	17.28	17.29
Crude fat, %	2.89	2.89	2.89
Crude fiber, %	2.83	2.83	2.83
Moisture, %	10.68	10.69	10.70
Ash, %	12.22	12.23	12.24
	------------------------Calculated -----------------		
ME, Kcal/kg	2,850	2,850	2,850
Dig Arg, %	0.762	0.762	0.762
Dig Lys, %	0.752	0.752	0.752
Dig Meth, %	0.298	0.298	0.298
Dig Thr, %	0.630	0.630	0.630
Dig Trp, %	0.158	0.158	0.158
Bacillus, CFU/g	-	1.60E+06	8.00E+05

Table 2. Summary of egg production.

	Treatment				
	Control	GalliPro® Tect	GalliPro® Max	SEM	P-value
HDEP (%)	75.0 [ab]	74.1 [b]	77.0 [a]	0.76	0.03
Feed intake (g/bird/day)	103.1	102.9	103.2	0.47	0.25
Egg weight (g)	67.5 [b]	68.2 [ab]	68.3 [a]	0.28	0.09
Egg mass (g/bird/day)	48.7	49.0	50.7	0.86	0.21
Feed efficiency (g/kg)	458 [b]	464 [ab]	483 [a]	8.10	0.08
Body weight (kg)	1.616	1.641	1.632	0.0165	0.57

[a-b]Least square means without a common superscript differ significantly ($P \leq 0.10$)

Table 3. Summary of egg quality.

	Treatment				
	Control	GalliPro® Tect	GalliPro® Max	SEM	P-value
Egg weight (g)	67.06	67.52	67.57	0.37	0.55
Albumen height (mm)	6.8 [b]	7.2 [a]	7.0 [ab]	0.11	0.10
Shell thickness (mm)	0.39	0.38	0.38	0.00	0.15
Shell weight (g)	8.68 [b]	8.95 [a]	8.70 [ab]	0.09	0.08
Yolk weight (g)	18.42	18.48	18.59	0.18	0.80
Albumen weight (g)	38.73	38.92	39.08	0.27	0.67
Haugh unit	80.0	82.3	80.8	0.78	0.13
Specific gravity	1.0754	1.0746	1.0748	0.0003	0.26

[a-b]Least square means without a common superscript differ significantly ($P \leq 0.10$)

Table 4. Energy conversion.

	Treatment				
	Control	GalliPro® Tect	GalliPro® Max	SEM	P-value
Feed intake (g/bird/day)	103.1	102.9	103.2	0.47	0.25
Egg mass (g/bird/day)	48.7	49.0	50.7	0.86	0.21
FCR (g feed/g egg)	2.12 [b]	2.10 [ab]	2.04 [a]	8.10	0.08
Calorie conversion (Kcal/kg egg)	6,034	5,985	5,801		
Difference from Control		49	232		
Calorie value (Kcal/kg feed)		23	114		

[a-b]Least square means without a common superscript differ significantly ($P \leq 0.10$)

THE GOOD, THE BAD, AND THE UGLY OF PERMITTED MOVEMENT OF POULTRY AND POULTRY PRODUCTS DURING AN HPAI OUTBREAK

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INTRODUCTION AND BACKGROUND

In the United States (US), emergency response efforts during a highly pathogenic avian influenza (HPAI) outbreak are focused on controlling and eliminating the disease, while simultaneously allowing essential poultry industry business operations to promote food security. Once HPAI is detected, a regulatory control area is established and quarantines and movement controls are instituted. Although these emergency response activities are critical to limit disease transmission, they can impede normal business operations and result in animal welfare issues, economic consequences, and food supply impacts. To address these unintended and negative effects, continuity of business plans has been created to manage the movement of non-infected live poultry and non-contaminated poultry products from, to, and within a control area.

The Secure Poultry Supply (SPS) Plans (i.e., Secure Egg Supply, Secure Broiler Supply, and Secure Turkey Supply Plans) have been developed over the last decade. Each of the SPS Plans was drafted separately through a public/private/academic partnership of individuals knowledgeable about HPAI or the poultry industry sector. Established science and risk based approaches were used as the basis for the plans, including risk assessments, premises-related information, biosecurity guidelines, cleaning and disinfection procedures, surveillance requirements, and permit guidance. All of these measures are designed to minimize the risk that HPAI transmission may occur through movement of poultry products such as eggs, egg products, broilers, and turkeys during an outbreak (5). The poultry industry sectors have different business operations, management practices, and mitigation strategies, and the different species have different disease dynamics as well. Taken together, these differences have resulted in different risks and permitted movement requirements, which, in turn, make the SPS Plans quite complex.

In the US, Minnesota (MN) is ranked first in turkey production, eighth in egg production, and nineteenth for broiler production; and consequently, the industry is densely populated in several areas of the state (1, 3). From March 4–June 5, 2015, a severe outbreak of H5N2 HPAI occurred in MN and affected 9,024,632 birds. A total of 104 HPAI cases were confirmed and included 98 commercial turkey premises, four commercial layer premises, one pullet premises, and one backyard premises. An additional six premises were designated as dangerous contacts (6). Overall, 23 of 87 MN counties had infected premises, and regulatory control areas encompassed 5,236 square miles. Over the course of the outbreak, 264 commercial and 1,599 backyard poultry premises were in control areas. For movement of poultry products on or off non-infected premises in these control areas, 931 permits were issued and 3,074 movements were associated with these permits. Additional permits were issued for other items (e.g., feed, finished products from processing plants, etc.). In excess of 5,048 hours were spent on permitting by state staff during the response period, which is equivalent to more than 41 hours per day (4). Likewise, industry members spent many hours on tasks related to permitted movement. All control areas were released on July 28, 2015 (2).

EVALUATION OF PERMITTED MOVEMENT DURING 2015 HPAI OUTBREAK IN MN

In December 2015, the University of Minnesota held a one-day meeting to facilitate a dialogue between state government and poultry industry sector leaders. Federal government and other academic representatives also were in attendance, primarily as

observers. The goal of the meeting was to evaluate permitted movement during the HPAI outbreak in MN in order to improve any future permitting. On the day of the event, an overview of continuity of business and the SPS Plans set the stage for discussion and was augmented by presentations from the state and the industry about their experiences and perspectives on permitting.

Ultimately, permitting was successful in MN because there was movement of a large number of poultry products and there is no evidence that risk-based permitted movements spread HPAI. Pre-movement testing also helped contribute to the detection of HPAI, along with cases found via mandated surveillance and industry-initiated voluntary surveillance.

Several important themes emerged from the discussion during the meeting. First, permitted movement is a tacit agreement between the state and the industry and it must be negotiated, particularly with respect to who should have responsibility for various aspects of the process. The knowledge, expertise, and resources that each of these parties possess should be considered in the negotiation. Second, the SPS Plans are complex. They need to be harmonized and simplified where possible to ensure that they are better understood and implemented by the state and the industry. Third, the SPS Plans provide the scientific foundation for permitted movement but they are not meant to be "one-size-fits all" plans for every state. Because each state is unique and each poultry industry sector within that state also is unique, a customized state permitting movement plan is needed. Additionally, the SPS Plans are not "recipes" for permitted movement. Therefore, as part of the customized plan, the responsibilities, processes, and details for permitted movement need to be specified in accordance with the requirements of the SPS Plans. However, the creation of the customized plan must be a cross-sector collaborative effort to ensure that it will be appropriate for the state's layer, broiler, and/or turkey industries collectively. Fourth, it is important to recognize that the issued permitted movement document is serving multiple purposes such as:

1) Enabling movement of poultry products in a timely manner within the parameters of the SPS Plans.
2) Facilitating accountability and adherence to SPS Plan conditions.
3) Providing animal disease and other traceability, education/information, and documentation.
4) Assisting in infection control.

Based on these themes, as well as other gaps and barriers that were identified during the meeting, two ad-hoc cross-sector work groups were formed to address the major permitting-related needs for MN. Both work groups already have convened and have made significant progress to date.

In summary, the one-day meeting was an efficient way to perform the continuous improvement process for permitted movement. The open, honest communication that took place between the state and the industry was vital to the success of the meeting. Such communication undoubtedly was made possible by the existing relationships among meeting participants. Thus, the importance of ongoing relationship-building between the state and the industry should not be underestimated.

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AN EXAMINATION OF *GALLIBACTERIUM ANATIS* IN COMMERCIAL POULTRY FLOCKS IN THE UNITED STATES

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SUMMARY

The objective of this paper is to present cases of *Gallibacterium anatis* septicemia and conduct analysis of *G. anatis* isolates obtained from fresh mortality in commercial poultry flocks from the United States. This organism was isolated from cultures taken from the respiratory tracts, reproductive tracts, abdomens, and viscera of fresh mortality. Gross lesions included moderate to severe salpingitis, peritonitis, sinusitis, pneumonia, airsacculitis, pericarditis, and hepatitis. Wrinkled, soft and thin egg shells were also observed and may be related to some infections of the reproductive tracts of egg-laying hens. Isolates from flocks in the U.S. were examined by several means and are discussed. Genome sequencing and phylogenetic relatedness were also examined. Control efforts focusing on environmental and water sanitation, *G. anatis* autogenous vaccines, and infectious bursal disease control were considered effective in reducing the severity of the problem.

INTRODUCTION

This study was initiated by respiratory infections in commercial broiler and breeder flocks and increased mortality associated with suspected bacterial infections in commercial layer flocks. *G. anatis* became of interest when it was recovered in pure culture from these "problem" flocks. Egg shell abnormalities were observed in some commercial layer flocks associated with *G. anatis* involvement. *G. anatis* was isolated from respiratory tracts, reproductive tracts, abdomens, and viscera of fresh mortality. Once classified as a Pasteurella, this organism has not typically been perceived as a major pathogen in commercial poultry, at least not in the meat-type poultry industry in the southern United States. *G. anatis* is known to colonize the upper respiratory tract and lower reproductive tract of normal chickens, but it has also been experimentally shown to induce natural infection in a chicken model (2).

In breeder flocks, it has been reported that *G. anatis* infection was similar to *Pasteurella multocida* (fowl cholera) systemic infections (ongoing mortality, decreased egg production, peritonitis, and airsacculitis)(4). *G. anatis* has been recovered in pure culture from a range of lesions in chickens including, septicemia, oophoritis, follicle degeneration, salpingitis, peritonitis, and respiratory tract infections (2). It has been shown that *G. anatis* isolates were prevalent in layers with reproductive disorders and that they played a role in the disorders (5). According to these authors, isolates of *G. anatis* from different locations in the body were highly similar; indicating that isolates residing in their natural habitat (upper respiratory system) may cause reproductive disorders and/or systemic disease under certain conditions. In a recent publication, it was shown that nasal inoculation with *G. anatis* caused microscopic lesions in trachea, lungs, air sacs, and liver (7). They concluded that *G. anatis* may be considered a primary pathogen for the respiratory tract of chickens and that liver damage detected may suggest the possibility of blood dissemination notwithstanding the nasal route of inoculation. *G. anatis* is globally distributed, having been isolated from poultry in countries within Europe, Africa, Asia, Australia and the Americas (6).

MATERIALS AND METHODS

***G. anatis* isolates.** *G. anatis* strains were isolated from farms associated with elevated mortality (up to 5x normal) due to obvious bacterial infections of the abdomen, reproductive or respiratory tract, and various viscera including liver and spleen. Fresh mortality was selected from

representative flocks for necropsy exam. Cultures were obtained via transport BBL CultureSwab™ plus Amies w/o CH single applicator swabs (Becton, Dickinson and Company, Sparks, MD) from obvious gross lesions (peritonitis; salpingitis; pneumonia; sinusitis; and airsaculitis). Swabs were refrigerated until they were shipped via overnight delivery with ice packs to the laboratory for processing.

Microbial growth and initial characterization. Initial isolation from clinical samples was conducted by traditional microbiology under CO_2 conditions at 37°C. For further analysis, all of the strains were cultured using blood agar and brain heart infusion (BHI) media. Strains were assessed for motility, biofilm formation and general biochemical characteristics (API 20E, API 20NE).

DNA isolation and PCR. *Gallibacterium* DNA was extracted using the Omega Cell & Tissue kits according to manufacturer's protocol on the KingFisher Flex magnetic purification processor (Thermofisher, Waltham, MA). *G. anatis* were analyzed by the PCR method described by Bojensen et al (1). Differences between isolates at the 16S-23S rRNA region in the *G. anatis* genome were assessed using the method described by Jabbari *et al.* (3).

Whole genome sequencing. Whole genome sequencing (WGS) was conducted on DNA sequencing libraries, constructed according to manufacturer's protocols and sequenced using the Ion Torrent PGM (Thermofisher, Waltham, MA). Briefly, DNA was sheared, bar-coded and amplified by emulsion PCR using the OneTouch v2(Thermofisher, Waltham, MA) . Libraries were quantified and pooled prior to being loaded into an Ion 318v2 sequencing chip.

Sequencing data analysis. NGS high quality sequence data (Q scores>20) and 16S-23S sequences were aligned and analyzed using the NGen and/or SeqMan Pro software (DNASTAR, Madison, WI) to reference sequence G. anatis UMN179 (GenBank accession CP002667). Sequences were aligned and a phylogenetic tree was constructed using the ClustalW statistic in MegaAlign (DNASTAR, Madison, WI). Draft assemblies for each strain were generated using CLC Genomics Workbench version 8. Genomes were aligned using MAUVE with other publicly available *Gallibacterium* assemblies. Single nucleotide polymorphisms were identified between the 41 genomes examined, and a phylogenetic tree was constructed from these data using Maximum Parsimony methods in MEGA 6.0.

Histology of affected tissues. Flocks that were also experiencing egg shell abnormalities (soft, misshapen or wrinkled shells) had tissues obtained and processed for histological examination using standard methods.

RESULTS

Microbial growth and initial characterization. All of the *G. anatis* strains grew in blood agar and produced the characteristic β hemolysis associated with *G. anatis* biovar haemolytica. All *G. anatis* strains produced biofilm with slight variations not associated to origin of isolation (farm, type of tissue). Variations were also observed between strains in sugar metabolism (mannitol, rhamnose, sucrose, melibiose, and arabinose).

Confirmation PCR and 16S-23S sequencing. The 790bp, and 1080bp PCR amplicons characteristic of *Gallibacterium anatis* were observed in all isolates. The 16S-23S sequencing data was blasted to NCBI data, confirming identity. Several of the isolates were also selected for further analysis by WGS based on 16S-23S sequencing similarities and origin of isolation.

Whole genome sequencing. Whole genome sequences were assembled for each isolate using UMN 179 (Genbank accession CP002667) as a reference. The quality of sequence was Q>20 and the coverage was greater than 30X for each isolate. Each of the isolates had approximately 1000 structural variants and 30,000 single nucleotide polymorphims (SNPs). The isolates broke out into varying clades with a variation of 0.01 in 100 bases.

Histology of affected tissues. Segments of the oviduct, including magnum and shell gland had bacterial salpingitis seen as disseminated bacterial thrombi within the glands, associated with foci of inspissated secretory material within the glandular parenchyma and ducts. The oviduct lumen had clumps of coagulated egg components mixed with bacteria. The bacteria in the glands and the bacteria in the lumen were of uniformly small rods, about 1 x 2 in dimension. Clumps of egg component coagulum were composed of laminated layers of egg membranes, interspersed with multifocal to confluent colonies of bacteria, of the same morphology of that identified in the oviduct.

Spleen had acute diffuse necrotizing splenitis with necrosis of the white pulp. Liver had large zones of hepatocellular necrosis with intralesional bacteria of the same morphology as identified in the oviduct. The zones of necrosis were circumscribed by multinucleated giant cell macrophages. Large blood vessels within the zones of necrosis had cellular thrombosis. Kidney had acute glomerulitis seen as enlargement glomeruli due to swelling of mesangial cells.

DISCUSSION

G. anatis isolated from clinical cases in commercial poultry showing increased mortality and/or effects in egg shell quality were assessed for their phenotypic and genomic characteristics. Even though some differences were observed in biofilm formation and sugar metabolism, all *G. anatis* strains were genetically similar as evidenced by 16S-23S sequencing analysis and WGS. The break out into separate phylogenetic clades has been noted, but the scale on the tree does not demonstrate any significant difference between isolates. Slight variations in phenotypes due to environmental conditions should be considered and further evaluated.

The lesion pattern of lesions in the oviduct and the occurrence of bacteria of similar morphology in coagulated egg components and in viscera was consistent with primary bacterial salpingitis. Alternatively, the oviduct was infected by hematogenous route.

Control efforts in some cases included cleaning and disinfection of the housing environment, water line sanitation to reduce biofilm, as well as, autogenous *G. anatis* vaccination and vectored vaccine for infectious bursal disease. All of these methods seemed to reduce mortality and clinical signs associated with *G. anatis* infections.

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EVALUATING HATCHERY SPRAY VACCINATION

B. Jordan

Avian infectious bronchitis (IB) is a highly contagious upper-respiratory tract disease of chickens. The disease results in major economic losses to the poultry industry worldwide due to poor feed conversion, decreased egg production, predisposition to secondary infections and condemnation at the processing plant. Infectious bronchitis virus (IBV) is the causative agent of IB. More than a dozen serotypes and hundreds of variants of IBV exist worldwide, with some serotypes more worldwide in distribution; Massachusetts (Mass), 793B (4/91), QX, and Q1 are found in many poultry producing areas across Europe, Asia and South America. Conversely, some serotypes are very regional in distribution; the Arkansas (Ark) serotype, for example, is found almost exclusively in the United States. Why some serotypes spread rapidly and with high efficiency while others remain localized to certain regions or countries is not well understood, but it can be said that all serotypes possess the highly infectious nature necessary for transmission.

Vaccination against IBV occurs primarily in the hatchery. Since IBV vaccines are live viruses, they cannot be given *in-ovo* as they cause decreases in hatchability. Administering them to individual chickens by eye-drop would be the gold standard for efficacy, but is logistically not possible considering the scale of poultry production. Therefore, mass application by spray is the only realistic method of vaccinating the millions of chicks hatched each week. Indeed, hatchery spray application of IBV has been an industry standard for quite some time. Original spray cabinets were manually operated; a hatchery employee would slide a box of chicks into the cabinet, which would activate the spraying mechanism and dose the chicks with vaccine. As hatcheries became more automated, “over the line” systems were developed that would spray the chicks with vaccine as they moved down a conveyor belt after being separated, counted, and placed into chick baskets. Once the chick basket moved into position, an air driven pump would depress a plastic syringe that sits on top of the cabinet and is filled with vaccine. This action would force the vaccine out through the nozzle(s) that are pointed at the chicks in the basket. The basket would be continually moving along the conveyor belt through the spray and when it cleared the cabinet, the syringes would be pulled back by the same air driven pump and refilled with vaccine stock. This has been the state of the technology for the past 30 years.

Recently, researchers have begun to revisit the IBV vaccine application process as a possible point of failure of vaccination. There are many parameters that can affect spray application efficacy, including droplet size, spray pattern, application volume, number of nozzles, nozzle flow rate, pressure, and syringe effects to name a few. One of the primary points of mechanical failure in the spray system is the plastic syringes used to dispel vaccine. This can be overcome by careful monitoring and maintenance of the spray equipment, but the syringes are also a point of vaccine destruction. The turbulence and shearing forces created by the depression and pulling back of the syringe plunger can destroy over a log of virus titer, depending on application volume and number of syringes. Application volume can also affect vaccination, as applying IBV vaccines in small volumes (7mL) creates smaller droplets that never reach the chicks in the box. Applying IBV vaccine in larger volumes (14 or 21mL) increases the amount of vaccine reaching the chicks. However, both of these scenarios are nullified if the flow rate of the spray nozzles or the total number of nozzles is not also changed to accommodate larger volumes of vaccine per chick basket. Increasing nozzle flow rate will also increase droplet size, while increasing the number of nozzles and decreasing the flow rate of each nozzle will decrease droplet size.

Identifying the best operating procedure for applying IBV vaccine is not simple or standard. As is described above, all of the individual pieces of the spray system are dependent on each other; if you change one setting it impacts all of the others. In reality, many of the choices related to optimizing spray vaccination are controlled by the hatchery itself. A set number of chicks are hatched and processed each day, and the systems in the hatchery must operate fast enough to maintain an economical pace. Thus, line speed is the only factor that cannot be changed. If your hatchery runs faster, you must vaccinate your chicks faster and vice versa. And since the line speed is set, the first optimization needs to be application volume. Once the application volume has been decided, the number of nozzles and the flow rate of the nozzles can be adjusted to match line speed. The only adjustment then left to make is the pressure applied to the vaccine in the syringes so that they expel all of the vaccine in the time it takes

the chick box to move through the cabinet. When all of these individual factors have been adjusted together, the spray system can operate at peak efficiency.

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

VACCINES FOR H5 HIGHLY PATHOGENIC AVIAN INFLUENZA CLADE 2.3.4.4

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SUMMARY

Avian influenza (AI) is a highly contagious, agriculturally–relevant disease that can severely affect the poultry industry and the national food supply. The viruses vary widely in their pathogenicity and the ability to cause disease among birds. The viruses are divided into low pathogenic AI (LPAI) and highly pathogenic AI (HPAI) strains. HPAI viruses, especially H5 subtype strains, cause widespread morbidity and mortality in domestic bird populations. Furthermore, with an increasing number of human infections of HPAI of the H5 subtype, there is a growing concern that this virus could cause a pandemic. One strategy to control AI including H5 HPAI is the development of effective vaccines for use in poultry industry.

Recently, a major outbreak of HPAI was detected in wild birds and commercial poultry in the U.S. During December 2014-June 2015, 21 states reported HPAI detections in commercial premises, backyard flocks, and wild birds with Asian-origin, HPAI type A influenza viruses of H5N8, H5N2, and H5N1 subtypes. Genetic characterization of the isolates determined they belonged to clade 2.3.4.4 of the Asian-origin H5 AI nomenclature. Greater than 50 million birds died or were depopulated during this time, such that there is need and interest in developing vaccines against these viruses.

Studies were performed to assess the potential use of vaccines as a control mechanism should future outbreaks occur. Inactivated and recombinant vaccines were tested in multiple types of poultry. Results demonstrated variability in terms of efficacy and reductions in viral shedding after challenge. As predicted, the best vaccine in terms of protection and reduced shedding was the homologous virus containing the identical hemagglutinin gene found in the HPAI virus. Vaccine testing included an inactivated H5 reverse genetic-derived low pathogenic virus vaccine, recombinant alphavirus RNA particle H5 vaccine and a herpesvirus of turkeys expressing the H5 hemagglutinin gene, as options for the control of HPAI. An overview of the results from multiple vaccine-challenge studies with North American lineage H5 HPAI clade 2.3.4.4 will be discussed.

MAPPING OF MULTIDRUG RESISTANCE IN BACTERIAL ISOLATES OF NON-VIABLE CHICKEN EMBRYOS

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ABSTRACT

Environmental and food safety issues have emerged as a major public health concerns worldwide, as animal products may be contaminated with antibiotics and harmful bacteria. In May 2014, chicken industry of Canada implemented a policy of antibiotic reduction and responsible use in chicken farms. Emergence of antibiotic resistant strains of bacteria and a lack of knowledge regarding antimicrobial drug resistance patterns of the bacterial pathogen associated with infection in chicken are the biggest challenges in implementing antibiotic-free poultry farming strategy. We obtained yolk from non-viable chicken embryos at 21 days of incubation from three commercial hatcheries in Western Canada, and were able to screen through 267 different *Enterococcus* isolates and 170 *E.coli* isolates. MALDI-TOF Mass Spectrometry was used to identify the bacteria to the species level. Disk diffusion was performed according to Clinical Laboratory Standards Institute standards for 20 antimicrobial agents. Among *Enterococcus* isolates, 98.5% (263/267) showed multi drug resistance phenotypes. Interestingly, 72.66 % of these isolates were resistant to ≥ five drug classes. Most of the *Enterococcus* isolates were resistant to apramycin, lincomycin, tetracycline and triple-sulfa drugs; whereas only 3.31% of the isolates were resistant to Vancomycin. Multi drug resistant phenotypes were also found in 61.18% (104/170) of the *E.coli* isolates. Of note, all the *E.coli* isolates were resistant to ≥ one antimicrobial drug tested. The most common resistance phenotypes among *E.coli* were to amoxicillin-clavulanic acid, ampicillin, penicillin, erythromycin, tetracycline and triple-sulfa. Present data highlight the need of future surveillance, antibiotic alternatives and better bio-security measures to alleviate public health concerns and to reduce economic loses to the poultry industry.

INTRODUCTION

Antimicrobial drug resistance has become an increasing public health concern in worldwide. Increasing incidence of nosocomial infections due to *Enterococcus* species in particular vancomycin resistance Enterococci (VRE) are difficult to treat (7). Multiple antimicrobial resistance is not only a problem in human health issues but also in food animal as well (6). Selective pressure exerted by antimicrobial drug use has been the major driving force behind the emergence and spread of drug-resistance traits among pathogenic and commensal bacteria (1).

In poultry industry, antimicrobials are used as therapeutics, preventive medications and as feed additives. Thus the prevalence of antimicrobial resistance in poultry is not uncommon (4).Prophylactic administration of ceftiofur in hatching eggs was voluntary withdrawn in Canada after May 15, 2014 once a strong correlation was identified between ceftiofur-resistant *Salmonella enterica* serovar Heidelberg isolated from retail chicken and incidence of ceftiofur-resistant *Salmonella* serovar Heidelberg infections in humans across Canada. However, ceftiofur resistance phenotypes are still prevalent even after the withdrawal of ceftiofur use in poultry (3).

Bacterial infections including yolk sac infections in chicks during the first week of life causes significant economic loses in poultry industry. Therefore, identification of prevailing resistance phenotypes in poultry bacterial pathogens is essential for determining the future resistance pattern and implementing alternative therapeutics including vaccine and improved biosecurity measures to minimize economic loses to poultry industry.

Present study aimed at identifying the resistance phenotypes of major poultry enteric bacteria like *E.coli* and *Enterococci* isolated from Western Canadian hatcheries.

MATERIALS AND METHODS

Experimental samples. Yolk samples of non-viable chicken embryos at the end of 21 days of incubation of hatching eggs were obtained from three commercial hatcheries in Western Canada during 2013 and 2014. *Enterococcus* and *E.coli* isolates were recovered on Colombia blood agar (Oxoid Company, Napean, Ontario, K2G 1E8, Canada)) and identified using matrix assisted laser desorption ionization time-of-flight mass spectrometry (Bruker Daltonik). Isolates were then frozen in brain heart infusion broth (DIFCO®, Detroit, MI) containing 40% glycerol (Fisher Scientific, Fair lawn, New Jersey) containing cryo vials -80°C for further testing.

Antimicrobial sensitivity. Each isolate was streaked on Colombia blood agar and incubated overnight before antimicrobial drug susceptibility testing. Fresh cultures were then tested for disk susceptibility to 20 antimicrobial agents using Clinical Laboratory Standards Institute Standards (CLSI M100-S24 and CLSI Vet01-S2 July, 2013) recommended methods and interpretive criteria, and reference strains. *E.coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as reference strains for *E.coli* and *Enterococcus* spp. respectively. Inhibition zone diameters were measured using Biomic-2014-Microbiology Digital Image Analysis system. The 20 antimicrobials used in this project. These antimicrobials were categorized into 10 antimicrobial classes. Multi drug resistance was determined as resistance to ≥ three classes of antimicrobial drugs.

RESULTS

We found that all the *E.coli* isolates were resistant to ≥ one antimicrobial drug tested. About 97% of the isolates showed resistance to bacitracin, lincomycin, tylosin and vancomycin. The highest resistance phenotypes were to erythromycin (91.23%), penicillin (97.08%), and tetracycline (54.39%). More than 20% but less than 30% of the *E.coli* isolates were resistant to ceftiofur (29.82%) and gentamycin-10 µg (28.65%). About 25% of the *Enterococcus* isolates showed resistance to nine antimicrobials where highest resistance phenotypes were observed for apramycin, lincomycin, tetracycline, and triple-sulfa drugs. Only 3.31% of the isolates were resistance to vancomycin. Interestingly 98.5% overall *Enterococcus* isolates showed multi drug resistant phenotypes.

DISCUSSION

In this study, antimicrobial resistance, including multi-drug resistance phenotypes, was observed in higher magnitude in *E.coli* and Enterococcus spp. isolated from non-viable chicken embryos. We found a significant percentage (29.82% resistance in *E.coli* and 51.10% in *Enterococcus* spp.) of ceftiofur resistance in *E.coli* and *Enterococcus* spp. even though ceftiofur is already withdrawn from prophylactic administration to hatching chicks.

These resistant bacteria can be potentially transmitted to the hatching chicks, causing severe bacterial infections in chicks then becoming human health threat via contaminated poultry products. It is believed that some resistance generally persists at low level and reintroduction of the antimicrobial can reselect resistant strains despite months or even years of non-use. Resistant bacteria may rapidly appear in the host or environment after antibiotic use, but they are slow to be lost, even in the absence of the selecting antibiotic (5).

Increasing incidence of human nosocomial infections due to *Enterococcus* spp., in particular vancomycin resistance *Enterococci* (VRE), which are difficult to treat, is becoming one of the biggest health concerns these days. VRE infection rate has increased since 2008 in Canada (Canadian Antimicrobial Resistance Surveillance System-report 2015).

Although, according to published literature vancomycin resistance phenotypes were more prevalent in human isolates, we observed only 3.31% of *Enterococcus* isolates resistant to vancomycin. A study conducted in 2010 in British Colombia using *Enterococcus* isolates obtained from fecal and cecal contents of commercial poultry demonstrated that none of the *Enterococcus* isolates were resistant to vancomycin (2).

Overall, present study clearly demonstrated a high prevalence of multi drug resistant bacteria for commonly used antimicrobials in human medicine and food animal husbandry. These findings highlight the need for a close monitoring of resistant patterns in food animal bacteria and controlled use and prevention of misuse of antimicrobials in food animal practices.

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MANAGING METABOLIC HEAT© TO IMPROVE CHICK QUALITY AND DECREASE INCIDENCE OF BACTERIAL DISEASE IN BROILER CHICKENS: CASE REPORTS AND OBSERVATIONS FROM THE FIELD

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It has been estimated that with modern genetics, today's meat yielding birds produce up to 20% more embryonic heat than in recent years (1). This increase is can be attributed to genetic advancements that result in faster growing birds with higher breast meat yields. Consequently, the risk of overheating broilers during incubation and hatch is high. To ensure optimal broiler development, performance and health, it is critical to ensure that damage due to overheating is prevented. This is particularly important during incubation and the first 10 days of life when the chick is poikilothermic and unable to control its own body temperature. Overheating chicks during incubation or during hatch may initiate a cascade of events that may ultimately result in poor performance and increased incidence of bacterial disease. This article will summarize why Managing Metabolic Heat© is a new challenge that should be recognized as a concept critical to the success of modern broiler production for its implications regarding disease prevention, animal welfare, food safety and economic sustainability.

An intact and functional intestinal tract along with optimal yolk-utilization is critical, especially as production trends result in more flocks being raised without the use of antibiotics. Studies have shown that overheating can damage tight junctions in the intestinal tract, allowing bacteria to gain access to the blood stream, resulting in bacteremia. This may manifest as colibacillosis, *Enterococcus cecorum* osteomyelitis, hepatitis, etc.

Overheated chicks are often undersize with large, unabsorbed yolk sacs. The large yolk sacs often translate into unhealed, open navels and an increased number of cull chicks. Additionally, overheated chicks hatch early and spend too much time in the hatch baskets, resulting in dehydration along with excess meconium residues on egg shells (dirty hatch debris). Furthermore, excessively dirty hatcher trays make cleaning and disinfection a challenge and add to microbial load in the hatchery. The combination of dirty hatcher trays and unhealed navels can result in omphalitis, yolksacculitis and septicemia. Additionally, the large yolk sacs (>10% of total body weight) are not absorbed and internalized appropriately so the early nutrition and maternal antibody the yolk sac should be providing, may be deficient. Poor yolk utilization may result in chicks that appear pale due to deficient pigment uptake (lack of carotenoids from the yolk sac). Overheated chicks may be lethargic and have mobility issues later in life (splay legs, osteomyelitis).

Overheating can result in poor flock performance with mortality, morbidity, body weight, and feed conversion being compromised. The probability of diagnosing infectious disease and requiring treatment are increased in these flocks.

Managing Metabolic Heat effectively can result in the following:

- Smaller hatch window
- Cleaner hatch debris
- Reduced cull chicks
- Increased number of saleable chicks hatched
- Improved chick vitality on-farm
- Reduced seven day mortality
- Reduced mobility issues (osteomyelitis, 'splay legs')
- Improved overall performance (final weights, feed conversion, livability)
- Reduced condemnations

In conclusion, Managing Metabolic Heat requires a multifaceted approach and a high level of precision and accuracy is required. Managing Metabolic Heat protocols in the hatchery involve a constant, disciplined and dynamic approach to incubation, hatching and chick comfort. Monitoring and adjusting based on eggshell temperatures (embryo temperatures) is critical to success. Managing Metabolic Heat must be skillfully monitored and managed to prevent disease and improve both animal welfare and food safety.

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EFFECT OF *BACILLUS SUBTILIS* AND *BACILLUS LICHENIFORMIS* ON *CLOSTRIDIUM PERFRINGES* IN BROILERS

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INTRODUCTION

The purpose of this trial was to observe and evaluate the performance and lesions scores of *C. perfringens* challenged broilers fed diets supplemented with GalliPro® MS (*B. subtilis* and *B. licheniformis*) or GalliPro® Tect *(B. licheniformis).*

MATERIALS AND METHODS

Accommodation/management. The study was carried out at Virginia Diversified Research Crop's building no. six which has a wood and cinder block structure with a metal roof and clay floor. Each study pen contained one water fountain and a 34 kg capacity feed tube. The dimensions of the pens were 1.2 x 1.5 m (Note: Pens started with 33 chicks but the stocking density was then reduced to 30 birds/pen by random removal of three chicks on day 21 for lesion scoring). Each pen was set on an elevated (1 inch) slatted floor covered with a three m1 thick plastic sheet topped with approximately three inches of new wood shavings prior to day 0. On day four, birds were exposed to 1.8 kg of used litter sourced from healthy chickens not exposed to dietary probiotics or enzymes. Prior to trial start all pens were carefully inspected to ensure that there were no openings to allow for pen to pen migration by broilers. Continuous lighting was provided until day 46.

Animals. Male chicks (Cobb x Cobb) were obtained on the day of hatch from Cobb-Vantress hatchery. The birds were sexed at the hatchery and received routine vaccinations (IBD/Newcastles Disease and Cocci-Vac vaccines administered at hatchery). A total of 1980 chicks were allocated to the study. At study initiation 33 chicks were allocated to each treatment pen by blocks.

EXPERIMENTAL DESIGN

The experiment consisted of 60 pens starting with 33 broiler chickens. The treatments were replicated in 12 blocks, randomized within blocks of 5 pens each. The study duration was 46 days. Treatments are shown in Table 1.

Each feed type, starter, grower, and finisher was prepared from a single large basal diet blended with the supplements shown in Table 1. Treatment feeds were mixed at VDR to assure a uniform distribution of respective test article. The mixer equipment was thoroughly cleaned between the mixing of different treatments.

Birds received feed appropriate to the treatment from day 0-46. A change from starter to grower in which all previous feed was removed and weighed occurred on day 21. Grower diet was removed and weighed from each pen on day 35 and replaced with the finisher diet. At each feed change, feeders were removed from pens by block, weighed, emptied, and refilled with the appropriate treatment diet. On the final day of the study (day 46), fed was weighed.

Pen observations. All pens were checked at least daily during the study. Observations included availability of feed and water, brooder control for attainment of desired temperature. All abnormal findings were recorded. Birds within each pen were visually observed and those morbid were counted and that value was divided by number of live birds at time of observation to determine percent morbidity at day 35. Morbidity was the subjective observation of unthrifty birds.

***Clostridium perfringens* challenge**. On Day 16, a carefully titrated (for 10% mortality) *C. perfringens* culture (isolated from an active commercial poultry operation in the Shenandoah Valley of Virginia, USA that was developed on clostridia specific media) was propagated in meat broth media approximately 24 h prior to use on day 17.

On day 17, approximately 24 h after initiation of propagation in meat broth media, the determined titrated level of desired use of *C. perfringens* was ahieved by serial dilution in sterile nutrient broth. The inoculum concentration was 8E+-9 cfu/ml and applied in a volume of one mL per bird (8E+08 cfu/bird).

After the inoculum was prepared, at least one-hour (no more than two hours) water fast was begun for all five treatment groups. After the fast, all birds of the pens to be inoculated (all but those of the 12 uCon pens) were caught and each orally inoculated

by use of a gavage tube attached to a graduated syringe.

Clostridia lesion scores. On day 21, three birds from each pen were randomly caught, euthanized and the intestines were examined for clostridial lesion scores. Lesion scores were based on a 0 to 3 scoring system, with 0 being normal and 3 being the most severe.

In addition, observations during the scoring showing complete sloughing off of the intestinal mucosa (epithelia lining) where the villi were no longer present resulted in a "severe" score of 3.

Statistical analysis. Live weight, feed conversion ratios (FCR), morbidity, mortality, and necrotic enteritis (NE) lesion score response variables were evaluated by Randomized Complete Block ANOVA and means separated by Tukey HSD Test (Statistic 10, Analytical Software, Tallahassee, FL). The p-value of any comparison found to be less than or equal to 0.05 ($p \leq 0.05$) by this analysis was considered statistically different.

CONCLUSIONS

The iCON birds were significantly lower in body weight at day 21, 35 and 46 than the uCON birds, indicating that the challenge model worked. Additionally, the mortality in challenged birds at day 28 ranged from 4.72 to 11.67 % indicating the target mortality level from *C. perfringens* challenge was achieved.

At 21 days, the probiotic treatments had significantly higher body weight than iCON.

At days 28, 35 and 46, body weights were significantly lower in all challenged treatments compared to uCON, but did not differ significantly between the challenged treatments. The day 21 and day 46, feed/gain ratios were significantly lower for challenged treatments compared to uCON.

The day 28 and 46, FCR mortality adjusted was significantly lower for challenged treatments compared to uCON.

All three probiotic treatments had significantly lower day 21 NE lesions scores compared to iCON.

Day 21 mortality % was significantly higher for the GalliPro MS half dose treatment than for the uCON treatment. Mortality was un-affected by treatments at day 28 and 35. The day 46 mortality was significantly higher for iCON and GalliPro MS half dose treatments, than for uCON, with other treatments intermediate.

Morbidity at day 35 was numerically higher in iCON (45.23%) compared to probiotic treatment (22.17-23.20%).

Although bacillus probiotics have several modes of action the above results occurred primarily due to the *B. licheniformis* anticlostridial action. A sufficient number of these spores must be present for maximum effect.

Table 1. Experimental design.

Treatment	Test Article	Probiotic dose	C. perfringens
1	Control (uCON)	-	-
2	Infected control (iCON)	-	+
3	GalliPro® MS	1.28E+06 CFU/g	+
4	GalliPro® Tect	1.60E+06 CFU/g	+
5	GalliPro® MS half dose	6.40E+05 CFU/g	+

Feeds and Test Article

Table 2. Weight gain.

Treatment	Day 0* g	Day 21 g	Day 28 g	Day 35 g	Day 46 g
Control (uCON)	44.0	842.8 a	1545.0 a	1976.8 a	2705.3 a
Infected control (iCON)	44.0	725.3 c	1281.9 b	1623.4 b	2097.4 b
GalliPro® MS	44.0	785.2 b	1316.8 b	1688.8 b	2170.0 b
GalliPro® Tect	44.0	780.2 b	1301.4 b	1616.6 b	2115.6 b
GalliPro® MS half dose	44.0	788.8 b	1299.1 b	1691.5 b	2153.7 b
P value	-	<0.001	<0.001	<0.001	<0.001

*At placement prior to treatment initiation

Table 3. Feed conversion ratio.

	Day 21 FCR	Day 28 FCR	Day 28 FCR mort adj	Day 35 FCR	Day 35 FCR mort adj	Day 46 FCR	Day 46 FCR mort adj
uCON	1.232 a	1.595 a	1.584 b	1.783 a	1.732 b	2.022 b	1.946 b
iCON	1.390 b	1.794 a	1.757 a	1.781 a	1.898 a	2.654 a	2.467 a
GalliPro® MS	1.288 b	1.913 a	1.774 a	1.754 a	1.874 ab	2.490 a	2.339 a
GalliPro® Tect	1.276 b	1.782 a	1.756 a	1.795 a	1.913 a	2.501 a	2.365 a
GalliPro® MS half dose	1.307 b	1.884 a	1.755 a	1.795 a	1.883 a	2.622 a	2.420 a
P value	<0.001	0.159	0.007	0.156	0.005	<0.001	<0.001

Table 4. Necrotic enteritis (NE) lesion scores (LSC).

	uCON	iCON	GalliPro® MS	GalliPro® Tect	GalliPro® MS half dose	P Value
Day 21 NE LSC	0.250 c	2.583 a	1.306 b	1.306 b	1.472 b	<0.001

NOTE: Lesion scores based on a scale of 0 to 3 with 0 being normal and 3 representing severe necrosis from *C. perfringens*.

Table 5. Mortality.*

Treatment	Day 21 %	Day 28 %	Day 35 %	Day 46 %
uCON	1.010 b	2.499 a	5.833 a	6.789 b
iCON	2.525 ab	4.722 a	9.444 a	17.90 a
GalliPro® MS	2.778 ab	7.499 a	10.56 a	10.18 ab
GalliPro® Tect	2.525 ab	4.721 a	9.722 a	12.03 ab
GalliPro® MS half dose	5.303 a	11.67 a	16.11 a	17.90 a
P value	<0.001	0.083	0.142	0.031

NOTE: D0-39 Mort (%) did not separate by Tukey's test but did by LSD at 0.05 level as listed above (a, b, ab).

Table 6. Morbidity.

	uCON	iCON	GalliPro® MS	GalliPro® Tect	GalliPro® MS half dose	P Value
Day 35 Morbidity, %	2.587 a	45.23 b	22.31 ab	23.20 ab	22.17 ab	<0.001

CHARACTERIZATION OF RECENT INFECTIOUS BRONCHITIS VIRUS ISOLATES FROM BROILERS AND BACKYARD FLOCKS OF GEORGIA

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SUMMARY

Infectious bronchitis virus (IBV) field isolates of GA07 and GA08 variants from commercial broiler flocks during 2014-15 cases of clinical respiratory and renal disease were characterized by reverse transcriptase-polymerase chain reaction and sequence analysis of the hypervariable region HVR-3 segment of the spike glycoprotein gene. All the 2014-15 isolates of GA07, regardless of their origin, formed two distinct groups. However, none of the isolates from this group showed genetic identity with previously characterized GA-07 isolates from 2007-2013 cases. In contrast, all the GA08 variants from 2014 cases showed high percentage of identity with each other as well as with those reported earlier from 2008-09 outbreaks in Northeast Georgia. Results indicate that wide-spread use of vaccines against GA08 probably led to re-isolation of vaccine viruses with altered pathogenicity that have been circulating in the field over short period of time.

IBV is highly prevalent in most countries of the world that affects poultry and produces severe economic losses. The disease manifestations of infectious bronchitis (IB) are mainly respiratory, renal, and reproductive forms. In the United States, both respiratory and nephropathogenic forms have been isolated in several poultry producing area for the past several years. IBV belongs to the order of *Nidovirales*, family *Coronaviridae* and to genus of Gamma-coronavirus group 3. The S1 spike glycoprotein contains serotype specific virus neutralizing epitopes. This S1 sub-unit of the spike gene is highly variable due to insertions, deletions and substitutions and recombination events (2, 9, 11). Due to this variability in nucleotide sequences many serotypes exist. It has been shown that the sequences of the hypervariable (HVR) of the S1 gene, particularly the HVR3 region (amino acid resides 274-387) are associated with serotype-specific neutralizing epitopes (14). For the past few years, the Georgia poultry industry has experienced outbreaks of IBV due to four different variants, designated as GA98, GA 07 and GA08 (6, 8, 10). Both, GA07 and GA08 were responsible for nephropathogenic and respiratory illness in broilers. In this study we extend our previous observations on resurgence of GA07 and GA08 variants (5) to selected isolates from 2014-15 cases from clinically affected broilers and backyard flock (byf) specimens to show the evolving patterns of these variants that have now become established in the region.

MATERIAL AND METHODS

Preparation of sample and virus isolation. The trachea, lung and kidneys were homogenized and processed for RNA extraction. Oropharyngeal swabs from byf were processed for virus isolation in 9-11 day-old specific pathogen-free embryos (6) only after the initial confirmation by RT-PCR for the presence of IB-HVR3-specific RNA (see below).

RNA isolation and RT-PCR. Total RNA was extracted from allantoic fluid of each isolate using the Rneasy mini kit (Qiagen Inc., Valencia, CA) according to manufacturer's instructions. The amplification of the HVR3 region of the S1 glycoprotein gene as well as full-length S1 glycoprotein gene was performed using SuperScript-III- RT with *Taq* polymerase kit (Thermo Fisher Scientific Inc., Waltham, MA) using the primers described earlier (6).

The PCR-amplified fragment of the HVR gene was purified and sequenced using BigDye Terminator v3.1 cycle sequencing kit (Life Technologies, Grand Island, NY) at the University of Georgia's Genomic Facility (6).

Sequence analysis. Sequence information was compiled with the SeqMan program (Lasergene v9.0, DNASTAR, Madison, WI). The alignment of sequence data and the construction phylogenetic tree was performed by MegAlign program (v9.0, DNASTAR) using the Clustal W multiple sequence alignment algorithm (7). The IBV reference strains that were closely related to IBV variants by basic local alignment search tool (BLAST) were selected for analysis. These include previously isolated GA08 (GU437858, GU37864), GA07 (JB160805), CA/1737/04 (EU925393), and DMV/5642/06 (EU694400). Previously reportedGA07 and GA08

isolates were also included in the analysis. GenBank accession numbers for these isolates are as follows: KM660638, KP085602, KP085603, KP085591, KP085592, KP085598, KP085600, KM0660636, KM263434, KM434263, KM434260, KM660630, KM660632, and KM660634.

RESULTS

Recent GA07 isolates from broilers and byf. Phylogenetic analysis was performed on the nucleotide sequence of the HVR3 region gene of the ten representative GA07 isolates from broilers and byf specimens obtained during 2014-15, and 11 published IBV sequences. All the 2014-15 isolates of GA07, regardless of their origin, formed a distinct group with varying branch distances (Figure 1, Group 1 and 2). Group 1 isolates, despite short branched distance, were only 88-93% identical to each other at amino acid (aa) level. Interestingly, both the byf isolates from this group (15-507 and 15-525) shared >90% aa identity with broiler isolates. However, none of the isolates from this group showed genetic identity with previously characterized GA-07 isolates shown in Group 2 and Group 3 (Figure 1) as well as with the reference strains, DMV5642/06 and CA1737/04. In Group 2, two byf isolates (15-015 and 15-558) shared 88-94% aa identity with a single broiler isolate 15-569, and three previously reported GA07 variants from byf (KP085600, KP085598, and KM0660636) detected in 2010. However, the branched distances in the phylogenetic tree were longer than the Group 1 isolates, indicating that these isolates were somewhat similar to each other. Finally, Group 3 comprised of eight out of 11 reference sequences selected for comparison. Of these, three sequences from byf were clustered with original GA07 (JN160805) and CA1737/04, reported from Georgia and California, respectively. Another byf isolate, 15-515 did not belong to any of the groups described above. Finally, none of the isolates of GA07 from 2014-15 cases were clustered in Group 1

Recent GA08 isolates from broilers. All the eight representative GA08 variants from 2014 cases showed high percentage of identity with each other as well as with those reported earlier from 2008-09 (GU437858, GU437864, 1) and 2012-13 (KM263434, KM434260) outbreaks in Northeast Georgia. The nucleotide and aa identities were 95-100% and 89-100%, respectively (Figure 2). This suggests that similar isolates are circulating in the broiler populations of Northeast Georgia. Comparison of three representative GA08 variants from byf showed a wide variation in the similarity with broiler isolates. Both nucleotide and aa identity for this group ranged between 88-98% and 80-98%, respectively.

DISCUSSION

In this study, we examined the HVR3 region of the S1 gene sequence of IBV-GA07 and GA08 sequence data from broilers and byf and compared that sequence data with previously isolated viruses during 2008 through 2013. Since 2000 Georgia poultry industry experienced recurrent outbreaks due to IBV variants like GA07, GA08, and GA-13. All the GA07 isolates of 2014-15 showed little or no genetic identity with previously characterized isolates of 2007 and 2010-2013. Isolates from byf were formed at least 3 different lineages. Interestingly, all the five broiler isolates of GA07 were grouped with byf isolates. This contrasts our earlier observation on GA07 isolates from 2010-2013 cases (5), in that, both the broiler and byf isolates formed two distinct clusters in the same phylogenetic tree exhibiting varying degree of similarity with the reference strains. This lack of genetic identity with the earlier variants, and clustering of broiler and byf isolates described in this report, needs further explanation. Two characteristics of byf are important in the evolution of variants such as GA07. First, given that isolated byf populations are not coordinated operations like commercial poultry, it is unlikely that these birds will be exposed to similar group of variants at the same time. In such flocks genetically distinct population of GA07-like viruses may evolve as independent lineages over a period of time. Second, oropharyngeal swabs from byf were collected from the auctions and flea market sites, where apparently healthy birds are brought for swaps. Natural reservoirs for RNA viruses are known to harbor the most genetic diversity while showing no symptoms in the host (3). Different lineages of coronaviruses in such birds may represent naturally occurring variants in non-commercial poultry, which show a constant population growth. Thus a high percentage of genetic identity with previously characterized isolates as well as concurrently isolated variants at any given time in several auction-market sites seems unlikely. Nonetheless, these viruses fall into a broader phylogenetic group as that of broilers. Such viruses may get quickly adapted to genetically different host, such as commercial broiler, and cause the disease. In this respect, GA07-like viruses from isolated byf populations mimic bat coronaviruses which were found in more than one phylogenetic group (11); whether such diversity occurs in other genes of GA07 variants remains to be seen. Unlike GA07 isolate, GA08 isolates showed 95-98% aa identity with most isolates from 2008-2010

outbreaks. This apparent discrepancy between GA07 and GA08 isolates is probably caused by wide-spread use of live-attenuated vaccine against GA08 that many poultry producers started using from 2011 onward. It has been reported that, in the absence of specific vaccine, the mutation rate of the hypervariable region in the S1 gene is far lower than the one observed due to selection pressure following the usage of live vaccines (1, 8). Introduction of live attenuated vaccine against GA08 could have provided stable populations of GA08 variants in high density poultry producing areas. In a commercial operation, it is known that both, vaccinated and contact-exposed birds (birds not getting enough vaccine doses) give rise to molecular evolution that can lead to the selection of virulent viruses as evidenced by recurrent outbreaks due to GA08. Therefore it is important to evaluate unintended consequences of introduction of live attenuated viruses in high poultry density population regions unless absolutely needed.

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Figure 1: The deduced amino acid sequences of thirteen GA07 isolates from broilers and backyard flocks were aligned with Clustal W and the phylogenetic tree was constructed using the neighbor joining method (7). The analysis was conducted using DNAStar v9.0. GenBank accession numbers for reference strains CA1737/04 and DMV5642/06 are EU925393 and EU694402, respectively.

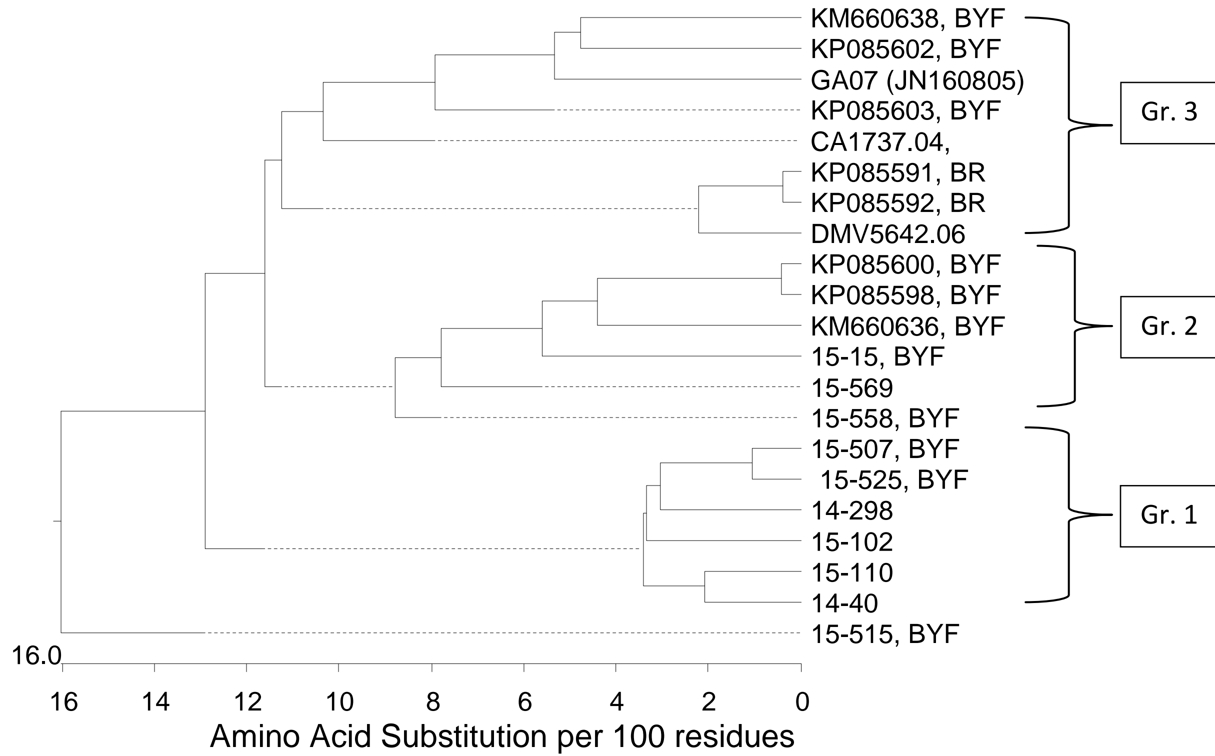

Figure 2: Percent S1 amino acid and nucleotide sequence identity values for the 2014 GA08 IBV broiler isolates and previously identified GA08 isolates from broilers and backyard flocks of Georgia.

% Amino Acid Identity																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
KM434263 [A]	1		98.7	96.5	95.2	**98.9**	**98.5**	**98.2**	**98.9**	**98.9**	**99.0**	**98.2**	**94.5**	96.3	90.8	84.4
KM434260 [A]	2	99.3		96.3	95.1	**98.9**	**98.5**	**98.2**	**98.9**	**98.9**	**99.0**	**98.2**	**94.5**	96.1	90.3	84.1
GU437858 [B]	3	97.3	97.5		98.0	**97.9**	**98.2**	**99.0**	**97.9**	**98.5**	**96.1**	**99.3**	**90.8**	98.2	92.2	85.9
GU437864 [B]	4	96.8	96.8	99.0		**96.9**	**96.8**	**97.9**	**96.6**	**97.3**	**95.1**	**97.9**	**89.8**	97.2	91.4	85.2
14-003 [C]	5	99.5	99.4	98.7	98.4		**98.2**	**97.2**	**98.6**	**98.9**	**100**	**97.2**	**94.5**	98.2	90.6	84.9
14-053 [C]	6	99.1	99.0	98.7	98.1	**99.0**		**97.1**	**98.2**	**97.6**	**97.5**	**97.5**	**91.4**	97.8	89.8	84.2
14-070 [C]	7	99.1	99.2	99.8	99.2	**98.6**	**98.4**		**96.9**	**97.7**	**96.9**	**99.7**	**92.1**	98.6	89.9	84.2
14-114 [C]	8	99.4	99.3	98.7	98.2	**99.3**	**99.0**	**98.5**		**98.1**	**97.9**	**97.3**	**92.1**	98.2	90.6	84.5
14-126 [C]	9	99.2	99.1	98.9	98.5	**99.5**	**98.8**	**98.6**	**99.1**		**98.9**	**97.7**	**97.7**	98.9	91.2	84.3
14-398 [C]	10	99.4	99.3	97.4	96.9	**99.8**	**98.6**	**98.5**	**98.7**	**99.4**		**96.6**	**93.1**	98.3	90.9	85.1
14-251 [C]	11	99.1	99.2	99.8	99.0	**98.5**	**98.4**	**100**	**98.5**	**98.6**	**98.1**		**91.4**	98.6	89.9	84.2
14-393 [C]	12	98.7	98.6	95.9	99.5	**98.7**	**97.2**	**97.1**	**97.5**	**99.6**	**98.0**	**96.8**		94.1	87.1	80.8
KM660632 [D]	13	97.0	97.2	99.1	98.4	98.7	98.3	99.3	98.6	98.9	98.6	99.3	97.9		92.2	85.9
KM660634 [D]	14	92.9	92.9	94.6	94.3	93.6	93.2	93.6	93.4	94.5	93.7	93.6	92.9	94.4		85.9
KM660630 [D]	15	85.2	85.2	86.8	86.4	88.9	88.2	88.9	88.5	89.0	89.1	88.9	88.3	86.9	87.2	
% Nucleotide Identity																

A GenBank accession ID. Representative broiler isolates from the outbreaks of 2010-13.
B GenBank accession ID. Representative broiler isolates of GA08 during 2008-2009
C Broilers isolates of 20014. Boldface type indicates the highest identity within this group.
D GenBank accession ID. GA08 isolates from the backyard flocks.

A VARIANT IBDV STRAIN-SK09 ISOLATED IN SASKATCHEWAN IS NOT AMENABLE TO CURRENT COMMERCIAL BROILER VACCINES

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ABSTRACT

In chickens, infectious bursal disease (IBD), also called Gumboro disease, is one the most important immunosuppressive diseases. Emergence of "variant" IBD virus (vIBDV) strains are of major concern and has been the cause of substantial losses in the poultry industry. In a previous study, we reported that a vIBDV strain-SK09 isolated in Saskatchewan can escape passive immunity as provided by the maternal antibodies. In this study, we investigated whether the active immunity induced by the current commercial broiler vaccines can provide protection against vIBDV strain-SK09. A group of broiler hatching eggs was vaccinated, at day 18 of incubation, with a turkey herpes virus-infectious bursal disease (HVT-IBD) vector vaccine by *in ovo* route. Another group, consisting of day old broiler chickens, were vaccinated with a commercially available live-attenuated IBD vaccine by the intramuscular route. All the chickens of vaccinated and non-vaccinated (control) groups received 3 $X10^3$ EID_{50} of vIBDV strain-SK09 at day six post-hatch. A randomly selected subset of each group (n=20) was sampled at days 19 and 35 post-hatch for bursal weight to body weight percentage (BBW) and bursal histopathology. The presence of live virus in bursa was confirmed by inoculating bursal tissue homogenate to specific pathogen free eggs and observed embryo lesions. The groups of broilers which received either HVT-IBD or live-attenuated vaccine had significantly lower BBW and severe lymphoid atrophy compared to non-challenged control group ($P<0.05$). Present study suggests that the current broiler vaccination programs are ineffective against vIBDV strain-SK09, thus there is an urgent need for new potent vaccine(s) against "variant" IBDV in Saskatchewan

INTRODUCTION

IBDV, a member of Birnaviridea, is a double stranded, non-enveloped, RNA virus which is a leading cause of immunosuppression in poultry. Hyperimmunization of broiler parents has long been practised as the main vaccine strategy for IBDV in broilers (2). The maternal antibodies transferred from parents are supposed to protect the broiler progeny from field exposure of IBDV. In a previous study, we reported that a vIBDV strain-SK09 isolated in Saskatchewan can escape passive immunity as provided by the maternal antibodies. In this study, we provide evidence that the active immunity induced by the current commercial broiler vaccines cannot provide protection against vIBDV strain-SK09.

MATERIALS AND METHODS

Challenge virus. A bursal homogenate derived field isolate of vIBDV strain SK09 was propagated in SPF chickens, titrated in embryonated SPF eggs used as the challenge virus in all experiments.

Vaccines. A commercially available rHVT-IBD vaccine (VAXXITEK® (Merial Canada inc, Baie-D'Urfe, QC) was used to vaccinate *in-ovo* route. Another commercially available vaccine MLV (Univax-BD, MERCK Animal Health) of IBD was used to vaccinate the one-day-old broilers with MAb

Birds and experimental design. Experiment 1 was designed to investigate the efficacy of *in ovo* administered recombinant herpes virus of turkey – infectious bursal disease vaccine. Fertile day 18 old broiler eggs purchased from a commercial hatchery were used for *in ovo* vaccination. The day old chicks hatched from the eggs received saline (un-vaccinated eggs), were allocated into 2 groups; Group 1= Negative control and Group 2= challenge alone. The birds hatched from rHVT-IBD vaccinated eggs, were

served as; Group 3 = rHVT-IBD alone and Group 4= rHVT-IBD + challenge (n=40). At six days of age Group 2 and 4 were orally challenged with 3 x 10^3 EID_{50} of vIBDV SK09 inoculum. Samples were collected at 19 and 35 days of age for serology, BBW, and histopathology.

Experiment 2 was designed to investigate the efficacy of an intermediate modified live vaccine (MLV). The one-day-old broilers with MAb received one dose (200µL) of vaccine or saline via the subcutaneous route. The birds who received saline were divided into 2 groups; Group 5= Negative control and Group 6= challenge alone. The birds vaccinated with MLV were served as; Group 7 = MLV alone and Group 8= MLV + challenge (n=40). Birds in Groups 6 and 8 were orally challenged with 3 x 10^3 EID_{50} viral particles of vIBDV strain SK09 inoculum at six days of age. Samples were collected at 19 and 35 days of age for serology, BBW, and histopathology.

RESULTS

Experiment 1. The efficacy of *in ovo* administered recombinant herpes virus of turkey–infectious bursal disease vaccine. IBDV un-challenged groups (Group 1and 3) had no evidence of a reduction in BBW or microscopical lymphoid depletions throughout the trial whereas IBDV challenged groups (Group 2 and 4) showed significant bursal atrophy in spite of the rHVT-IBD vaccination.

Experiment 2. Efficacy of an intermediate modified live vaccine (MLV). IBDV un-challenged groups (Group 5 and 7) had no evidence of seroconversion, reduction in BBW or bursal lymphoid depletion whereas IBDV challenged groups (Group 6 and 8) showed a substantial reduction in BBW and a severe lymphoid depletion despite IBD – MLV vaccination.

DISCUSSION

Economic losses caused by the IBDV-induced immunosuppression have been a common problem worldwide. Control of IBDV is one of the most difficult challenges in current poultry disease management (4). Although hyperimmunization of broiler breeders has been recognized as the most common strategy to control IBDV, some poultry producers combine broiler vaccination using live attenuated IBDV or vectored vaccine (1). Recently, we have shown that maternal antibodies passively acquired through current broiler breeder IBDV-vaccinations are not able to provide protection in chicks against vIBDV field isolate. The aim of the current study was to investigate whether active immunity as induced by the broiler vaccination can provide protection against vIBDV. Our study shows that despite the vaccination against IBDV, vIBDV strain SK09 challenge caused significant bursal atrophy and lymphocytic depletion in the birds vaccinated with commercially available IBDV vaccines. In this study, we evaluated two different commercial vaccines. Our results suggest that the immune responses produced by both vaccines are not able to prevent the viral pathogenesis and ultimately causing severe lymphoid degeneration and necrosis (3). Overall, this study demonstrates that rHVT-IBD and MLV vaccines are not efficiently controlling vIBDV strain SK09 infection in broilers and highlights the need for identifying antigenically appropriate vaccines for controlling circulating strains of vIBDV in Canada.

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ANTIGENIC AND IMMUNOGENIC CHARACTERIZATION OF INFECTIOUS BURSAL DISEASE VIRUS-LIKE PARTICLES PRODUCED BY A RECOMBINANT BACULOVIRUS CO-EXPRESSING PRECURSOR POLYPROTEIN AND VP4

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SUMMARY

In the global poultry industry, the control of Infectious bursal disease virus (IBDV) is based mainly on the immunization of chickens with live, inactivated, or recombinant vaccines. In many cases, oil-emulsion inactivated vaccines are manufactured using BF-derived IBDV antigen extracted from specific pathogen-free (SPF) chicks infected with virulent IBDV, since it is considered to be more immunogenic than egg-based or cell culture-based IBDV antigen. However, this process is time-consuming and requires the handling of infectious virus and a large number of SPF animals.

To overcome these limitations, various approaches for producing recombinant IBDV proteins *in vitro* have been developed to create alternative vaccines to the killed IBDV vaccine. In particular, recombinant vaccines based on VLP technology hold great promise for the development of highly efficacious vaccines, due to the intrinsic immunogenic properties as well as high safety profile of VLPs. One strategy for producing IBDV VLPs is the expression of precursor polyprotein (PP), where the VP4 protease drives the maturation process of VP2 and VP3, which self-assemble into VLPs. However, the production of VLPs resembling authentic IBDV has proved to be unsatisfactory due to inefficient processing and maturation. Another approach involves the co-expression of two structural proteins pVP2 and VP3 using two different recombinant baculoviruses, but a precise adjustment of the MOI for both viruses is required for efficient assembly of the VLPs.

In this study, we developed a VLP vaccine against IBDV by expressing a baculovirus in insect cells. To improve the self-proteolytic processing of PP of IBDV, VP4 protease of IBDV were co-expressed with the PP in insect cells by single recombinant baculovirus containing the PP and VP4 protein genes. As examined by ELISA and western blotting, simultaneous expression of PP and VP4 protease recombinant proteins resulted in increase in the yields of VP2 and VP3. Electron microscopic observation showed that the VLPs had an authentic IBDV structure in terms of morphology, and the size of the particles was approximately 60 nm. Moreover, The VLPs produced showed antigenicity close to naïve IBDV when tested by DAS-ELISA and a commercial IC kit.

In SPF chickens, vaccination with the recombinant VLP induced strong and uniform humoral immunity and provided complete protection against challenge with very virulent (vv) IBDV in SPF chickens (n = 12). As determined by the bursa of Fabricius (BF)/body weight (B/BW) ratio, the protection against post-challenge bursal atrophy was significantly higher ($P < 0.001$) in VLP-vaccinated birds than in non-vaccinated controls.

Collectively, the protective efficacy of the VLP vaccine was comparable to that of a commercially available inactivated vaccine, and possibly due to the presence of the additional VP4. The recombinant VLP merits further investigation as an alternative means of protection against vvIBD.

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

A NOVEL, TRANSLATIONAL, MULTIDISCIPLINARY APPROACH TO CONTROL POULTRY RESPIRATORY DISEASES IN THE UNITED STATES

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INTRODUCTION

Respiratory diseases continue to be a major concern to poultry producers because losses induced by respiratory diseases have significant local and national economic impact to the industry. Protection of poultry by effective prevention and control of diseases is critical to maintain wholesome poultry products, which is the number one animal protein consumed in the United States. Such efforts make a significant contribution towards national food security. The goal of USDA-NIFA funded Poultry Respiratory Disease Coordinated Agricultural Project (PRD-CAP) is to develop knowledge-based integrated approaches to detect, control and prevent endemic, emerging and re-emerging poultry respiratory diseases in the US.

OBJECTIVES

In this project, the efforts of multiple institutions across the country will concentrate on the following four specific objectives.

Objective 1: Understand the ecology of poultry respiratory diseases. The surveillance effort will be built upon the foundation laid by the existing multistate research program (NC-1180). Participants will gather disease surveillance information from each state and share the data among participants and institutions. A standardized and easily accessible data reporting and dissemination system will be developed. Metagenomic-based approaches will be incorporated to better understand the multi-etiological nature of respiratory diseases and also to assess the contributions of the commensal respiratory microbiota towards the establishment of disease.

Objective 2: Investigate the multifactorial etiology involving poultry respiratory diseases. Research on pathogenesis of respiratory diseases involves exploration of the intricate and complex interactions among pathogen, host, and environment. Collaborative efforts will be taken emphasizing contemporary approaches to understand multifactorial interactions of infections impacting respiratory disease of poultry. Participants will collaborate to determine co-infecting viral and bacterial agents involved in respiratory disease, and host status predisposing to disease.

Objective 3: Develop new and improved diagnostic tools, vaccines, and novel preventive measures. We will focus on validation of diagnostic tests, and development and commercialization of new vaccines and antibiotic-free antimicrobial approaches. Effort will be made for standardization of molecular diagnostic tests among laboratories. Novel vaccines for viral pathogens and small molecule-based alternative control strategies for bacterial pathogens will be developed.

Objective 4: Educate stakeholders for prevention and control of respiratory diseases. Our efforts will focus on translating research findings into practices that will reach both industry and government stakeholders under real world conditions. We will work closely with producers to adopt and implement effective intervention strategies and evaluate their effect on overall respiratory disease prevalence. Extension activities will be designed to work with individuals and groups, including educational programs developed to help producers better understand new management practices as well as preventative and emergency procedures so that appropriate measures can be taken to reduce disease risk and transmission of disease agents. Relevant government stakeholders will be engaged in establishing standardized test protocols, and in validating and applying newly developed diagnostic tests and vaccines.

INDIVIDUAL PROJECTS

Currently, 35 investigators from 10 different institutions around the U.S. are involved in 18 individual projects. Two-thirds of the projects is basic and applied research projects and one third is extension and outreach projects. Detailed information on individual projects can be found at PRDCAP.COM.

YEARLY REQUEST FOR SUBMISSION OF PROPOSALS

The purpose of the Annual Grant Competition is to stimulate new and innovative research in critical areas of poultry respiratory disease which are not included in the current proposal and also to support expansion of the most successful activities suggested in this proposal that has the merit to be expanded further for validation, commercialization, nation-wide activity, etc. The priority will be given to innovative extension and outreach activities and highly applied research that will generate direct impact in short period of time. Two proposals (maximum of $50,000 per award) will be selected upon the recommendations of the advisory board.

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MALARIA CONTROL VIA COCCIDIOSIS CONTROL IN COMMERCIAL POULTRY

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Exposure to mosquito bites repeated at monthly intervals to protect human volunteers against subsequent heavy challenges of *Plasmodium falciparum* (1) has established the essential role of live vaccines for malaria control. However, there is still no successful use of such live vaccines in the field reported to date. Unlike malaria control, a single dose of live vaccine with repeated parasite exposure through natural parasite cycling (recycling) have been used for the immune protection of commercial poultry against coccidiosis for decades. The success of these live vaccines is self-evident by the billions of doses used uneventfully every year, in protecting commercial chickens and turkeys against coccidiosis.

The commercial poultry industry was fortunate that coccidiosis researchers, such as Dr. Allen Edgar (2), recognized more than half a century ago that live vaccines were effective in the control of coccidiosis. However, these vaccines were used later on mainly on breeder flocks when breaks became more frequent. It was not until a new approach of administering low level of oocysts suspended in an edible gum (carrageenan) was devised to achieve uniform exposures (3) that live coccidiosis vaccines were accepted for use in both breeder and broiler flocks. Since then, this increased use of live coccidiosis vaccines relying on various methods of achieving uniform exposure have met with such success that now billions of doses are used on commercial chickens and turkeys each year.

Useful findings derived from the uses of live coccidiosis vaccines are:

1) Two or more exposures to the complete parasite life cycles were needed for immune protection. This has been observed as early as 1987 (4). Even when just one application of vaccine was used on each flock as a general practice to save cost, protective immunity was not in evident until after two life cycles were completed (5).

2) Almost all existing or available coccidiosis vaccines are multivalent or multi-species vaccines. Therefore, most likely multivalent, either multi-species or multi-strain vaccines should work equally well for live malaria vaccines, as long as they are exposed simultaneously and repeated at least once.

3) These repeated exposures can be telescoped backward to consecutive days as shown in Tables 1 and 2 (6) and to achieve protective immunity faster.

4) When drug sensitive oocysts were used, these live vaccines can be used to salvage one or more anticoccidials drugs that had become obsolescent because of the emergence of drug resistant strains in the field. And

5) The commercial poultry industry is so comfortable in using live vaccines for coccidiosis control, it almost go unnoticed that there has been no new anticoccidials drugs being introduced for the past 24 years .If live malaria vaccines are as successful as coccidiosis vaccines, if they are good for nothing else, there may not be a need to search for anymore new antimalarial drugs.

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Table 1: Induction of early immunity in *E. acervulina* by repeated gavages of low doses of oocysts

Treatment Groups	Challenge/Hours Post vaccination	Averaged duodenal Lesion scours
Controls	(+) 114	2.5 (N=20)
3-Gavages (Inoculations)	(+) 114	2.6 (N=20)
Controls	(+) 120 (5 days)	2.4 (N=20)
3- Gavages (Inoculations)	(+) 120 (5 days)	1.7 (N=20)

Table 2. Induction of early immunity in chickens against mortality with the same dose *of Eimeria tenella*

Treatment group	Days of Challenge	# Dead birds/total	% Mortality
Control unvaccinated	7	15/20	75%
3 Gavages	7	5/20	25%
Control unvaccinated	9	13/20	65%
3 Gavages	9	1/20	5%

DETECTION OF H5N2 HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS ON INFECTED LAYER FARMS IN MINNESOTA AND IOWA

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SUMMARY

The Minnesota and Iowa commercial poultry industries experienced great losses during the 2015 H5N2 highly pathogenic avian influenza (HPAI) outbreak in the United States. To evaluate the extent of HPAI virus distribution on infected farms, 930 environmental samples were taken from bird and non-bird areas of infected Minnesota and Iowa layer farms during various points in the depopulation and cleaning and disinfection (C&D) processes (including heat and formaldehyde trials in certain barns). Samples were tested for the presence of H5Nx viral RNA using real-time reverse transcriptase polymerase chain reaction (rRT-PCR). Manure-associated barn locations, egg belts, and barn floors were, on average, the three areas sampled with greatest RNA amplification; mean cycle threshold (Ct) for gloves/dust masks found on infected farms was second highest of all general locations. Neither heat nor formaldehyde treatments appeared to substantially reduce amplification of RNA compared to routine C&D. A statistically significant difference was observed between mean Ct values for samples taken on farms with high vs. normal mortality. All rRT-PCR-positive samples were virus isolation negative. Our results may aid in guiding C&D efforts and methodology on HPAI-infected farms, and also support active surveillance for early detection of infection to reduce viral contamination of the farm environment.

INTRODUCTION

Between January and June 2015, H5N8 and H5N2 HPAI affected 49.7 million birds in 211 commercial poultry flocks and 21 backyard flocks in nine states (10). The two most severely affected states were Minnesota (MN) and Iowa (IA), with losses comprising nine million birds on 110 farms in MN and 31.5 million birds on 77 farms in IA (2, 3, 10).

In infected chickens, HPAI viruses are shed in high concentrations in respiratory secretions, feces, and via feathers (4, 7). H5N1 virus has been isolated from the trachea and feces of infected chickens at concentrations of $10^{3.5}$/mL and >$10^{4.5}$/mL, respectively (5). Fecal virus titers were found to be upwards of 10^9 median embryo lethal dose (ELD_{50})/g in white leghorn hens experimentally infected with the 1983/1984 Pennsylvania H5N2 HPAI strain (1). A median titer of $10^{6.5}$ median tissue culture infective dose ($TCID_{50}$)/0.1mL has been reported for H5N1 HPAI in chicken feathers (4). H5N2 HPAI has been isolated in the following ranges from eggs laid by non-vaccinated and sham-vaccinated layers: 0.97 to $10^{5.9}$ median embryo infective dose (EID_{50})/mL eggshell surface; 0.97 to $10^{6.1}$ EID_{50}/mL in albumin; and 0.93 to $10^{4.8}$ EID_{50}/mL in yolk (8). A separate study reported virus concentrations >10^4 ELD_{50}/mL in the yolks and albumen of eggs laid by H5N2-experimentally infected chickens (1). Thus, opportunities for contamination of the egg layer barn and associated environments abound.

The purpose of this study was to assess the extent of HPAI viral RNA distribution in various bird (biologic) and non-bird (non-biologic) locations of infected MN and IA layer farms, and the effect of heat and formaldehyde decontamination (decon) treatments on presence of viral RNA. Findings could be used to guide environmental sampling prior to restocking, and focus C&D within HPAI-infected barns. Additionally, this study examined the difference in Ct values in barns with normal vs. high mortality at the time of depopulation, as a proxy for virus shed by birds and contamination of the barn environment.

MATERIALS AND METHODS

A total of eight H5N2 HPAI-infected farms were conveniently chosen for sampling by the veterinarians performing sample collection. In an effort to first determine optimal sampling for virus detection, samples taken from barns not subjected to decon treatments were collected randomly inside barns, egg processing, the feed mill, and outdoors on farm property. From these same "proof-of-concept" premises (POCP), samples were collected from barns with confirmed infection and barns that were not known to be infected. Sample location sites were characterized as biologic and non-biologic and further categorized as follows: barn other, cage, door, egg belt, egg processing, exterior, fan, feed mill, feed trough, floor, manure associated, office, and wall; gloves and dust masks found on farms were also sampled. POCP barns received dry cleaning with or without additional wet cleaning and disinfection depending on the facility, and did not receive any of the subsequent decon treatments described below. HPAI detection dates for these POCP barns ranged from April 19 to May 26, 2015, with sample collection occurring between May 4 and July 10, 2015.

Once the optimal sampling sites were determined, barns on three of the eight farms underwent decon treatment trials; one infected barn on each of the selected farms was assigned randomly to the heat decon, formaldehyde decon, or no decon (control) treatment. Environmental sample locations by farm were intended to match the optimal locations determined by the POCP testing and also to be consistent across the heat decon, formaldehyde decon, and control barns as much as possible. The treatment barns on Farm 1 received a preliminary dry clean and initial environmental samples were taken prior to treatment (pre-trial); on Farm 2, trial barns were initially swabbed while dirty, and then underwent a preliminary dry clean followed by treatment; and on Farm 3, treatment barns were fully dry cleaned at the time of initial environmental sampling, and then the decon treatment was applied. Heat treatment consisted of increasing the temperature in the assigned barns to 90-100°F with portable propane heaters for seven days. Formaldehyde treatment consisted of application of formaldehyde as a 4% solution of formaldehyde gas in water. Re-sampling (mid-trial) in heat treatment, formaldehyde, and control barns occurred 3-4 days after 90°F had been reached in the heat-treated barns. Final sampling (post-trial) on heat, formaldehyde, and control barns was performed seven days after 90°F had been reached in heat treatment barns. Dates of HPAI detection for trial barns occurred between April 19 and May 5, 2015, with sample collection occurring between June 4 and July 1, 2015.

Environmental sampling technique included immersion of 4" x 4" gauze sponges (Oasis Medical, Glendora, California) in approximately 20 mL of buffered peptone solution (Hardy Diagnostics, Santa Maria, California) in a quart-sized Ziploc zipper storage bag (S.C. Johnson & Son, Inc., Racine, Wisconsin). While wearing non-sterile exam gloves, a gauze square was removed from the bag, used to swab the sample location, and enclosed in a labeled Whirl-Pak (Nasco, Fort Atkinson, Wisconsin). Exam gloves were removed and changed between individual sample collections. Samples were stored at 4°C for less than 48 hr and transported to the Mid-Central Research and Outreach Center, Avian Research Center (Willmar, MN) for influenza A virus (IAV) matrix gene rRT-PCR testing. The IAV rRT-PCR Ct value results were categorized as positive if Ct $\leq$ 36.99; intermediate if 37 $\leq$ Ct $\leq$ 39.99; and negative if Ct $\geq$ 40 or no Ct obtained. For purposes of performing statistical analyses, if a sample was negative with no Ct obtained, it was arbitrarily assigned the value of Ct = 40.976 (the average of the three highest recorded Ct values greater than the negative cut-off value of 40).

RESULTS

A total of 930 samples were collected: 628 samples from POCP barns and 302 samples from decon treatment barns. There were 543 positive, 31 intermediate, and 356 negative rRT-PCR results. Mean Ct values were calculated for general sampling location. Results are available in Table 1. Manure-associated barn locations, egg belts, and barn floors, were, on average, the three barn areas sampled with greatest RNA amplification; mean Ct for gloves/dust masks found on infected farms was second highest of all general locations.

Mean Ct values were calculated for the pre-, mid-, and post-trial samples in the control, heat, and formaldehyde decon treatment barns. Results are available in Table 2. Baseline Ct values for each trial farm were found to be statistically different, and so no comparisons of mean Ct values were made between barns on different farms. Pre- and post-trial mean Cts were compared for each barn sampled. No statistically significant differences were seen in mean Ct values between pre- and post-trial samples for any of the heat decon or control barns. The formaldehyde-treated barn on Farm 1 did show a statistically different mean Ct value between pre- and post-trial samples (p-value <0.0001), however, the post-trial Ct value was still positive.

Mean Ct values also were calculated for all samples taken on farms with high vs. normal mortality at the time of HPAI detection. High mortality farm samples had a mean Ct of 32.23 (std. dev. 3.70; range 23.48 – 42.06) and normal mortality farm samples had a mean Ct of 40.62 (std. dev. 1.61; range 26.39 to no Ct). A two-sample t-test to evaluate the difference of means was performed, and this difference was found to be statistically significant (p-value <0.0001).

All samples with Ct values <40 were confirmed to be negative for viable virus by virus isolation in accordance with United States Department of Agriculture, Animal and Plant Health Inspection Service environmental sampling and restocking guidelines (9).

DISCUSSION

With the exception of the feed mill and farm office, results of environmental sampling indicate that all biologic/bird areas of the farm, as well as egg processing areas exterior to barns, do become heavily contaminated with virus during an HPAI outbreak. When considering evaluation of the efficacy of C&D of the farm, viral RNA detection alone has limited utility. A negative rRT-PCR test is valuable in indicating absence of virus (whether viable or inactive). Especially in a high mortality situation where contamination of the farm environment is extensive, a positive Ct value has limited usefulness in assessing C&D practices. In this study, neither heat nor formaldehyde decon treatments appear to have a significant effect on the quantity of viral RNA within barns. Only one formaldehyde-treated barn had a statistically different mean post-trial Ct value compared to the pre-trial mean Ct, and the post-trial Ct value was still positive. However, only certain disinfectants are effective at both inactivating HPAI virus and preventing virus amplification by rRT-PCR. While phenolic disinfectants, a quaternary ammonia compound, a peroxygen compound, and sodium hypochlorite (bleach) were all found to effectively inactive avian influenza virus in one study, only the peroxygen compound and bleach could also render viral RNA undetectable by rRT-PCR (6). One of the limitations to this study was the use of adaptive sampling throughout the course of the outbreak. In the future, sample locations, quantity, and timing should be standardized and systematic throughout the trials, and not adjusted during a trial. Serial rRT-PCR and viral isolation assays of matched samples would have allowed for better determination of the ability of heat and formaldehyde to disrupt viral RNA beyond amplification and inactivate virus in our study.

As there was a statistically significant difference noted between mean Ct values for samples taken from farms with high vs. normal mortality at the time of disease detection, our findings support that early detection of HPAI on layer farms coincides with decreased viral contamination of the farm environment. Producers should be encouraged to institute active surveillance for clinical signs, changes in feed/water intake and egg production, as well as daily testing of mortality for HPAI infection. These steps will not only support disease control, but also business continuity for the operation.

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

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Table 1. Mean Ct values of all 930 samples by sampling location.

General sampling location	Number of observations	Mean Ct value	Standard deviation	Minimum	Maximum
Barn other	19	35.38	5.65	27.90	No Ct*
Cage	18	38.88	4.83	25.65	No Ct
Door	9	35.09	4.57	29.75	No Ct
Egg belt	14	33.33	5.55	25.97	No Ct
Egg processing	24	40.40	1.51	34.46	No Ct
Exterior	24	40.07	2.44	33.65	No Ct
Fan	116	37.09	3.88	28.57	No Ct
Feed mill	9	No detectable Ct value obtained for any sample			
Feed trough	100	35.10	5.07	27.37	No Ct
Floor	99	34.40	5.32	26.08	No Ct
Gloves/dust masks**	20	32.52	2.84	28.82	No Ct
Manure associated	175	32.27	4.81	23.48	No Ct
Office	5	No detectable Ct value obtained for any sample			
Wall	96	36.55	4.09	26.47	42.06

*No Ct = No detectable Ct value obtained.

**Gloves/dust masks = gloves and a dust mask collected from barns undergoing cleaning and disinfection (exact barn locations unknown).

Table 2. Mean Ct values for pre-, mid-, and post-trial samples in the control, heat, and formaldehyde decon treatment barns.

<table>
<tr><th>Farm</th><th>Sample time</th><th>Control</th><th>Heat</th><th>Formaldehyde</th></tr>
<tr><td rowspan="3">1</td><td>Pre-trial</td><td>30.56</td><td>30.89</td><td>29.82</td></tr>
<tr><td>Mid-trial</td><td>31.37</td><td>31.06</td><td>34.24</td></tr>
<tr><td>Post-trial</td><td>Not performed</td><td>31.81</td><td>33.53</td></tr>
<tr><td rowspan="3">2</td><td>Pre-trial</td><td>37.02</td><td>35.32</td><td>31.89</td></tr>
<tr><td>Mid-trial</td><td>38.17</td><td>36.28</td><td>33.61</td></tr>
<tr><td>Post-trial</td><td>36.83</td><td>36.63</td><td>34.29</td></tr>
<tr><td rowspan="3">3</td><td>Pre-trial</td><td>No Ct value obtained for any sample</td><td>40.85</td><td>Not performed</td></tr>
<tr><td>Mid-trial</td><td>No Ct value obtained for any sample</td><td>No Ct value obtained for any sample</td><td>Not performed</td></tr>
<tr><td>Post-trial</td><td>No Ct value obtained for any sample</td><td>40.76</td><td>Not performed</td></tr>
</table>

*No Ct = No detectable Ct value obtained.

OUTBREAK OF *SARCOCYSTIS FALCATULA* IN OUTDOOR HOUSED COCKATOOS

G.A Lossie, P. Wakenell, W Wigle, and T.L Lin

INTRODUCTION

There are at least six species of Sarcocystis that infect birds, with *S. falcatula* appearing to be the most significant in susceptible birds (3). The North American opossum is the definitive host, with a wide range of intermediate hosts from multiple orders including Psittaciformes, Passeriformes, and Columbiformes (1), with cowbirds and grackles as the natural intermediate host (3). Transmission to the intermediate host is often via insects such as flies and cockroaches (1). In aberrant intermediate hosts, *S. falcatula* causes acute pulmonary disease with severe dyspnea and often acute death (1). Cardiac disease can occur if cysts localize in the myocardium (1).

CASE HISTORY

Over a one month period from 9/18/15-10/7/15 three cockatoos (one Galah and two Major Mitchell's) were submitted to the Indiana ADDL for necropsy. All three birds were kept in the same outdoor enclosure at a public zoo. One cockatoo lost 60 g over a three week period prior to death with no other signs of illness/disease being reported.

GROSS NECROPSY

The first bird that was submitted had the following gross findings: serous atrophy of fat, hepatomegaly, splenomegaly, pulmonary edema, and hydroperitoneum. The second bird had pectoral atrophy, hepatomegaly, and splenomegaly with the third bird exhibiting: ascites, hepatomegaly, splenomegaly, hydropericardium with focal epicardial hemorrhage, and pulmonary edema.

HISTOPATHOLOGY

Histopathologic findings on the first bird were consistent with lymphosarcoma of the liver and spleen, pulmonary congestion and edema, and skeletal sarcocystosis. Findings from the second bird revealed: interstitial and parabronchial pneumonia with intra-endothelial protozoal schizonts, hepatic-sinusoidal-leukocytosis, pericarditis and epicarditis with intra-endothelial protozoal schizonts, nephrosis, airsacculitis, and skeletal sarcocystosis. Intra-endothelial schizonts measured approximately five µm in width and 25 µm in length and contain numerous small, basophilic, spherical merozoites. Histopathology from the third bird revealed: necrotizing hepatitis, splenic necrosis, epicarditis, interstitial pneumonia with suspect intra-endothelial protozoal schizonts, skeletal myonecrosis, airsacculitis, anterior uveitis, meningitis, and encephalitis.

FURTHER DIAGNOSTICS

Based on the histopathologic findings of the second case, non-stained slides of the pulmonary tissue from all three birds, at the zoo's request, were sent to the California Animal Health and Food Safety Laboratory System for polyclonal Sarcocystis IHC. Pulmonary tissue from all three birds was positive for *Sarcocystis* spp. (Fig 1). A diagnosis of Sarcocystosis was made with *S. falcatula* being the most likely *Sarcocystis* spp. based on gross and histopathologic findings.

FOLLOW UP

Remaining birds within the aviary were started on prophylactic treatment for Sarcocystis. It was recommended that insect populations (roaches and flies) and exposure to wild possum feces be addressed.

DISCUSSION

This case demonstrates the importance of preventing exposure of captive aviary birds (particularly old world psittacines) to wild animals and insects. Monitoring and elimination programs for insects and nuisance wildlife must be in place to keep birds housed in outdoor aviary systems healthy.

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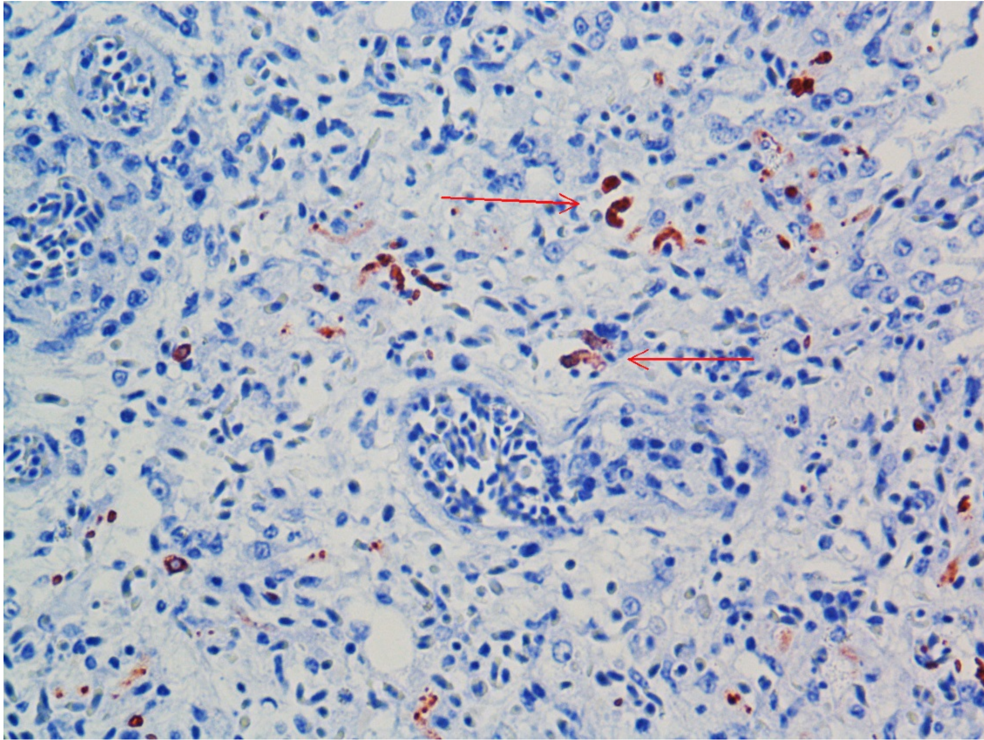
Figure 1. Immunohistochemical staining of pulmonary tissue. Red arrows highlight intra-endothelial *Sarcocystis* spp. schizonts (brown/orange in color).



DETERMINATION OF THE INJECTION DEPOSITION SITE IN CHICKEN EMBRYOS BY A NOVEL *IN OVO* INJECTION SYSTEM

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Ceva Animal Health – France[A]; Spain[B]; Hungary[C], South Africa[D]

ABSTRACT

A novel *in-ovo* vaccination method with a dual pressure injection system (EGGINJECT®) was used to evaluate under field-like conditions the *in ovo* injection deposition site in the chicken embryo compartments at three different Embryo Development Levels (EDL) (17.5; 18.5 and 19 days of incubation) using three different broiler breeder hen ages (28; 45 and 56 weeks of age) for each EDL evaluated. Embryonating chicken eggs were randomly distributed among the nine treatment groups designed in a 3×3 table design study. A total of 4,050 embryonating eggs were evaluated for the injection deposition site, conducted by three different teams, assigning randomly egg trays (holding 150 embryonating eggs each) to each team for each one of the three EDLs corresponding to each breeder hen age. Each treatment group (breeder age and EDL) had 450 eggs evaluated. The injection deposition site classification in this study was divided into three embryo compartments: Air chamber/undefined, intra-embryonic, and external-embryonic deposition. A logistic model analysis for the independent variables, EDL and breeder flock age, was used for the evaluation of the embryo deposition site. Results of this study will be presented.

ASSESSMENT OF THE SEROLOGICAL RESPONSE INDUCED BY A FOWL POXVIRUS VECTOR

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ABSTRACT

An evaluation of the serological response induced by a recombinant fowl poxvirus (FPV) vectored vaccine carrying the gB gene of the infectious laryngotracheitis virus (ILTV) (rFPV-LT) was conducted in specific-pathogen-free (SPF) chickens to assess, under controlled conditions, the kinetics of the humoral immune response to the FPV vector and the expressed envelope surface glycoprotein B of the inserted gene of ILTV. Two serological tests were used for the detection of the humoral immune response to the FPV vector and the glycoprotein B expressed from the ILTV gene inserted in its genome. An indirect Elisa prepared in-house for FPV and an indirect immunofluorescent assay (IFA) were used to detect the antibody response specific for FPV. Also, a commercial indirect Elisa for the detection of antibodies to ILTV and IFA to detect the antibody response specific for ILTV were used in this study. Birds from the same hatch and source were randomly distributed into four groups and vaccinated via subcutaneous route, as follows:

Group 1: Non-vaccinated negative control
Group 2: Vaccinated at day of age
Group 3: Vaccinated at 28 days of age
Group 4: Vaccinated at 1 day of age and 28 days of age

Each vaccinated group had in total 15 birds. Samplings were conducted on a weekly basis until seven weeks of age. Using indirect ELISA and IFA, FPV-specific antibodies were detectable beginning at 14-21 days post-vaccination in chickens vaccinated at one day of age, and beginning at seven days post-vaccination (35 days of age) in chickens vaccinated at 28 days of age. A secondary antibody response to FPV characterized by an increase in antibody titer was observed in chickens vaccinated twice at one and 28 days of age. ILTV-specific antibodies were detectable using the Synbiotics ELISA beginning at 14 days post-vaccination in chickens vaccinated at one day of age, beginning at seven days post-vaccination (35 days of age) in chickens vaccinated at 28 days of age. These findings demonstrate immunogenicity of the rFPV-LT vaccine.

OBJECTIVE

The objective of this study was to evaluate specific antibody responses to FPV and ILTV in specific-pathogen-free chickens after vaccination with a fowlpox vector vaccine (Cevac Vectormune FP-LT).

MATERIALS & METHODS

A total of 60 specific-pathogen-free chickens were used.

A fowl poxvirus vectored vaccine carrying the glycoprotein B gene of ILTV (Vectormune®FP-LT) was used for this evaluation. The vector vaccine was administered at full dose by the sub-cutaneous route.

Birds were randomly distributed into four treatment groups, as follows:

Group 1: Negative control
Group 2: Vaccinated at day of age
Group 3: Vaccinated at day 28
Group 4: Vaccinated at day of age and at 28 days of age.

Weekly blood sampling was performed starting at day seven until day 49 of life.

The tests used were in-house ELISA for the fowl poxvirus vector; an indirect immunofluorescent assay specific for either fowlpox virus or laryngotracheitis virus; a commercial ELISA (Fowl Laryngotracheitis Antibody Test Kit - Synbiotics) specific to detect antibodies to Laryngotracheitis virus.

RESULTS

Using indirect ELISA and IFA, FPV-specific antibodies were detectable beginning at 14-21 days post-vaccination in chickens vaccinated at one day of age, and beginning at seven days post-vaccination (35 days of age) in chickens vaccinated at 28 days of age. A secondary antibody response to FPV characterized by an increase in antibody titer was observed in the treatment Group 4 where chickens were vaccinated twice at one and 28 days of age. ILTV-specific antibodies were detectable using the a commercial ELISA (Synbiotics) beginning at 14 days

post-vaccination in chickens vaccinated at one day of age, beginning at seven days post-vaccination (35 days of age) in chickens vaccinated at 28 days of age. These findings demonstrate immunogenicity of the rFPV-LT vaccine.

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Fig. 1. Percentage positive of FPV-specific antibody detected in rFPV-LT-vaccinated SPF chickens as determined by indirect ELISA using purified FPV as antigen.

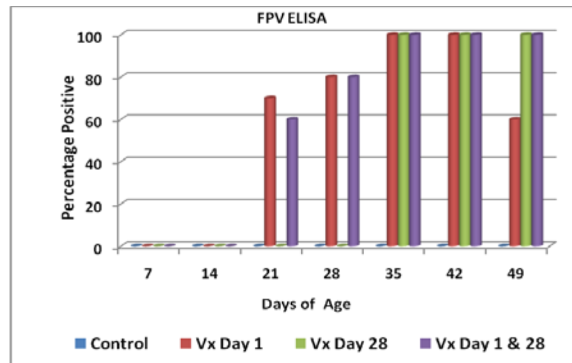

Fig. 2. Percentage positive of FPV-specific antibodies in rFPV-LT-vaccinated SPF chickens as determined by indirect immunofluorescence.

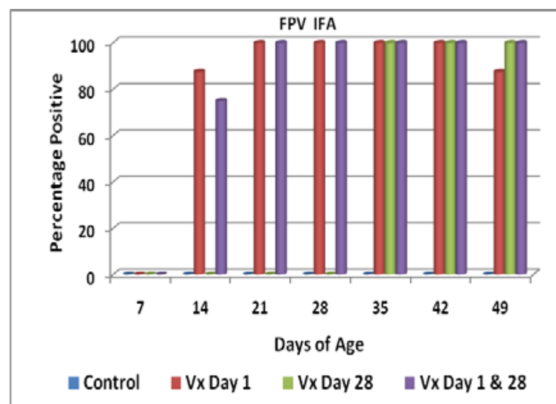

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Fig. 3. Percentage positive of ILTV-specific antibodies detected in rFPV-LT-vaccinated SPF chickens as determined by ELISA (Synbiotics ILT ELISA).

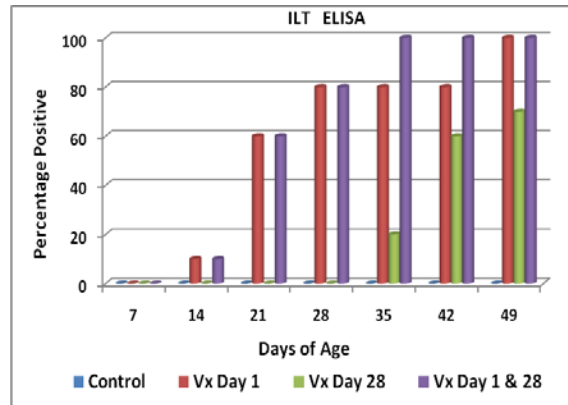
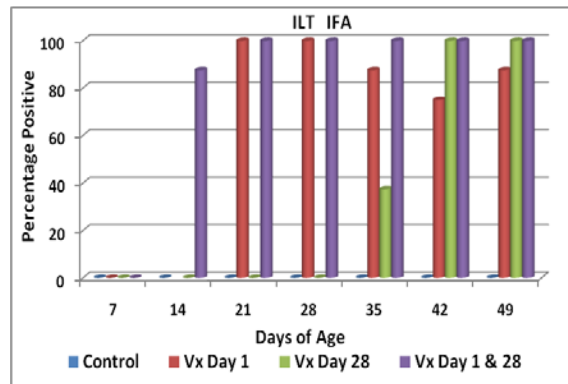


Fig. 4. Percentage positive of ILTV-specific antibodies detected in rFPV-LT-vaccinated SPF chickens as determined by indirect immunofluorescence.



A COMPARISON OF *SALMONELLA* ENTERITIDIS COLONIZATION IN BROILER CHICKENS FOLLOWING AN INTERTRACHEAL AND SUBCUTANEOUS CHALLENGE

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SUMMARY

Salmonella Enteritidis (SE) is an important food borne pathogen that is typically associated with poultry. This study is part of a larger study to determine the incidence of SE in broiler chickens when challenged via different routes, times and doses. In this study $1x10^4$ cfu of SE was given either as a 100uL intertracheal gavage or as a 500uL subcutaneous injection at day zero. After 35 days the following samples were collected: bursa and thymus (pooled), breast, ceca, crop, kidney, liver and spleen (pooled), skin, spinal cord, thigh, and trachea. A sterile swab was utilized to take samples from the abdominal cavity, bone marrow, cloaca and lung. Birds given the intertracheal challenge were positive for SE in 1-49% of the collected samples, while the birds administered the subcutaneous challenge were positive in 0-20% of the samples. The conclusion of this study was that if the bird is exposed to SE at day zero there is a very strong chance of it being recovered at market age.

INTRODUCTION

According to estimates by the Centers for Disease Control and Prevention, 48 million cases of foodborne illness are reported each year causing over 128,000 people to become hospitalized and approximately 3,000 deaths (2). The cost associated with issues surrounding foodborne illnesses has been estimated at $51 billion annually (4). Poultry alone is attributed with causing approximately $2.4 billion in lost productivity annually (1). One of the most common causes of bacteria related a foodborne illness is *Salmonella* (2). National *Salmonella* surveillance estimates indicate that this agent causes more than 1 million illnesses in humans yearly in the United States, with more than 19,000 hospitalizations and 370 deaths (4). Accounting for medical costs and lost productivity the estimated costs associated with salmonellosis is approximately $4.4 billion (4). The new criteria set forth by the United Stated Department of Agriculture's Food Safety and Inspection Service (6) states that *Salmonella* levels in broiler (meat bird) processing plants be less then 7.5% (five positive samples out of 51). Most poultry companies comply with this standard (6) and according to this same data, the prevalence of *Salmonella* in ground chicken is 28%.

Salmonella enterica serovars are predominantly associated with poultry, although it infects other animals, including humans, and may contaminate produce. *Salmonella* that causes foodborne illness in humans readily colonizes chickens but usually produces no disease (3). In 2012, *S.* Enteritidis was the most common clinical *Salmonella* isolate from chickens in the United States, followed by *S.* Kentucky and *S.* Typhimurium. The three most common serotypes isolated from non-clinically infected chickens were *S.* Heidelberg, *S.* Kentucky and *S.* Enteritidis (5).

MATERIALS AND METHODS

Birds and management. SE was administered either by an intertracheal challenge or subcutaneously on the back of the neck on day zero. Each challenge consisted of 150 straight run broiler chicks that were obtained at day of hatch from a commercial broiler company and then transported to the Auburn University Poultry Research Farm. Upon arrival the unsexed chicks were randomly assigned into floor pens containing fresh pine shavings. Standard husbandry and biosecurity procedures were followed for the duration of the trial. All birds were fed a standard diet containing an ionophore at the appropriate level in a three phase feeding program consisting of a starter (from zero to seven days), a grower (from eight to 21 days) and a finisher (from 22 days- termination) feed.

***Salmonella* isolate, dose, and challenge**. The SE utilized in this trial was nalidixic acid as well as novobiocin resistant, which made recovery and confirmation of the dosed bacteria relatively easy. After growing the bacteria the stock was diluted to give a final dose per birds of approximately $1x10^4$ cfu. Birds were challenged on day zero either by an intertracheal or subcutaneous challenge. The intertracheal challenge consisted of delivering 100uL

of SE into the chick's trachea using a syringe fitted with a blunted "feeding" needle. The subcutaneous challenge consisted of delivering 500uL of SE under the skin on the back of the chick's neck.

Sampling. A total of 182 birds (80 subcutaneous and 102 intertracheal challenged birds) were necropsied on days 35-38. From these necropsies the following tissue samples were aseptically collected and placed in buffered peptone water (BPW): trachea, ceca, kidney, skin, breast meat, thigh meat, crop, spinal cord, plus two pooled tissue samples (liver, spleen as one sample and thymus, bursa as the other). In addition swabs were taken of the cloaca, intra abdominal cavity, bone marrow and lung after the swabs were taken they were placed into sterile BPW tubes. It cannot be over emphasized that strict aseptic techniques were followed for every sample collected. This ensured that little or no cross contamination occurred and the results were of high reliability.

***Salmonella* culture and isolation**. Since this necropsy occurred over four days, samples collected on days one through three were placed into BPW and stored at 4°C until the last sampling day. At that time each sample (tissue and swab) was allowed to incubate overnight at 37°C. These BPW pre-enriched samples had a subsample taken which were then enriched in tetrathionate broth (TTB) tubes. TTB tubes were incubated overnight at 41°C. From the TTB tubes selective plating was performed using XLT4 for the native *Salmonella* population. In addition XLT4+nn plates were utilized to track the SE utilized in the challenge. The streaked plates were allowed to incubate for 24-48 h at 37°C. Characteristic colonies, which appear black centered on XLT4 agar will then be confirmed as *Salmonella* using polyvalent antisera.

Data analysis. Collected data is reported as a percent positive for the challenge SE in regards to each organ as well as to the number of positive samples SE and analyzed using GLM.

RESULTS AND DISCUSSION

Figure 1 shows the results for how many collected samples were positive for SE per bird. As can be observed the subcutaneous route of challenge elicited significantly ($p<0.05$) less positive samples (61%) then did the intertracheal challenged birds (18%). The intertracheal challenged birds had significantly more birds that had 1-3 (52%) and 4-7 (30%) samples positive for SE then did the subcutaneously challenged birds 35% and 4% respectively. Figure 2 is a representative of the data collected for each challenge route. For the intertacheally challenged bird's ceca content was highly significantly ($p<0.001$) more likely to be harboring SE then birds challenged subcutaneously. The statistically significant ($p<0.05$) SE samples collected that are shown in that figure: cloaca, crop and trachea again had the intertracheal challenged birds having a higher incidence of SE positive samples then the subcutaneous challenged birds. There was no statistical difference observed between breast muscle or skin samples; however the trend of having more SE positive samples is still observable.

Based on the results shown here as well as those to be presented the conclusion can be made that if SE is introduced into the birds lungs via the trachea at day zero there is a high chance that the majority of birds (in this case 82%) will have SE when they are processed. The data also shows that if the birds are scratched at day 0 and SE is introduced into the wound there is a significantly less chance that SE will be present; however the likelihood that SE will be present is still there (39%). Considering the percent incidence for the two challenge routes (82% and 39%) and the fact the birds were challenged on day zero with relatively few SE cells ($1x10^4$) and that we were still able to recover SE when the birds were necropsied between 35-38 days, it implies that the best strategy for controlling this foodborne pathogen is to take an aggressive approach in keeping this pathogen out of the poultry house and/or using a feed additive or vaccine that has efficacy against this foodborne pathogen. As for the challenge route and the organs affected it is apparent in Figure 2 as well as the data to be presented that the intertracheally challenged birds have a higher incidence of colonization with SE compared to the birds challenged subcutaneously. In birds that are heavily colonized with SE, the bacterium can be isolated in every tissue sample that was collected during he necropsy.

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Figure 1. Percentage of birds with no (0) SE positive tissue samples, 1-3 SE positive tissue samples, 4-7 SE positive tissue samples and 8-11 SE positive tissue samples when challenged either subcutaneously or intertracheally. Significant differences ($p<0.05$) were detected between all the categories for the two challenges except for the 8-11 samples.

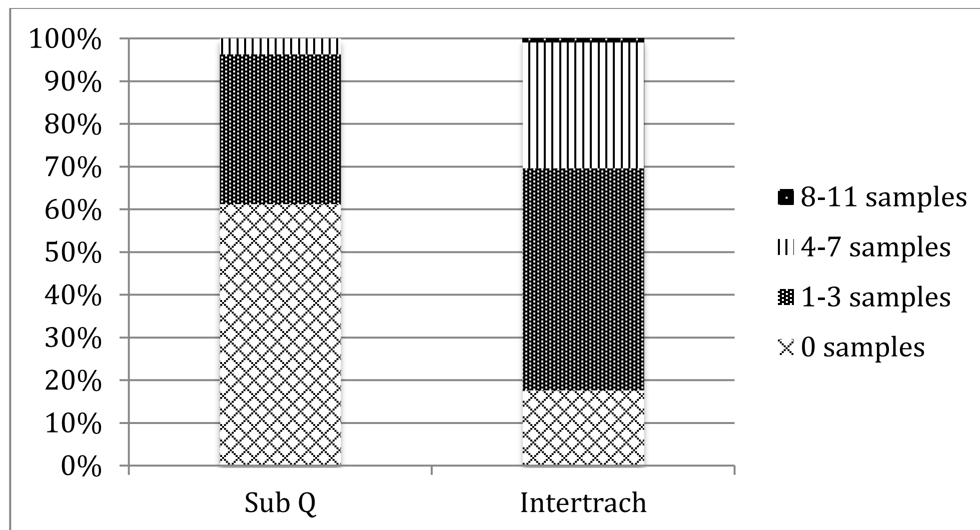
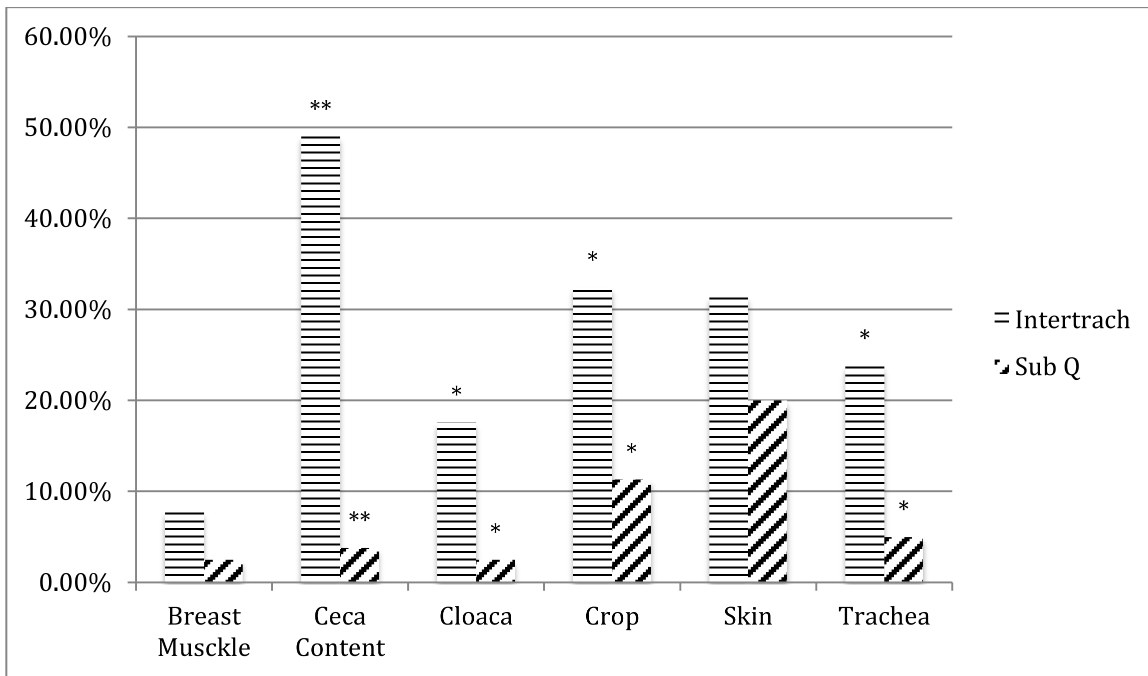


Figure 2. Percentage of samples positive for SE when challenged either subcutaneously or intertracheally. * signify significant differences (P,0.05), ** signify highly significant differences (p<0.001) between the two challenge routes.



HETEROLOGOUS ANTIBODY RESPONSE OF CHICKENS VACCINATED WITH AN INFECTIOUS BRONCHITIS (IB) INACTIVATED VACCINE OF THE MASSACHUSETTS TYPE AFTER PRIMING WITH LIVE ATTENUATED VACCINES OF DIFFERENT SEROTYPES

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SUMMARY

Four vaccination programs using different combinations of live attenuated vaccines of the Massachusetts and 793B serotypes with or without a booster vaccination with inactivated infectious bronchitis virus (IBV) in the form of a multivalent inactivated vaccine (Newcastle disease virus, IBV Massachusetts serotype and egg drop syndrome virus) were compared in their ability to induce virus neutralizing antibodies (VNA) against seven serotypes of IBV (Massachusetts-M41, D274, 793B, Israel Variant 2, Q1, Arkansas and D388/QX) in specific pathogen free (SPF) layer pullets.

The vaccination programs resulted in different levels of VNA against the tested strains of IBV. The results showed that heterologous priming using live attenuated vaccines of the Massachusetts as well as 793B serotypes induced higher titre of VNA against more strains and that the boost with the used inactivated vaccine was very effective in increasing this VNA titre further.

The use of only live vaccines (homologous or heterologous) resulted in all groups in the lowest levels of VNA. This was followed by the group that received the homologous live priming and a vaccination with an inactivated vaccine containing the homologous Massachusetts IBV serotype antigen. The highest antibody response in form of the VNA was observed in the group that received heterologous priming with the two live vaccines followed by vaccination with an inactivated vaccine containing the homologous Massachusetts IBV serotype antigen.

INTRODUCTION

The effective control of infectious bronchitis (IB) in layers and breeders is only possible with the use of either live or inactivated vaccines or combinations thereof in conjunction with biosafety measures (6). Layers and breeders need long-lasting protection against relevant field strains in the field. Moreover protection against losses in egg production and egg quality is an important purpose of the vaccination programs.

Future layers and breeders will receive their first IB vaccination in the form of live vaccines during the first day(s) of life and will subsequently receive one or more further vaccinations with live vaccines during the rearing period. This is mostly followed by the administration of an inactivated vaccine before the onset of lay. In general live attenuated vaccines of the Massachusetts serotype are used as the base vaccination for an effective vaccination program.

The control of IB in many poultry producing regions of the world has become complex due to the presence of variant types of IBV, such as 793B, QX, Q1, Arkansas and Variant 2, just to name a few. However research has shown that several variant IBV subtypes can be readily controlled without the need of newly developed vaccines (4,6).

The currently available range of live and inactivated vaccines can be successfully used against variant IBV by carefully selecting the strains to include in the vaccination program and by administering them correctly at the appropriate moment. The principle of providing broad protection is based on the immunization of chickens with vaccines of different IBV serotypes, e.g. with live attenuated vaccines of the Massachusetts and 793B serotypes (4).

Much has been published about the benefits of using live vaccines of different serotypes for broad protection against variant strains of IBV in chickens. Nevertheless not much in this regard has been investigated after the additional administration of inactivated vaccines.

An indirect way to evaluate the performance of a vaccination program against IBV strains, here with the focus on variant strains for which a limited range of vaccines is available, is to measure the level of VNA that result from vaccination against one specific virus or a combination of vaccines. This can be done

my means of the virus neutralization (VN) test (7). With this test it is possible to assess the presence and level of antibodies that prevent the infection of cells and the subsequent replication of IBV. The higher the level of VNA found in the serum of immunized birds, the higher is the chance of neutralizing the virus and preventing the damages that result from infection.

MATERIALS AND METHODS

SPF leghorn birds were divided in four groups (15 birds/each) and kept in separated isolators. Each group was vaccinated with a different program as described in Table 1. They were vaccinated at one day of age with a live attenuated vaccine of the Massachusetts serotype, followed by revaccination at 28 weeks of age with either a heterologous (Groups 1 and 2) or homologous (Groups 3 and 4) live attenuated vaccine. The live attenuated vaccines were administered using one vaccine dose by the eye drop route. The inactivated IBV component was provided in the form of a multivalent inactivated vaccine (a combination of Newcastle disease virus, infectious bronchitis virus of the Massachusetts serotype and egg drop syndrome virus) which was applied by subcutaneous injection in the neck to Groups 2 and 4 at 56 days of age.

Virus neutralization test. Blood samples for serological evaluation were collected in all groups at 99 days of age and the VN test was carried out using the β-method. The serum was obtained from clotted blood samples using standard procedures. This method employs a constant amount of virus and two-fold dilutions of the serum.

Sera of each group were pooled and tested in four-fold for the presence and amount of VNA against a constant amount of 100 - 300 median tissue culture infectious dose ($TCID_{50}$) of IBV using strains M41, D274, 793B, Variant 2, Q1, Arkansas and D388. The virus was mixed with two-fold dilutions of the antisera and incubated for one hour at room temperature. Subsequently, the virus-serum mixtures were transferred in duplicate to wells of microtitre plates which contained monolayers of primary chicken embryo kidney cells. The plates were placed in a CO_2 incubator at 38-39°C for 72 hours. The VN titers were expressed as the reciprocal of the highest dilution of serum that prevented cytopathic effects. The test was performed in presence of the appropriate controls.

The serotypes included in the VN test were chosen because of their widespread geographical importance and the problems they cause in the field. Moreover, IBV Massachusetts (M41) and IBV 793B were included in the comparison because they represent the serotypes used in the different vaccination setups.

RESULTS

The vaccination programs in the layer pullets induced different levels of VNA against the tested variants. The average level of VNA titre against IBV serotypes Massachusetts, D274, 793B, D388, Q1, Arkansas and Variant 2 of the birds vaccinated twice with a live attenuated vaccine of the Massachusetts serotype (Group 3) was $2^{4.1}$ (see Table 2) which can be considered as low.

Birds vaccinated only with heterologous live vaccines (Group 1) showed a mean VNA titre of $2^{6.5}$. The other vaccination programs resulted in higher mean levels of VNA against the tested IBV strains. The highest average levels of VNA against the tested IBV strains were achieved in the group that received heterologous priming (Group 2; $2^{8.3}$). In comparison the use of homologous live priming followed by a booster vaccination with the inactivated multivalent vaccine resulted in an average VNA titre of $2^{6.9}$ (Group 4).

The prime-boost vaccination with the attenuated live Massachusetts serotype vaccine was taken as a base line. The combination of Massachusetts serotype vaccine with a live attenuated vaccine of the 793B serotype increased the average level of VNA titre against the seven IBV strains by a factor of 5.4. Adding the inactivated multivalent vaccine containing the Massachusetts antigen to the program of the vaccination with the two live Massachusetts serotype vaccinations increased the average level of VNA titre against the seven strains by a factor of 4.9. Combining the live Massachusetts serotype vaccination with a live attenuated vaccine of the 793B serotype and the inactivated vaccine containing Massachusetts serotype antigen in a vaccination program increased the average titre of VNA against the seven strains by a factor of 18.8.

DISCUSSION

In the work reported here a comparison was made between groups of SPF pullets in their response to different vaccination programs against IBV using different combinations of live attenuated vaccines of the Massachusetts and 793B serotypes with or without a booster vaccination with inactivated IBV containing the homologous Massachusetts serotype antigen.

The VN test was used as a measure to assess the performance of the vaccination programs against IBV. It is expected that a higher level of antibodies in the serum of immunized birds will result in better

protection against IB. The possibility of inducing VN antibodies against an IBV strain by the administration of combinations of two or more heterologous IBV has been reported before (5, 8).

The results show that broad priming using live attenuated vaccines of different serotypes induces higher VNA titres against more strains and that the boost with the inactivated multivalent vaccine was very effective in increasing this further. This is especially important since there are only a limited number of vaccine strains available in contrast to the wide number of IBV in the field. As shown here, mid-high level of neutralizing antibodies against variant viruses can be obtained with the right combination of live and inactivated vaccines.

Previous work described in several experiments the importance of different vaccination set ups and the relation of the serological response, measured by using the hemagglutination inhibition test, in providing protection against virulent IBV (1, 2, 3).

Especially remarkable were the high levels of antibodies induced by the program including heterologous priming followed by the administration of the inactivated multivalent vaccine against two of the most important IBV serotypes in the field, namely IBV 793B and IBV QX (2^{11} and 2^{10}, respectively). As in a previous study, high antibody levels in breeders (of between 2log 9-10) were relevant in the protection of internal organs, such as oviduct and kidneys, after infection with a more invasive strain, such as IBV D388/QX (7).

The work presented here provides useful information on vaccinations programs to increase antibody levels in laying birds using a limited number of commercially available vaccines. Further work would be required to establish the relation between the levels of antibodies obtained with the most suitable program and their meaning in relation to protection against IBV challenge infections in the field.

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Table 1. Vaccination program in the different groups.

Group	Day 1	Day 28	Day 56
1	Live attenuated Massachusetts type[a]	Live attenuated 793B type[b]	-
2	Live attenuated Massachusetts type	Live attenuated 793B type	Inactivated ND+IB+EDS[c]
3	Live attenuated Massachusetts type	Live attenuated Massachusetts type	-
4	Live attenuated Massachusetts type	Live attenuated Massachusetts type	Inactivated ND+IB+EDS

[a] *Volvac® IB Mass MLV*
[b] *Nobilis® IB 491*
[c] *Volvac® ND+IB+EDS KV*

Table 2. Mean VN titers of pooled sera against different IB serotypes.

Group	All*	Mass	793B	Arkansas	D388	D274	Q1	Israel Variant 2
1	6.5	5.5	9.5	5.5	7.3	5.8	6.3	6.0
2	8.3	9.8	11.1	6.0	10.0	6.8	8.0	6.8
3	4.1	4.8	4.3	4.8	4.8	3.8	3.5	3.5
4	6.9	9.3	7.0	8.0	8.0	5.5	7.0	5.8

*All (clustered mean VN titer against all serotypes)

THE EFFECT OF A PLANT DERIVED ALKALOID, SANGROVIT®, FED TO BROILER CHICKENS TO REDUCE *CLOSTRIDIUM PERFRINGENS* INDUCED NECROTIC ENTERITIS

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INTRODUCTION

There is an increased demand for the production of poultry without using antibiotics. This is in part driven by regulations withdrawing the growth promotant antibiotics from the market (U.S. and E.U.) or by food service companies requesting no antibiotics to be used in poultry produced for their restaurants. For over 50 years, poultry producers have used low doses of antibiotics, termed growth promotants, as a means of controlling subclinical necrotic enteritis (NE) due to *Clostridium perfringens* (6). Since *C. perfringens* is a ubiquitous bacterium in poultry houses (7), it is necessary to find methods to control this bacterium without antibiotics. In addition to controlling *C. perfringens*, it is also necessary to control the coccidia *Eimeria maxima*, which has been shown to predispose broilers to NE (4).

One class of natural compounds that have both antibacterial and anticoccidial efficacy are plant derived alkaloids. These non-volatile alkaloids extracted from plants have been shown to have anti-inflammatory properties (1), antimicrobial properties (2) and antiprotozoal effects (8, 9). This paper presents the results of two floor pen studies comparing a plant extract Sangrovit® to Bactracin Methylene Disalycilate (BMD®) for prevention of both clinical NE (mortality) and subclinical NE (depressed body weight and elevated feed efficiency) in broiler chickens grown to 42 days of age.

MATERIALS AND METHODS

Experimental design and treatments. Two studies were done in a 48 pen (1.5 m x 3 m) dirt floor house. Both studies were performed to 42 days of broiler age. The same five treatments were used in both studies with the only difference Study 1 had new softwood pine bedding and Study 2 had the reused bedding from Study 1. The treatments can be found in Table 1.

Feed and water were given *ad* libitum. Commerical grade diets (starter D0-20, grower D20-35, and finisher D35-42) were formulated with corn and soybean meal.

Necrotic enteritis challenge model. Briefly the model was coccidia vaccine birds on day of hatch at two times label dose. Then *C. perfringens* broth (strain CP#6) was poured onto nonmedicated feed after two hours of feed withdrawal (1.0 x 10^8 CFU/mL dose). After 30 minutes treatment feeds were returned. This was done on days 18, 19 and 20. NE lesion scores were performed on day 20 as follows: Score 0 = normal jejunum; score 1 = slight mucus; score 2 = Necrotizing enteritis (classic NE lesion); score 3 = sloughed mucosa with blood. NE lesion score of 5 birds at 20 days was by the method of Hofacre, et al. (5). Birds were weighed by pen on days 0, 20, and 42.

RESULTS AND DISCUSSION

Subclinical necrotic enteritis primarily affects broiler growth and feed efficiency, Table 2. In both studies the phytogenic product, Sangrovit, at 60 gm/ton had a significant reduction in the adjusted FCR vs. the nonmedicated challenged broilers at the end of the 42 day growout. The lower dose of Sangrovit (30 gm/ton) had higher FCR than the 60 gm treatment however the 30 gm Sangrovit was statistically the same FCR as the antibiotic BMD. There were no significant differences in body weight gain between any of the treatments in either the new litter or reused litter study. However, the higher dose of Sangrovit (60 gm/ton) did have numerically higher body weights in both studies. As expected, the body weight gains in the reused litter study were lower since these birds would have been exposed to the coccidia and *C. perfringens* from the previous study.

Clinical NE seen by lesions in the small intestine and mortality due to NE was evident in both studies with the highest mortality in the nonmedicated C.P. challenged treatment (Table 3). In both studies, there was significant reduction in mortality due to NE in the birds fed the 60 gm/ton of the Sangrovit. The 30

gm/ton of Sangrovit was statistically the same in both the new and reused litter study to the antibiotic BMD in preventing clinical NE mortality. NE lesions in coccidia vaccinated broiler floor pen studies are normally very mild. This was evidenced in both the new and reused litter study with the only significant difference found between the nomedicated /nonchallenged and all other treatments.

Since this study was not designed to measure coccidia lesions, it is not possible to determine if the higher dose (60 gm/ton) of the plant alkaloid, Sangrovit had the improvement in adjusted FCR and lower NE mortality due to the anticoccidia effect or the antimicrobial effect. However, the effects are clearly dose related since in two consecutive studies the higher dose significantly reduced both clinical and subclinical necrotic enteritis.

As poultry producers raise more broiler chickens without using antibiotics, new methods to control *C. perfringens* will need to be found. Dahiya et al. stated there may not be a single satisfactory replacement for the antibiotics (3). That a combination of non-antiobiotic measures such as probiotics, prebiotics, organic acids, enzymes and plant extracts may be candidate replacements for the antibiotics. It is clear from these two studies that the plant alkaloid, Sangrovit may be effective in improving performance and reducing NE in an antibiotic-free broiler program.

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Table 1. Experimental design.

ID	Treatment Description[1]	Cocci-Vaccine[2]	*Clostridium perfringens* challenge	Pens/ Treatment	Birds/ Pen
T1	Non-medicated, Cocci Vaccine	DOT 0[3]	No	9	50
T2	Non-medicated, Cocci Vaccine	DOT 0	DOT 18, 19, 20[1]	9	50
T3	Sangrovit Dose (30 gm/ton), Cocci Vaccine	DOT 0	DOT 18, 19, 20[1]	9	50
T4	Sangrovit Dose (60 gm/ton), Cocci Vaccine	DOT 0	DOT 18, 19, 20[1]	9	50
T5	BMD (50 grams/ton), Cocci Vaccine	DOT 0	DOT 18, 19, 20[1]	9	50

[1]DOT 18, 19, & 20: *Clostridium perfringens* was added to a complete unmedicated feed at a dose of approximately $1x10^8$ CFU/ml/bird.
[2]Huvepharma Advent® at 2 times label dose
[3]DOT = Day of trial

Table 2. Study 1 and Study 2 performance results post NE challenge at day 42.

Treatment	Adjusted Feed Conversion*		Average Weight Gain (Kg)	
	Study 1	Study 2	Study 1	Study 2
Non-medicated, No C.P. challenge	1.755[a]**	1.760[b]	2.046[a]	1.996[ab]
Non-medicated, C.P. challenge	1.747[ab]	1.850[a]	2.056[a]	1.918[b]
Sangrovit (30 gm/ton), C.P. challenged	1.723[abc]	1.805[ab]	2.072[a]	1.998[ab]
Sangrovit (60 gm/ton), C.P. challenged	1.688[a]	1.786[b]	2.105[a]	2.037[a]
BMD (50 gm/ton), C.P. challenged	1.704[bc]	1.802[ab]	2.056[a]	2.007[a]

*FCR adjusted for non N.E. mortality
** All differences in columns are significantly different at $P \leq 0.05$.

Table 3. Study 1 and 2 necrotic enteritis lesion scores and NE mortality.

TREATMENT	NE		NE (PERCENT) MORTALITY	
	STUDY 1	STUDY 2	STUDY 1	STUDY 2
Non-medicated, No C.P. challenge	0.00[b] **	0.00[b]	0.00[b]	0.00[b]
Non-medicated, C.P. challenge	0.23[ab]	0.4[a]	3.19[a]	2.90[a]
Sangrovit (30 gm/ton), C.P. challenged	0.41[a]	0.3[a]	1.28[ab]	1.00[b]
Sangrovit (60 gm/ton), C.P. challenged	0.4[a]	0.4[a]	0.64[b]	0.00[b]
BMD (50 gm/ton), C.P. challenged	0.41[a]	0.4[a]	1.28[ab]	0.60[b]

** All differing letters in columns are significantly different at $P \leq 0.05$.

EFFECT OF CALCIUM LEVEL AND A DIRECT FED MICROBIAL ON BROILERS EXPERIENCING NATURALLY OCCURRING NECROTIC ENTERITIS

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SUMMARY

Evaluation of dietary approaches to maintain intestinal integrity and prevent or minimize performance losses and mortality associated with necrotic enteritis (NE) is essential with increased prevalence of antibiotic free broiler production. This trial evaluated if a direct fed microbial could mediate the effects of NE when birds were fed different Ca levels. Nine treatments were arranged in a three by three factorial using eight replicate pens. The factors were dietary Ca (0.60, 0.75 and 0.90%) and feed additive (0.5lb/ton Probiotic, 50g/ton BMD, or no additive). Broilers were vaccinated with a live coccidia vaccine and raised on used litter. Increased NE mortality was seen for the 0.9% Ca diets compared to 0.6% Ca diets. An interaction between Ca and additive occurred for BW, ADG and FCR. Decreasing dietary level of Ca to 0.6% resulted in reduced mortality from NE, and with the inclusion of the probiotic, resulted in heavier BW.

INTRODUCTION

The intestinal environment, including the mucosal structure and function, is influenced by many factors including dietary ingredients, non-antibiotic alternatives, dietary composition, and diet quality among others. The health of the intestinal tract is extremely important as nutrient digestion and assimilation for growth occur here. Intestinal integrity is based on the system functioning properly for digestion, absorption, secretion, and immunity. Subsequently, when disruption of this system occurs, major consequences in terms of bird health and growth follow. The poultry industry is increasingly in need of non-antibiotic alternatives to improve gut health or decrease enteric pathogen impacts in commercial broilers. Options being investigated include among many, enzymes, and prebiotics and probiotics. Additionally, there may be numerous opportunities to preserve a healthy intestinal environment by alterations in existing nutritional strategies including adjustment in levels of amino acids, dietary minerals, or protein. While for years dietary enzymes have been used to improve nutrient availability and bird performance, less research has been done to investigate modulation of intestinal health and gut integrity by dietary enzymes to improve intestinal response during a disease challenge in relation to its ability to function optimally for immune defense, digestion, absorption, secretion and transport. Dietary nutrient levels may also play a pivotal role in intestinal health during enteric disease challenges, and altering of levels could influence the response to and severity of the disease. Lastly, the usage of dietary supplements, including probiotics, can alter the host response to intestinal challenges depending on the timing of application, the specific challenge, and other factors in the intestinal environment. The objective of the current research was to evaluate the effect of dietary Ca level with and without probiotic inclusion on the development and pathogenicity of naturally occurring necrotic enteritis.

MATERIALS AND METHODS

Birds. On day of hatch, 2,592 Cobb 500 straight-run broilers were administered a commercial coccidia vaccine in a spray cabinet and placed on used litter from a previous flock with necrotic enteritis. Chicks were selected at random from the hatchery chick boxes, weighed by pen (n=36 birds), and placed into the pens relative to treatment group. Feed and water were administered *ad libitum* throughout the study. During the first three days, supplemental feed of the appropriate treatment diet was provided in disposable cardboard feed trays.

Diets. Diets were formulated according to commercial based nutrient specifications for male Cobb 500 broilers. Diets were mixed at the Virginia Tech feed mill, pelleted and crumbed at Big Spring Mill, bagged (50 lbs) and transported to the Virginia Tech research farm. The same diet was fed from days 0 to 28. Each dietary treatment was replicated by

eight pens with 35 birds per pen. All diets contained 0.02% phytase. The probiotic evaluated was Sporulin® (Novus), containing a proprietary combination of three live strains of *Bacillus subtilis.* Dietary treatments were as indicated in Table 1.

Parameters Evaluated. Feed intake, body weight, body weight gain, and feed conversion ratio on a per pen basis were adjusted for mortality and calculated for the periods of 0 to 9 days, 9 to 18 days, 0 to18 days, 18 to 28 days, 9 to 28 days, and 0 to 28 days. Total percent mortality and NE related mortality were determined for each period on a pen basis. On day 9 and day 18, one bird/pen (n=12/treatment) was euthanized for collection of right tibia. Tibias were dried, fat extracted, and ashed. Total weight of ashed bones, as well as percent ash of bones (ashed weight/dy matter weight), was determined.

Analysis of data was completed in SAS (version 9.3) using the Glimmix procedure. Presence of a significant interaction ($P \leq 0.05$) between calcium level and additives was first determined. If no significant interaction ($P > 0.05$) was present then further statistical analysis was completed to determine differences for the main affects (calcium level or additive). When significant differences for an interaction or main effect were discovered, differences between the treatments were determined comparing the least square mean differences at $P \leq 0.05$. A Tukey-Kramer adjustment was used to correct for multiple comparisons when looking at the lsmean differences. Percent mortality is reported but statistical analysis was completed using converted data (arcsine of the square root of percent mortality).

RESULTS AND DISCUSSION

At 28 days, an interaction for BW was apparent between calcium level and additive (P < 0.033). Birds fed the low calcium (0.60%) with Sporulin diet had increased BW of 1.57 kg compared to the high calcium (0.90%) with no additive diet birds with BW averaging 1.45 kg. No interaction was observed between Ca level and diet additive for mortality associated with necrotic enteritis from days 0-28. Mortalities with NE lesions from 10-28 and 0-28 days were reduced in the low calcium (0.60%) diets compared to the high (0.90%) calcium diets (P < 0.020), with mortality of birds on the 0.75% dietary Ca intermediate to the 0.6% and 0.9%. Tibia bone ash differences were seen between levels of calcium. At 18 days, percent bone ash was higher at 52.77% in the medium calcium (0.75%) diet compared to 51.43% in the high calcium (0.90%) diet with neither being different from the medium calcium (0.75%) diet (P<0.013)

Similar to results previously reported by our laboratory, decreasing dietary calcium to 0.6% as compared to 0.9% resulted in reduced mortality associated with necrotic enteritis (1,2). Dietary calcium at 0.75% resulted in intermediate mortality compared to 0.6 or 0.9%. Reducing dietary calcium had no negative impact on tibia bone ash percent. Based on the performance data, inclusion of Sporulin, a proprietary combination of *Bacillus subtilis* strains, in the 0.6% calcium diet resulted in improved body weight. Combined approaches of dietary calcium modification and probiotic inclusion may be beneficial during naturally occurring necrotic enteritis.

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Table 1.

Trt	Calcium %	Probiotic	BMD
1	0.60	-	-
2	0.75	-	-
3	0.90	-	-
4	0.60	0.5 lb/ton	-
5	0.75	0.5 lb/ton	-
6	0.90	0.5 lb/ton	-
7	0.60	-	50 g/ton
8	0.75	-	50 g/ton
9	0.90	-	50 g/ton

PRELIMINARY DATA FROM A NOVEL PROBIOTIC PRODUCT APPLIED DURING THE HATCHING PERIOD IN COMMERCIAL POULTRY

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SUMMARY

Probiotics have been used for food safety improvement and performance enhancement. These live bacterial products have been applied in the drinking water, in the feed, and as spray post hatch. Our object was to evaluate the application of a novel probiotic product in commercial poultry hatcher cabinets. The novel probiotic formulation showed reduction on the aerobic Gram negative bacteria during an increase of bacterial levels "microbial bloom" that occurs in hatcher cabinets. Moreover, probiotic improved body weight, feed conversion, and livability of market age broilers under commercial conditions.

INTRODUCTION

Modern poultry hatcheries have expanded in size and scale due to increased consumer demand. An increase in hatchery output demands an increase in related operations, such as movement of personnel and vehicles and near-continuous use of hatchery facilities. To ensure the production of healthy disease-free chicks, maximum sanitary standards must be observed, especially with respect to eggs and any equipment that will come into contact with eggs.

One area of the hatchery that requires special care is the hatching cabinets, the first environment to which the chicks are exposed, and it is the first environment to influence the chicks' microbial colonization and make-up, since gastro-intestinal tract (GIT) colonization starts immediately after hatch (1). Under commercial conditions, maternal microflora transference is minimal and the chicks are exposed to uncontrolled bacterial colonization from machinery, boxes, trucks, personnel, as well as other sources (2). Often the bacteria present in a hatching cabinet may not be beneficial to the newborn chicks and may even be harmful; common bacterial found in hatcher cabinets include: *Salmonella*, *E. coli*, *Pseudomonas*, *Streptococcus*, *Staphlococcus*, *Enterococcus*, among others.

The warm temperature and humidity of the hatching cabinets is favorable to the growth of these organisms; therefore, it is important to reduce contamination and prevent a potential increase in the bacterial levels "microbial blooms" within the hatcher. Additionally, the GIT of poultry is dependent on beneficial microbiota to stimulate intestinal and immune development (3). The sooner the neonatal poultry are exposed to beneficial bacteria, the less likely they are to be colonized by pathogenic bacteria and the sooner their GIT will begin to develop. For this reason, our objective was to evaluate the application of a novel probiotic combination on poultry hatcher cabinets and its effect on performance parameters such as mortality, body weight, and feed conversion under commercial field conditions.

MATERIALS AND METHODS

Probiotic culture and application. The probiotic product used is a poultry-derived, generally recognized as safe (GRAS) probiotic culture, consisting of two strains of lactic acid bacterial isolates and three strains of *Bacillus* spp. spores in equal amounts in a dry powder form. For application in commercial hatcher cabinets, the formulation was administered at transfer, 20% hatch, and 50% hatch, and 75% hatch via a mechanical pneumatic dispenser. The four doses were adjusted to supply $1x10^9$ spores per bird and $1x10^7$ cfu of lactic acid bacteria per bird over the treatment period.

Experimental design. The powder was applied into the ventilation system of the hatcher and the stir fans were used to distribute the beneficial bacteria from the formulation around the hatcher cabinet. Eggs were randomized between control and treated groups to account for incoming bacterial load and egg size differences due to breeder source variation. A single hatcher hallway was divided between control and treated groups to control for hatcher environment. Further contract commercial farms were divided between control and treated groups to control for growout house environment and

management. A total of 16 houses (400,000 broilers) were evaluated (eight treated and eight control houses). Seven day body weights and mortality were measured. In each house, treated or control, at least 400 chicks were weighed in 10 bird lots. Chicks were taken from different points within the house to reduce sampling error. Seven day mortality was taken from records maintained for each house by the flock manager. Performance data was reported by the commercial poultry company (broiler integrator) according to its methods of data analysis.

RESULTS AND DISCUSSION

Upon arrival at the hatchery, eggs are sometimes sanitized to remove microbes transferred from the hen and fecal material. This does not remove all bacteria, which usually continue to grow in the warm, humid environment of the setters and may result in a market increase of bacterial levels after transfer into the hatching cabinets. The bacterial level could increase in magnitude as the chicks begin to hatch presumably due to an increase in humidity within the cabinet and more nutrient availability in the form of amniotic fluid. Traditionally hatchery sanitation has been accomplished by the use of antimicrobial chemicals to reduce the pathogens from the hatcher. The fumigation of the hatching cabinets with formaldehyde is a common practice to prevent an increase in bacterial levels. Nevertheless, formaldehyde may cause some ill effects to the chicks, as it is a known irritant, and is a potential safety hazard to hatchery personnel. Therefore, several countries have moved to ban or drastically lower the short-term-exposure-limit for formaldehyde, so various alternatives have been tested over the past several years.

Moreover, formaldehyde and other chemical disinfectants do not address the need for exposure to helpful, commensal bacteria. To accomplish this, treatments with probiotic bacteria have been extensively studied and applied post hatch. These have generally been applied either at the hatchery during processing of the chicks (4, 5) or soon after placement at the poultry farm (6, 7). However, the time from hatch to first exposure to these probiotic bacteria can range up to 48 h post hatch depending on actual hatch time of a given chick and when the probiotic is applied post hatch. Since broiler chickens only live an average of 42 days, 48 h is a significant period of time before they are provided with beneficial microorganisms. Therefore, the application of a probiotic product during the hatching process may be a better choice for application of a probiotic product to poultry during the neonatal period.

Viability, application, and dosage rate in the hatcher cabinet environment of the probiotic product studied was determined by previous experiments (8). Furthermore, in previous studies this probiotic combination showed reduction on the aerobic Gram negative bacteria in the hatcher cabinet "microbial bloom". In the present study, probiotic treated groups from a commercial trial showed improved seven days body weight by 4.4 g on average when compared to control groups. Seven day mortality was reported as 1.19% for control and 1.64% for treated group. However, livability at market age was improved by over 1% on average in treatment groups. Market age body weight was on average 23 g higher for treated groups. Moreover, feed conversion of the treated flocks was seven points higher. These results indicate that the bacterial formulation improved performance at both seven days and at market age. The seven points of feed conversion improvement showed a 3.5% improvement utilization of feed, the major cost of growing broilers and an indicator of flock health and performance.

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COCCIDIOSIS AND VIRAL RESPIRATORY VACCINES: COMPATIBILITY AFTER SIMULTANEOUS APPLICATION AT THE HATCHERY

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SUMMARY

Respiratory (infectious bronchitis and Newcastle) and coccidiosis vaccines are routinely administered separately at the hatchery at one day of age, using different spray cabinets. However, these are not the only vaccines administered at the hatchery in this manner.

The aims of these studies were to evaluate efficiency of Coccivac-B52 application with a constant pressure spray cabinet, in addition to assessing the viability of the live attenuated respiratory vaccines Mildvac Mass+Ark, Newhatch C2, and Ma5 alone and in combination with Coccivac B52 vaccine (without potassium dichromate).

The effect of Coccivac B52 on Ma5 infectious bronchitis virus vaccine (IBV vaccine), in addition to the application efficiency using a constant pressure spray cabinet was measured. Titration, oocyst count, and IBV vaccine replication data, showed that there was no effect on IBV from Coccivac B52. This data also showed that application of both vaccines with a constant pressure spray application system is very efficient and effective. In addition, vaccine titer determination of the other respiratory vaccines alone and in combination with Coccivac B-52 at different times was assessed.

Additional information on the most important findings of the two separate studies performed at the University of Georgia and Mississippi State University will be presented.

A DOUBLE RECOMBINANT HERPES VIRUS OF TURKEYS FOR THE PROTECTION OF CHICKENS AGAINST NEWCASTLE, INFECTIOUS LARYNGEOTRACHEITIS, AND MAREK'S DISEASES

M. Morsey

INTRODUCTION

Immunization of chickens with live, inactivated or recombinant vaccines, is extensively used in the poultry industry world-wide and vaccination is considered the cornerstone of infectious disease control in all segments of poultry production (6). The present report describes the efficacy of a herpesvirus of turkeys (HVT)-based double recombinant vaccine in protection of susceptible chickens against the diseases caused by challenge with virulent Newcastle disease virus (NDV), infectious laryngeotracheitis virus (ILTV) and Marek's disease virus (MDV). The recombinant HVT vector vaccine, designated HVT-NDV-ILT, contains both the ILTV gD plus gI genes, and the NDV-F gene. The vaccine virus was constructed using a cosmid-based strategy and the plaques obtained following transfection of CEF with a set of cosmid clones were purified on CEF cells to assure vaccine purity. For evaluation of vaccine efficacy, white leghorn chickens hatched from a specific pathogen free flock (Charles River SPAFAS, Storrs, Connecticut) were used in all studies. Chickens were housed in biosecurity level two isolators. Food and water were provided *ad libitum*. All experimental procedures were performed in compliance with institutional animal use guidelines. Viable eggs, at 18 days of embryonation, were inoculated by the *in ovo* route with 0.1 mL of the respective vaccine or diluent placebo. The inoculum was administered into the amniotic sac using a one mL syringe fitted with a 22G x 1¼" needle. One day old chicks were vaccinated by the subcutaneous route with 0.2 mL in the back of the neck using a 22G x 1" needle using the respective vaccine or diluent placebo. All challenge viruses were titrated in chickens to calculate the virus dilution that causes disease in at least 80% (MDV GA 5 strain) or at least 90% (NDV Texas GB strain, ILTV 96-3 USDA strain) of non-vaccinated chickens. ILTV challenge was conducted using USDA challenge strain by the intratracheal route using a dose of $10^{4.5}$ 50% egg infective dose (EID_{50}) in 0.25 mL. NDV challenge was conducted using the velogenic Texas GB strain by the intramuscular (IM) route with 10^4 embryo lethal dose (ELD_{50}) in 0.2 mL dose. Marek's challenge was carried out using GA 5 strain by the intraperitoneal (IP) route.

RESULTS

Evaluation of protective immunity to NDV. The efficacy of the double recombinant vaccine in protection against challenge with the velogenic NDV strain Texas GB was evaluated by calculation of the percent of animals with clinical signs of NDV infection over a 14 day period and the results are shown in Table 1.

Evaluation of protective immunity to ILTV. The efficacy of the double recombinant vaccine in protection against challenge with a virulent ILTV strain (USDA challenge strain) was evaluated in SPF chickens which were vaccinated by the *in ovo* or SC routes and the results are shown in Table 2. The extent of protection is expressed as the percent of animals with clinical signs of ILTV infection over a 10 day period following challenge.

Evaluation of protective immunity against MDV. The protection afforded by the HVT-NDV-ILT vaccine was assessed following challenge of chicken with a virulent MDV (GA 5) and the results are presented in Table 3. The extent of protection is expressed as the percent of animals with clinical signs of MDV infection over a 49 day period following challenge. The results demonstrate that the insertion of multiple genes from two different pathogens did not alter the ability of HVT backbone to provide solid protection against challenge with a virulent MDV.

DISCUSSION

Vaccination is considered the most effective strategy for control of many infectious agents causing severe economic losses in poultry flocks world-wide (5, 6). One of the key obstacles for poultry vaccination, however, is the development of recombinant vaccines that can protect against multiple diseases from one vector with a single dose delivered by mass application. Several recombinant HVT-based vaccines have been developed and used successfully to protect poultry flocks against a

number of important individual avian pathogens (1, 2, 3, 4). However, to date, these vaccines offer a limited range of protection and are seldom combined and used for concurrent vaccination against multiple diseases due to the suspected interference among different HVT-based vaccines. To overcome these shortcomings, we constructed a double recombinant HVT-vectored vaccine (HVT-NDV-ILT) and assessed its potential as a polyvalent vaccine for protection against three key avian pathogens; MDV, NDV, and ILTV.

The efficacy of the recombinant HVT-NDV-ILT as a polyvalent vaccine was assessed in vaccination/challenge studies as prescribed in 9CFR. For all three pathogens (ILTV, NDV, and MDV), the double recombinant vaccine provided excellent protection after a single dose given by the *in ovo* or SC routes. The protection afforded by the double recombinant HVT-NDV-ILT vector vaccine against virulent MDV challenge indicates that the insertion of several foreign genes did not adversely affect the capacity of HVT to protect against Marek's disease.

In summary, the present report ushers in a new generation of safe and well-characterized HVT-based recombinant vaccines that provide excellent protection against three key poultry pathogens from a single HVT backbone with a single dose given by mass application.

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Table 1. Protection of SPF chicken against challenge with Texas GB NDV.

Group*	Vaccine route	Positive/total	% Protection
Placebo	*in ovo*	10/10	0
HVT-NDV-ILT group-1	*in ovo*	1/21	95
HVT-NDV-ILT group-2	*in ovo*	1/30	97
Placebo	SC	10/10	0
HVT-NDV-ILT group-1	SC	1/30	97
HVT-NDV-ILT group-2	SC	0/30	100

*Groups were either vaccinated with a diluent placebo or with different vaccine doses

Table 2. Protection of SPF chicken against challenge with ILTV strain LT 96-3.

Group	Vaccine route	Positive/total	% Protection
Placebo	*in ovo*	13/14	7
HVT-NDV-ILT group-1	*in ovo*	5/34	85
HVT-NDV-ILT group-2	*in ovo*	5/35	86
HVT-NDV-ILT group-3	*in ovo*	2/35	94.3
Placebo	SC	12/15	20
HVT-NDV-ILT group-1	SC	0/34	100
HVT-NDV-ILT group-2	SC	0/35	100

*Groups were either vaccinated with a diluent placebo or with different vaccine doses

Table 3. Protection of SPF chicken against challenge with virulent MDV GA 5 strain.

Group	Vaccine route	Positive/total	% Protection
Placebo	*in ovo*	31/35	11
HVT-NDV-ILT group-1	*in ovo*	6/30	80
HVT-NDV-ILT group-2	*in ovo*	1/34	97
Placebo	SC	33/34	3
HVT-NDV-ILT	SC	4/34	88

*Groups were either vaccinated with a diluent placebo or with different vaccine doses

A HIGHLY STABLE, HOMOGENOUS SUSPENSION OF FENBENDAZOLE (SAFE-GUARD® AQUASOL) FOR THE TREATMENT OF GASTRO-INTESTINAL NEMATODES

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SUMMARY

Intestinal parasitic worms are a common problem in the poultry industry. These parasites have a significant impact, contributing to the transmission of disease, decreasing yield, and subsequently, increasing costs. This parasitism adds to the total price of production and results in significantly lower producer profits. Due to the systematic impact of intestinal worms, a water soluble fenbendazole (Safe-Guard® AquaSol) has been developed by Merck Animal Health and approved for use in poultry. This new product is a highly stable, farm-friendly, water-administered suspension. The stability of Safe-Guard AquaSol does not require frequent agitation and provides a high level of efficacy as demonstrated in repeated animal tests. Gastro-intestinal nematodes including *Ascaridia galli* (L5 and adult stages) and *Heterakis gallinarum* (L5 and adult stages) are susceptible to treatment with Safe-Guard AquaSol. A review of stability characteristics over time and efficacy in layers, broilers, and breeders will be discussed.

INTRODUCTION

Like most vertebrates, chickens are vulnerable to parasites that can live on or in their bodies. Internal gastrointestinal helminths are a costly global problem for poultry production, capable of causing clinical disease, poor meat quality, and other negative economic impacts that severely erode the efficiency and profitability of producing meat and eggs. Parasitic nematodes populating the gastrointestinal system of production birds are by far the most significant group of helminths affecting poultry (2). The prevalence of gastrointestinal nematode infections within modern commercial poultry facilities are directly related to the environmental management, market age of the bird, production system, and, if utilized, effective parasiticides. These facilities in the United States (US) employ intervention strategies based predominantly on prophylactic treatment of long-lived bird populations and treat broilers on an as-needed basis on farms with a history of heavy nematodiasis (industry survey by author). Administration of these intervention strategies via drinking water is advantageous over in-feed medications due to the ability to treat poultry upon diagnosis of an active worm infestation. Prior to the recent introduction of AquaSol, piperazine was the only product labeled for use in poultry to treat nematodes. However, when used at the recommended dosage over 20 years ago, piperazine was shown to be 57.5% efficacious (1). This poor efficacy resulted in a significant percentage of the US poultry industry utilizing other anthelmintics off-label, requiring a veterinary prescription. These off-label products have been effective, but the physical stability and homogeneity of these treatments have been less than ideal, often resulting in sediment that obstructs bulk tanks, pipes, and watering system equipment resulting in potentially under dosed birds within the flock.

Fenbendazole (FBZ) is an excellent broad spectrum anthelmintic with proven efficacy in a variety of animal species (2). The new formulation of FBZ, Safe-Guard AquaSol, recently released by Merck Animal Health in the United States is labeled for the treatment of gastrointestinal nematodes in chickens infected with the preadult L5 and adult stages of *Ascaridia galli* (roundworms) and the preadult L5 and adult stages of *Heterakis gallinarum* (cecal worms), two of the most important helminth species in poultry (2). A patented wet-milling process is utilized to reduce the active ingredient of Safe-Guard AquaSol to a refined, homogenous, and submicron particle size allowing it to remain in suspension without agitation for up to 24 h. Safe-Guard AquaSol provides a safe and unique combination of dosing flexibility, efficacy, and convenience in a farm-friendly formulation developed for water-administration. Results are described herein.

MATERIALS AND METHODS

Efficacy. A negatively controlled, partially blinded, randomized, single-site dose-confirmation study was conducted to confirm the efficacy of three

Safe-Guard AquaSol dosage regimens administered in drinking water (one and two mg/kg BW daily for five days, and 3.5 mg/kg BW daily for three days) for treating natural infections of *A. galli* and *H. gallinarum* in chickens. Experimental birds consisted of 105 female chickens, approximately two years of age, weighing 1.3 to 2.5 kg.

On day 10, chickens were ranked by weight into 21 blocks of five. Birds were then randomly divided into five treatment groups (20/group) with five sentinel birds randomly allocated for day one necropsy and worm counts. Treatments were administered via drinking water as follows:

Group 1. Untreated control group (5-day dosing regimen)

Group 2. Untreated control group (3-day dosing regimen)

Group 3. 1 mg FBZ/kg BW/d for 5 days (days 1-5)

Group 4. 2 mg FBZ/kg BW/d for 5 days (days 1-5)

Group 5. 3.5 mg FBZ/kg BW/d for 3 days (days 1-3).

Fecal sampling was performed on days seven, six, four, and three for all groups, then on days nine and 10 for Group 2 and Group 5, and on days 11 and 12 for groups 1, 3, and 4. Chickens were then euthanized and necropsied seven days after last treatment administration (day 10 and day 12) for parasite collection. The nominal FBZ concentrations for the medicated water were calculated using the mean of individual BW and number of chickens per pen on day three and the average daily water consumption measured on day six to day four. To ensure complete consumption of the medicated water, dosages were provided in approximately 50% of the normal daily amount of drinking water. Efficacy assessment was based on percent worm count reduction of the treated animals as compared to the negative controls at necropsy. Chickens used in this study were naturally infected with *A. galli* and *H. gallinarum*, as confirmed by the statistical and parasitological approaches (required at least six chickens in the two control groups for adequacy of infection). For statistical approaches, adequacy of infection was achieved for adult *A. galli* in study Group 1 and *H. gallinarum* in study Groups 1 and 2, but not for adult *A. galli* in study Group 2. For parasitological approaches, adequacy of infection was achieved for pre-adult and adult stages of *A. galli* and *H. gallinarum*.

The study was not fully blinded due to the different number of days over which treatment was administered, but the level of blinding was deemed sufficient because two control groups were used (treated for three or five days). Three adverse events were reported in chickens treated with FBZ during the study but none were considered likely to be related to anthelmintic administration.

Safety. Test birds were randomly divided into four treatment groups and administered Safe-Guard AquaSol in the drinking water at daily dose rates of 0, 1, 3, or 5 mg FBZ/kg BW, corresponding to dose rates of 0, 1×, 3×, or 5× the recommended dose rate, respectively. In addition, each dose rate was administered daily for 15 consecutive days, three times the recommended five day treatment duration. As a result, the total net doses of FBZ administered were 3×, 9×, or 15× the recommended total dose (Figure 1).

This study involved 480 healthy broiler chickens (Ross 308, 240 males/240 females) housed in 48 single-sex pens holding 10 chickens/pen (each pen comprised an experimental unit). Six pens of each gender were randomly allocated to each of the four treatment groups (12 pens/treatment). Prior to treatment, two chickens per pen were randomly selected for hematology assessments, two per pen for clinical chemistry samplings, and four per pen for later necropsy. Treatment with Safe-Guard AquaSol at the various dose rates commenced when chickens were 21 to 23 days of age, with daily treatment administered in drinking water for voluntary uptake over a full 24 h period.

Clinical health was monitored throughout the study and feed and water intake was recorded. In addition, hematology and clinical chemistry parameters were measured pre-treatment (study day -3) and after commencement of treatment on study days six and 16. On study day 17, gross necropsies were performed on chickens identified before treatment and histopathology samples were collected. Data collections were performed by personnel blinded to treatment, with the exception of histopathology assessments.

RESULTS

Efficacy. All three Safe-Guard AquaSol treatment groups provided significant ($P < 0.05$) worm count reductions compared to controls for both nematode species (Table 1). Efficacy ≥ 96.9% was demonstrated in all treated groups. Results from groups treated with a five day regimen (1 or 2 mg/kg BW/day) were consistent with results from other dose-determination and dose-confirmation studies, while the three day dosage regimen (3.5 mg/kg BW/day) also demonstrated similarly high efficacy against the target parasites.

Safety. Analysis of the medicated water samples confirmed that FBZ concentrations were 100% to 104% of the expected concentration for each

treatment group. Evaluations of the massive quantity of collected data revealed no clinically significant differences for any safety or toxicological parameters between the control group and all medicated groups, including birds treated at the five times the daily dose rate. Thus, outcomes for feed consumption, body weights, hematology, clinical chemistry, organ weights, and histopathology were similar between all groups, and any occasional detected differences were not related to drug administration. Water intake was also similar between all treatment groups (Figure 2), indicating that normal palatability was maintained for drinking water medicated with Safe-Guard AquaSol

DISCUSSION

Multiple dose-confirmation studies have demonstrated potent and consistent efficacy of Safe-Guard AquaSol treatment when provided in the drinking water of chickens at the most cost-effective daily dose and duration of 1 mg/kg BW for five consecutive days. In all trials, Safe-Guard AquaSol treatment at the 1 mg/kg BW/day dosage provided significant ($P < 0.05$) worm count reductions compared to controls for both *A. galli* and/or *H. gallinarum*. Dosages greater than 1 mg/kg BW or for durations exceeding five days did not significantly improve efficacy. Thus, 1 mg FBZ/kg BW/day for five days represents the cost-effective recommended dosage for Safe-Guard AquaSol, providing high and consistent efficacy against *A. galli* and *H. gallinarum* parasites. In addition, while *H. gallinarum* infections alone may not exert severe direct impacts on poultry health and production, the parasites pose their greatest risk as carriers of Histomonas meleagridis, a protozoal agent that causes blackhead disease in chickens and turkeys. As an important transmission vector for *H. meleagridis* and cecal worms thus present a considerable threat to ongoing flock health and profitability that requires appropriate prevention and control measures, as such Safe-Guard AquaSol stands as one of the few remaining tools to control this economically important disease.

The target animal safety study demonstrated that Safe-Guard AquaSol is highly palatable and well tolerated in growing broiler chickens, even when grossly overdosed at five times the intended daily dose rate for three times the intended duration. Use of Safe-Guard AquaSol at the recommended daily dose rate of 1 mg/kg BW for five days clearly offers a wide margin of safety for growing broiler chickens (and all classes of growing chickens) and does not impact rates of water consumption.

FBZ has been used for decades in multiple species and is known to be extremely safe for animals. Safe-Guard AquaSol has been tested extensively in chickens, including during intense periods of growth and peak egg production. These and other studies have demonstrated the product is safe for use in all classes of chickens and that it has no adverse impact on fertility, hatchability, survival of progeny or water consumption. Safe-Guard AquaSol clearly offers safe and consistent anthelmintic efficacy against two economically significant parasites affecting all classes.

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Figure 1. Experiment design of target animal safety studies.

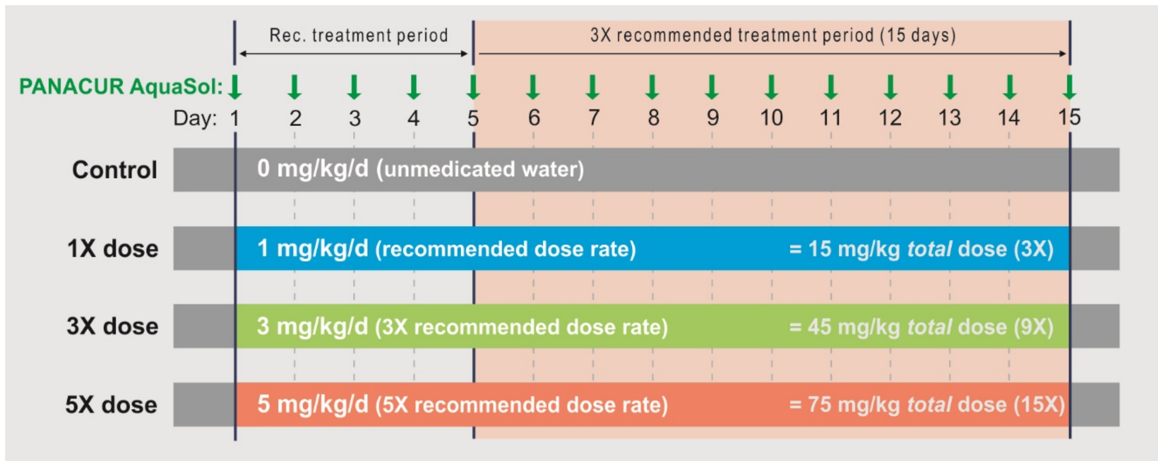

Figure 2. Similar intake of non-medicated and Safe-Guard® AquaSol -medicated drinking water by growing broiler chickens.

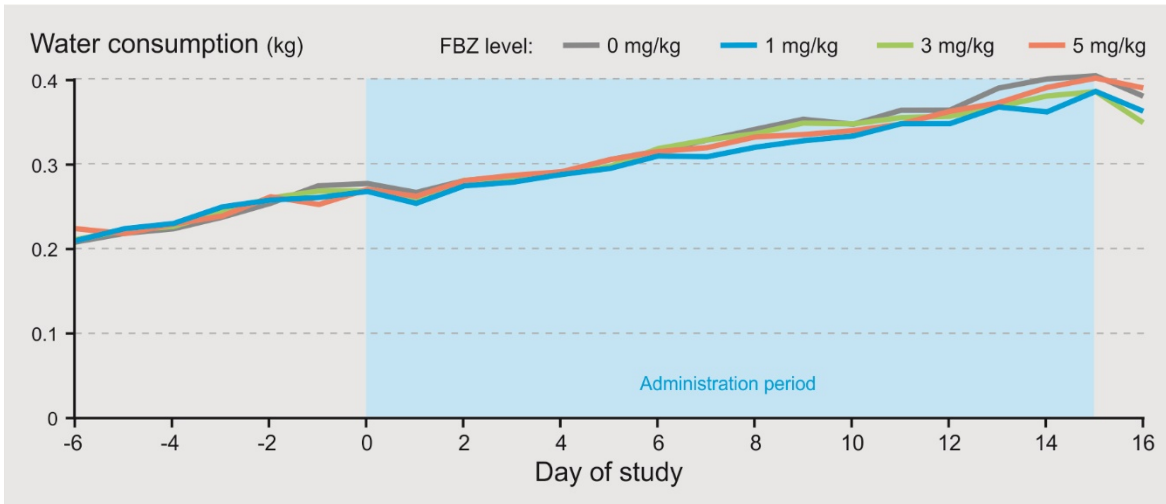

Table 1. Efficacy results of Safe-Guard® AquaSol against *A. galli* and *H. gallinarum*.

Study group (FBZ dose regimen)[a]	Pre-adult and adult worm count reduction (%)	
	A. galli	*H. gallinarum*
1,2 Untreated controls	---	---
3 1 mg/kg BW/d for 5 days*	**97.3**	**96.9**
4 2 mg/kg BW/d for 5 days	100.0	99.3
5 3.5 mg/kg BW/d for 3 days	100.0	99.4

[a]Worm count reductions vs controls significant ($P < 0.05$) for both nematode species

*Recommended Safe-Guard AquaSol dose

MONITORING, EVALUATING, AND AUDITING A BIOSECURITY PROGRAM WITH ACCUPOINT ADVANCED

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SUMMARY

In animal production, the most common and important processes of biosecurity programs are cleaning (removal of organic matter and dry cleaning) and disinfection. But as important as these processes are, there is no widely used routine method to monitor and evaluate the effectiveness of an animal production facility's cleaning and disinfection processes.

When asked how the effectiveness of these routine processes are evaluated, answers vary. Some use surface sampling with culture plates to determine the presence of bacterial contamination. However, even when this process is used, it is only used periodically. And, in most cases, there are no monitoring programs available in such critical areas as at hatcheries for quarterly evaluations, as they are the initial sites for early exposure of hatching eggs.

The limitations of using culture media methods. When using culture media, it is important to take into account its importance, as well as its limitations. The identification of bacterial colonies through culture media is very important because it provides very specific results. These methods can often narrow down bacteria by name, and aided by additional techniques, they can be identified by "last name" of the microbial organisms that are most prevalent in animal operation facilities — even after the use of cleaning and disinfection protocols.

However, the sensitivity of culture media methods is somewhat limited, since it is dependent on the media that is used, as well as external factors. These factors include temperature, which may promote the growth of thermophilic bacteria at temperatures of at least 37.5°C, and the number of bacteria alive on a specific surface when the sampling or the stamp were taken. This number will determine whether the minimal amount of bacteria is present during sampling to form the colonies needed to accurately identify the bacteria.

These factors limit the ability of culture media methods to accurately evaluate the effectiveness of biosecurity protocols. The development of effective thresholds and parameters for cleanliness baselines, and a reliable method to evaluate a program's ability to meet those thresholds and parameters, could help demonstrate if the existing cleaning and disinfection procedures are meeting their expectations. It could also ensure that the way they are being applied or used (e.g., mechanical action, dilutions and exposure time) are best suited for each type of surface and application.

The bacterial culture methodology contributes very important information, but these results would increase its usefulness if we complement the monitoring, evaluation and auditing programs with a tool that has a higher accuracy in terms of better defining true clean surfaces.

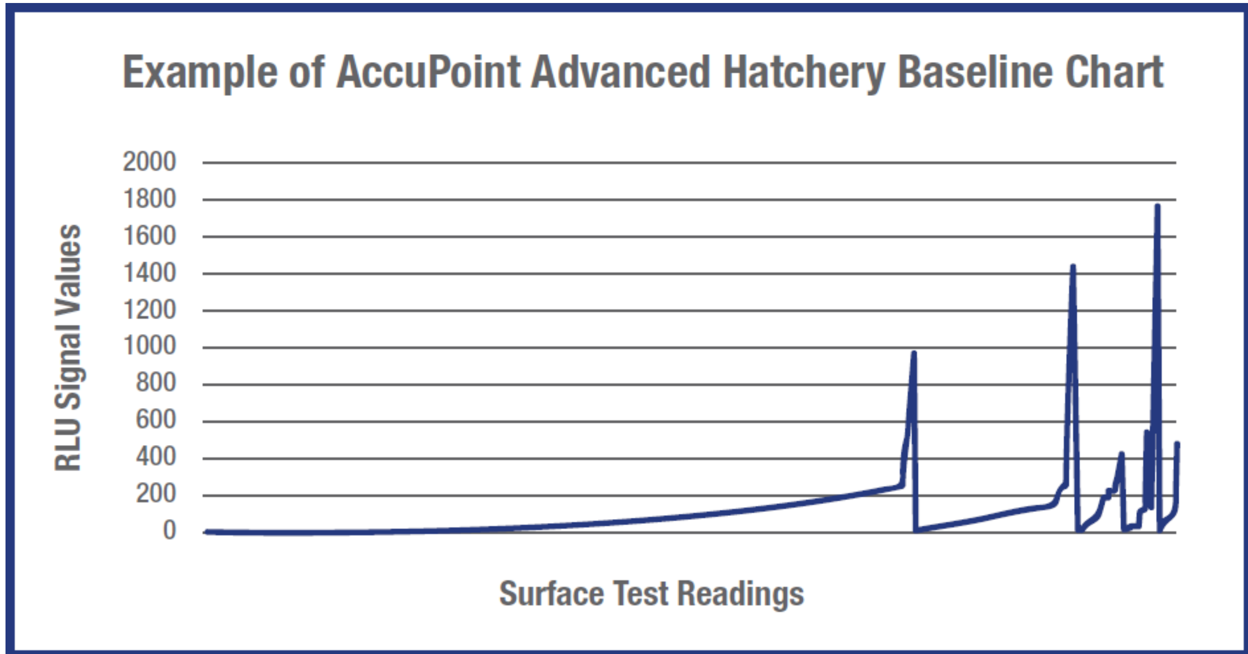
The AccuPoint Advanced ATP Sanitation Monitoring system. The AccuPoint Advanced ATP Sanitation Monitoring system is based on the detection of adenosine triphosphate (ATP), which is like the gasoline of all living cells. The AccuPoint system consists of a reader that detects the presence of ATP on surfaces through the production of light when ATP is combined with two enzymes in the system's samplers, luciferin and luciferase. The AccuPoint reader calculates the light produced in relative light units (RLU), and the sample's RLU results are directly correlated with the level of cleanliness of the sampled surface — the higher the RLU result, the more ATP that was detected on the surface.

This technology can be used by any animal production unit to establish their baselines to establish more objective and accurate audits. These results can be obtained in less than 20 sec and the sampling can be performed right there after the surface dries. After being subjected to a detergent wash sampling should take place in the same manner after the use of disinfectants.

AccuPoint Advanced can help establish permissible ATP levels for each type of surface and to establish allowable ranges (biosecurity indexes and cleaning and disinfection indexes) to correlate with best performance results and health programs. Thus, it can allow the performance of more regular audits according to existing protocols adopted on different production units, or to those protocols intended to be adopted in the future. It can also perform product evaluations in a more objective way, and generate data specific to an operation.

Examples of results received from AccuPoint Advanced. Assessments were conducted in some incubator surfaces, such as plastic, fiberglass, metal and aluminum, as they are considered particularly critical to reduce or eliminate the presence of biofilm and/or organic material such surfaces. These results may vary from company to company, their baseline could be in the range from 300 to 400 on acceptable levels for a cleaning and disinfection protocol results.

The AccuPoint Advanced is able to show activity between 0 to 99.999.



VARIATION IN ADSORPTION OF LAYER *SALMONELLA* ISOLATES FROM DIFFERENT MANNAN OLIGOSACCHARIDE

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SUMMARY

Mannan oligosaccharides (MOS) have been used to reduce or eliminate pathogen loads in poultry species. Reactive mannan receptor sites on the MOS provide binding sites for pathogens thus reducing or eliminating the ability of the pathogen to colonize the bird's GI tract. Seven commercial yeast-based products were examined for the ability in adsorb layer *Salmonella* isolates *in vitro*. Only two of the seven were found to absorb the *Salmonella* isolates. In vitro adsorption of Salmonella isolates has been shown to be predictive of in vivo activity in terms of reducing or eliminating Salmonella in poultry.

Variation in MOS products exists in terms of adsorption of *Salmonella* isolates from commercial operations.

INTRODUCTION

Salmonella continues to be the major foodborne pathogen in poultry and other raw meat products. Although many cases of salmonellosis in humans are not reported, it is estimated that more than 1.2 million people are infected per year in the United States. Reducing the prevalence of *Salmonella* and other pathogens in foods continues to be a primary directive. MOS is a mannose-based complex carbohydrate fraction obtained by certains strains of yeast that have been shown to have certain effects on gut health, pathogen colonization and immune function(1-3). A previous study demonstrated that chicks supplemented with a specific type of mannan oligosaccharide had a lower incidence of *Salmonella* infection than unsupplemented birds (4). Further studies demonstrated that an in vitro agglutination assay was predictive of in vivo activity. Subsequent studies have shown the form of the mannan oligosaccharide impacts the ability of this molecule to agglutinate pathogens. The purpose of this study was to examine commercial MOS products for in vitro agglutination of *Salmonella* strains obtained from US commercial operations.

MATERIALS AND METHODS

MOS products were obtained from commercial sources. *Salmonella* isolates were obtained by isolation from US layer operations. The total mannan and glucan content of the MOS products was determined by the method of Freimund and coworkers (5). The in vitro agglutination assay is based upon the methods described by Mirelman et al (6). This method is semiquantitative and results are expressed as "yes" for positive agglutination of the *Salmonella* isolate to the MOS product, "weak" for slight agglutination and "none" for no visible agglutination between the pathogen and the MOS product.

RESULTS

Table 1 shows the mannan and glucan content of the samples examined. The commercial products from source A have higher mannan fractions than source B. It should be noted that the products from source A are marketed as mannan oligosaccharides while source B products imply mannan oligosaccharide effects on *Salmonella* species. The percentage of mannan in products from source A are numerically higher than those from source B. Table 2 examines the ability of each commercial product to bind (agglutinate) to the *Salmonella* isolate. Where "Yes" is reported, the commercial MOS product agglutinated to that *Salmonella* isolate. "Weak" denotes a low affinity of that *Salmonella* strain for the MOS product and "None" means that no agglutination of the *Salmonella* strain to the commercial product was observed.

DISCUSSION

One of the first observed modes effects of MOS was the ability to agglutinate with certain pathogens that have mannose receptors. This agglutination seems to occupy the binding sites of the bacteria preventing colonization of the bird. A previous study demonstrated that chicks supplemented with a specific mannan oligosaccharide had a lower

incidence of *Salmonella* infection than unsupplemented birds (3). These authors went on to show that an in vitro agglutination assay was predictive of in vivo activity.

The low content of mannan in the products from source B may explain the inability of the products from source B to agglutinate any of the *Salmonella* strains examined. It should be noted that the products from source A are marketed as mannan oligosaccharide while only implications of mannan oligosaccharide-like properties are used with the products from source B. From these results it appears that agglutination of *Salmonella* strains for products from source B does not appear to occur. The weak agglutination of certain *Salmonella* strains from source A may indicate that these strains contain glucose-receptors, since there is a glucan fraction in MOS products. The actual carbohydrate receptors present on these isolates requires further investigation as there may be mannan and glucan receptors present or only glucan receptors on these *Salmonella* isolates.

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Table 1. Glucan and mannan content of MOS product examined.

Sample ID	Glucan Content (%) DWB	Mannan Content (%) DWB
A-1	28.2	17.2
A-2	22.9	15.7
B-1	42.6	5.4
B-2	42.5	6.4
B-3	30.7	11.3
B-4	28.1	10.4
B-5	28.8	10.5

Table 2. Agglutination characteristics of mannan oligosaccharide products on *Salmonella* isolates obtained from commercial poultry facilities.

Sample ID	Product A-1	Product A-2	Product B-1	Product B-2	Product B-3	Product B-4	Product B-5
Salmonella Grp C2 (S. Kentucky)	**Yes**	**Yes**	**None**	**None**	**None**	**None**	**None**
Salmonella Grp C1	**Yes**	**Yes**	**None**	**None**	**None**	**None**	**None**
Salmonella poly (S. Kentucky)	**Yes**	**Yes**	**None**	**None**	**None**	**None**	**None**
Salmonella Grp B	**Weak**	**Weak**	**None**	**None**	**None**	**None**	**None**
Salmonella Grp C2	**Yes**	**Yes**	**None**	**None**	**None**	**None**	**None**
Salmonella poly (S. kentucky)	**Yes**	**Yes**	**None**	**None**	**None**	**None**	**None**
Salmonella Grp D (S. enteritidis)	**Weak**	**Weak**	**None**	**None**	**None**	**None**	**None**
Salmonella Grp C1 (S. mbandaka)	**Yes**	**Yes**	**None**	**None**	**None**	**None**	**None**
Salmonella Grp B	**Yes**	**Yes**	**None**	**None**	**None**	**None**	**None**
Salmonella Grp B	**Weak**	**Yes**	**None**	**None**	**None**	**None**	**None**
Salmonella Grp C2 (S. kentucky)	**Yes**	**Yes**	**None**	**None**	**None**	**None**	**None**
Salmonella Grp D (S. enteritidis)	**Yes**	**Yes**	**None**	**None**	**None**	**None**	**None**
Salmonella Grp F (S. anatum)	**Yes**	**Yes**	**None**	**None**	**None**	**None**	**None**
Salmonella Grp D	**Yes**	**Yes**	**None**	**None**	**None**	**None**	**None**
Salmonella Poly (S. kentucky)	**Yes**	**Yes**	**None**	**None**	**None**	**None**	**None**
Salmonella Grp B (S. Heidelberg)	**Yes**	**Yes**	**None**	**None**	**None**	**None**	**None**
Salmonella Grp B (S. Heidelberg)	**Yes**	**Yes**	**None**	**None**	**None**	**None**	**None**
Salmonella Grp B (S. enteritidis)	**Weak**	**Yes**	**None**	**None**	**None**	**None**	**None**
Salmonella Grp C2 (S. kentucky)	**Yes**	**Yes**	**None**	**None**	**None**	**None**	**None**
Salmonella Grp D (S. enteritidis)	**Yes**	**Yes**	**None**	**None**	**None**	**None**	**None**

PATHOGENESIS OF EMERGING TURKEY ARTHRITIS REOVIRUS

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INTRODUCTION

Reoviral arthritis emerged in the U.S. commercial turkeys in 2011 and outbreaks have continued to occur in the Midwest (1). This has led to significant financial losses to the turkey industry (2). Turkey arthritis reovirus is genetically and antigenically different from vaccine strains being used in chickens (S1133, 1733, and 2048).

Two types of turkey reoviruses have been reported: turkey enteric reoviruses (TERVs) and turkey arthritis reoviruses (TARVs). TERVs and TARVs cannot be differentiated based on full genome sequence analysis. In recent studies conducted by a research group in Minnesota, reoviruses that were originally isolated from hock joints were re-isolated from tendons of commercial poults challenged by different routes at one week of age (3, 4, 5). Our main objective is to establish an SPF turkey model to assess TARV pathogenesis. This is an important step toward developing preventive measures including vaccines.

MATERIALS AND METHODS

Viruses. TARV-O'Neil and TERV-MN1 were provided by Drs. Sagar Goyal and Jack Rosenberger. They were grown in QT-35 cells.

RNA extraction and RT-PCR. RNA was extracted from intestinal and tendon homogenates using RNeasy Mini Kit (QIAGEN). RT-PCR was done as previously described (6).

Serology. Samples were sent to the Animal Disease Diagnostic Laboratory at the Ohio Department of Agriculture for detection and titration of reovirus antibodies by ELISA (IDEXX, Westbrook, Maine, USA).

Histologic inflammation scoring. Formalin-fixed and decalcified hock joints with attached tendons were sent to the Comparative Pathology and Mouse Phenotyping facility at the Ohio State University for H&E slide preparation. The slides were read by a pathologist (Dr. R. E. Porter, University of Minnesota) who was blinded to the grouping and the infection status of individual birds.

Experimental design. Groups of one week old SPF poults from an egg line flock (n=24 per group) were inoculated via the oral route with 0.2 mL of medium or virus preparation. Each virus dose contained about 5×10^6 $TCID_{50}$. This flock is maintained free from known turkey pathogens including reovirus. At one week post infection (WPI), eight poults from each group (control, TARV-O'Neil, and TERV-MN1) were euthanized to collect tendon tissues (gastrocnemius and digital flexor tendons) and intestinal contents for virus detection by RT-PCR and isolation (up to three blind passages) in QT-35 cells. In addition, the hock joint and intestines were collected and fixed in 10% neutral-buffered formalin for histopathological examination. At four and nine WPI, the remaining poults were bled, euthanized, and samples collected as done at one WPI.

RESULTS AND DISCUSSION

In vivo studies were conducted in SPF turkeys to provide experimental baseline data to better understand the pathogenesis of reovirus and develop appropriate control strategies.

Virus detection. Table 1 shows reovirus detection from distal part of gastrocnemius and digital flexor tendons and intestines by RT-PCR using primers that amplify full-length S4 gene (6) and by virus isolation in QT35 cells. The RT-PCR was less sensitive in detecting reovirus compared to the QT-35 cell-based virus isolation. None of the samples from mock-infected birds was virus RT-PCR positive at one and four WPI and no attempt was made to isolate virus from these samples. Intestinal tissues and contents sampled at one and four WPI were found to be RT-PCR negative and were not subjected to virus isolation. TARV O'Neil was detected in the gastrocnemius tendon more frequently than TERV-MN1 at the early time points (one and four WPI). Notably, TARV-O'Neil was detected in tendon samples from all eight poults sampled at four WPI suggesting that orally inoculated virus may take

up to four weeks to spread to the hock joint. Both viruses were detected at similar levels at nine WPI (five or six of eight birds). Although TARV-O'Neil detection in tendons was previously shown to peak at four to eight weeks of age (three to seven WPI) the rate of detection from individual birds was 50% or less (5). A follow up experiment will shed more light on how SPF turkeys compare with commercial turkeys as a model for reoviral arthritis. Since both TARV and TERV were detected in the intestines in previous studies (3, 4, 5, 7), it is possible that some of the intestinal samples generated in this study will test positive in QT-35 cells.

Seroconversion. Serum antibody titers were measured at four and nine WPI to confirm virus infection and to provide baseline data for future vaccine development. As expected, none of the uninfected controls had reovirus antibodies at four and nine WPI. All infected birds had seroconverted by four WPI. However, the rate of seroconversion and increase in antibody titers was slower in birds infected with TERV-MN1 compared to the TARV-O'Neil group. The levels of antibody titers correlate well with the rates of virus isolation at both time points (Table 1). Future research should examine the role of humoral immune response in TARV pathogenesis.

Body weight. Reovirus infection has been associated with growth depression in turkey poults (8, 9). In this study, weight gain depression was not observed at one and four WPI. However, at nine WPI, TARV-O'Neil-infected turkeys had significantly less weight compared to the uninfected control group. A similar observation was reported in commercial poults where weight gain depression by TARV-O'Neil occurred at 12 weeks of age (11 WPI) onward (5).

Lameness and swollen hock joints. For the entire duration of the experiment (infection for nine weeks), none of the turkeys in any of the three groups developed lameness or swollen hock joint. TARV-O'Neil was reported to cause significant lameness in commercial turkeys at 8-16 weeks of age (i.e., 7-15 WPI) (5). Further, under field conditions, the onset of reovirus-associated lameness occurs at 10 week-old or older turkeys (2). It is possible that the disease can be reproduced in the SPF turkeys if the experimental timeline is extended beyond nine WPI.

Histologic inflammation. The control and TERV-MN1 groups did not show histologic inflammation at one and four WPI. However, and in line with previous observations (3, 4, 5), inflammation started to emerge in TARV-O'Neil infected poults at one WPI and progressed to higher level at four WPI.

Additional effort will also be made to reproduce the disease in younger (one day-old) or immunosuppressed (dexamethasone-treated) SPF turkeys.

(The full article will be published in *Avian Diseases* or elsewhere.)

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Table 1. Detection of reovirus from gastrocnemius tendon and intestines.

Detection Method	Group	1 week p.i.		4 weeks p.i.		9 weeks p.i.	
		Intestine	**Tendon**	**Intestine**	**Tendon**	**Intestine**	**Tendon**
RT-PCR	TERV MN1	0/8*	1/8	0/8	2/8	NT	2/8
	TARV O'NEIL	0/8	1/8	0/8	7/8	NT	2/8
	Mock	0/4	0/4	0/4	0/4	NT	0/5
Isolation	TERV MN1	NT	2/8	NT	2/8	NT	6/8
	TARV O'NEIL	NT	5/8	NT	8/8	NT	5/8
	Mock	NT	NT	NT	NT	NT	NT

*Number of birds positive for reovirus by RT-PCR or CPE/total number of birds; NT, Not Tested; p.i., post infection

ASSESSMENT OF THE COMPATIBILITY BETWEEN THE VECTOR RHVT-F AND RHVT-H5 VACCINES IN SPF AND COMMERCIAL CHICKENS

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Avian influenza (AI) and Newcastle disease (ND) are the most devastating diseases affecting the poultry industry. Traditionally, the prevention of these two diseases, in endemic countries, is based on biosecurity and vaccination.

Conventional inactivated vaccines against avian influenza present some limitations such as interference with maternally derived antibodies (MDA-AIV), narrow spectrum of protection and relatively short duration of immunity. Likewise, inactivated vaccines against Newcastle disease are affected by MDA-NDV. Additionally, live attenuated ND vaccines can induce undesirable vaccine reactions with negative consequences to the performance of the flocks.

Vector HVT-based vaccines against these two diseases were developed to overcome the passive immunity and induce a more reliable, broader spectrum and longer lasting immunity than classical, live or inactivated, vaccines. Due to these advantages, the field use of the rHVT-H5 (avian influenza) and rHVT-F (Newcastle disease) vector vaccines has increased remarkably over the past few years.

When applied separately, it has been shown these vector vaccines induce very high levels of protection against ND and AI of H5 subtype, respectively (1, 5, 6, 7). However, as both vaccines are indicated for in-ovo or subcutaneous injection at the hatchery, questions regarding their compatibility have arisen.

Studies have already demonstrated some degree of interference between the rHVT-F and a rHVT-VP2 (infectious bursal disease) or between the rHVT-F and rHVT-LT (infectious laryngotracheitis) when administered simultaneously to commercial broilers (3, 4).

Therefore, several trials have been conducted to evaluate the compatibility of the simultaneous use of rHVT-F and rHVT-H5 vaccines in SPF chickens and commercial broilers and the results of clinical protection are summarized in the Table 1.

In addition to the clinical protection, other parameters such as humoral and cell-mediated immune responses and re-excretion of the challenge virus were investigated (data not shown). Based on these criteria, although reduced values of some of the parameters investigated were observed, it can be concluded that the interferences between rHVT-F and rHVT-H5 were acceptable hence the vaccines can be administered simultaneously.

The reasons for compatibility or interference between HVT-based vector vaccines are not fully understood. In the case of the rHVT-F and rHVT-H5 vaccines, it seems that the compatibility is linked to the very high immunogenicity of the immunogenic epitopes (F and HA) expressed by these vaccines. Further investigation is needed.

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Table 1: Protection rates of SPF birds and commercial broilers vaccinated with rHVT-F, rHVT-H5 and combination of rHVT-F & rHVT-H5 and challenged with velogenic ND virus and HPAIV H5N1

Trial	Type of bird	MDA^{NDV} (log_2)	MDA^{AIV} (log_2)	Vaccine	Challenge						Clinical Protection		References
					Age (weeks)	Route	Newcastle Disease		Avian Influenza		Newcastle Disease	Avian Influenza	
							Strain	Dose ($log_{10}ELD_{50}$/bird)	Strain	Dose ($log_{10}ELD_{50}$/bird)			
1	SPF chickens	-	-	rHVT-F rHVT-H5 rHVT-F & rHVT-H5 Control	4 / 8	Oculo-nasal	Malayian genotype VIIh isolate	5.0	Egypt 2008 H5N1 HPAIV Clade 2.2	6.0	100 / 100 - 100 / 100 0 / 0	- 100 / 100 100 / 90 0 / 0	Rauw *et al.* 2013 (WVPA Nantes)
2	Commercial broilers	yes	yes	rHVT-F rHVT-H5 rHVT-F & rHVT-H5 Control	3 / 4 / 7 / 10	Oculo-nasal	Malayian genotype VIIh isolate	5.0	Egypt 2008 H5N1 HPAIV Clade 2.2	6.0	90 / 100 / 100 / 100 - 85 / 85 / 95 / 100 0 / 0	- nd / 0 / 100 / 80 nd / 20 / 950 / 90 0 / 0	Rauw *et al.* 2013 (WVPA Nantes)
3	Commercial broilers (?)	yes	yes	rHVT-F rHVT-H5 rHVT-F & rHVT-H5 Control	3 / 4	intra-nasal	Genotype VII isolate	6.0	Egypt H5N1 HPAIV Clade 2.2.1	6.0	94 / 100 - 40 / 60 0 / 0	- 86 / 94 87 / 94 0 / 0	Setta (Unpublished data)
4	Commercial broilers	yes	yes	rHVT-F & rHVT-H5 (+ live ND at 14 days) Control	4.5	intra-nasal	vvNDV	6.0	HPAIV H5N1	6.0	79 0	100 0	Elbestawy *et al.* 2015 (9th ISAI, Athens GA)

ASSESSMENT OF THE PROTECTION INDUCED BY A VECTOR RHVT-F VACCINE AGAINST DIFFERENT GENOTYPES OF NEWCASTLE DISEASE VIRUS

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ABSTRACT

Although all Newcastle disease virus (NDV) isolates belong to one single serotype, with the development of molecular diagnostic tools and their routine use in the research laboratories and in the field, NDV isolates have been classified based on genotyping.

Recently, a vector vaccine (rHVT-F) that expresses the F gene obtained from a genotype I NDV has been made commercially available in different regions where a broad range of NDV genotypes are prevalent.

Studies on birds exclusively vaccinated with this vector vaccine (rHVT-F) and challenged with virulent NDV isolates from Asia, Latin America, Africa, Middle East, and reference challenge strains from the USA and European Union showed that this vector vaccine induces strong clinical protection regardless of the genotype used.

INTRODUCTION

Newcastle disease (ND) is a highly contagious and often severe disease found worldwide that affects birds including domestic poultry and is caused by a virus belonging to the family of paramyxoviruses (APMV-1).

Different isolates of NDV may induce strong variation in the severity of disease and they have even been divided into categories according to tropism, pathotype and virulence such as viscerotropic velogenic, neurotropic velogenic, mesogenic, lentogenic and asymptomatic-enteric strains.

More recently, with the development of molecular diagnostic tools and their routine use in the research laboratories and in the field, NDV isolates have been classified based on genotyping. Today, the most prevalent genotypes are V and VII. Genotype V is widespread in Central America and genotype VII in Middle-East, Asia, Africa, and also South-America.

On the other hand, the rHVT-F ND vaccine expresses the F gene obtained from a genotype I NDV. Although all ND isolates belong to one single serotype, questions about cross-protection against different genotypes have been raised. In order to determine the protection induced by this vector vaccine against these genotypes, several challenge studies were conducted using commercial broilers, commercial layers, commercial turkeys and SPF birds.

MATERIALS AND METHODS

The vaccine. The rHVT-F ND vaccine uses the herpesvirus of turkeys, strain FC 126, as the vector and in which genome the Fusion (F) gene extracted from the a genotype I, D-26 strain of NDV has been inserted in between UL 45 & 46 genes and the promoter Pec ensures the expression of the F gene. This vaccine is recommended to be applied either by in-ovo or subcutaneous routes.

Animals and vaccination. Day-old chicks of different types (SPF, commercial broilers and commercial layers) and commercial day-old poults were vaccinated at day of age with a rHVT-F ND vector vaccine by subcutaneous route. No other ND vaccine was applied to these birds. In all trials, a group of birds were left unvaccinated to serve as controls.

Challenges. At different challenge ages, which varied from one to 72 weeks, these birds were challenged by different routes, including intramuscular, intra-nasal, oculo-nasal and choanal routes and doses varying from 3.5 to 5.0 $log_{10}ELD_{50}$/bird, with ND virus classified as belonging to different genotypes (Figure 1):

a. Genotype II: Hitchner B1 vaccine strain and Texas GB strain;
b. Genotype IV: Herts 33/56 and JEL strains;
c. Genotype V: APMV1/chicken/Mexico/D516/1/2008 isolate and Chimalhuacan strain;
d. Genotype VII: Lopburi strain;

e. Genotype VIIa: D1598/1/11/PH and D1675/1/11/ID isolates;
f. Genotype VIIb: D575/6/05/PE isolate;
g. Genotype VIId: D1435/3/3/SA/10, D1500/2/1/10/CN and GPMV 171/06/ZA (Goose Paramyxovirus) isolates;
h. Genotype VIIh: D1524/1/1,2/MY/10 isolate;
i. Genotype VIII: RB Daagstam ND/01/ZA isolate.

RESULTS

The Table 1 summarizes the challenge results with a lentogenic and a velogenic strains belonging to genotype II and velogenic isolates belonging to genotypes IV, V, VIIa, VIIb, VIId, VIIh and VIII that were obtained from different parts of the world.

The onset of immunity induced by the rHVT-F vaccine depends directly on the replication of its vector (HVT) and consequently on the gradual expression of the F gene of NDV. From these challenge results, the clinical protection was detected as early as two weeks after vaccination and progressively increased with time reaching very significant levels at three weeks and full protection at four weeks of age onwards (Table 1, trials 3, 4, 9, 10, 12, 14, 18, 20, 21, 22). Because of this progressive induction of immunity, it is necessary to reinforce the early protection in those areas where the ND challenge is high and this needs to be done by spraying a live ND vaccine at day of age (in the hatchery). Depending on the case, a field booster at around 10-15 days of age can be recommended.

Additionally, HVT remains in the birds for their whole life and its replication constantly boosts the ND protection inducing long-lasting immunity. From the trials 19 and 22, 100% of clinical protection was reached up to 72 weeks of age.

Finally, the rHVT-F vaccine induced complete clinical protection against ND isolates from genotypes II (Texas GB strain and HB1 vaccine strain), genotype IV (Herts 33/56 and JEL strains), genotypes V, VII, VIIa, VIIb, VIId, VIIh and VIII, from Morocco, Mexico, Thailand, Philippines, Peru, China, Malaysia, Saudi Arabia and South Africa, demonstrating its ability to induce cross-protection against distinct genotypes. In fact, these results confirm what is well known: despite of been classified into different genotypes, NDV isolates belong to one single serotype.

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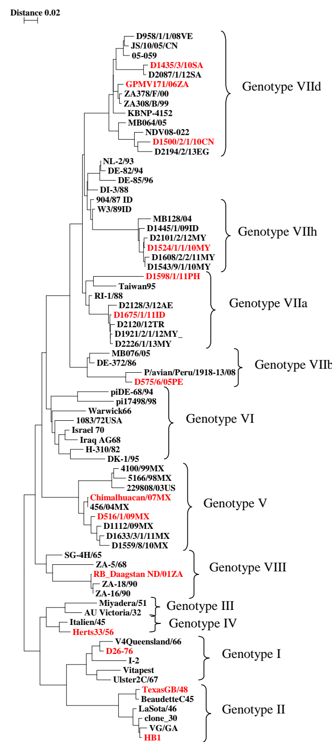
Table 1: Protection rates of SPF birds, commercial broilers, commercial layers and commercial turkeys vaccinated with rHVT-F and challenged with different ND genotype isolates

Trial	Genotype	Strain	Type of bird	Challenge			Protection rate (%)	
				Age (weeks)	Dose ($log_{10}ELD_{50}$/bird)	Route	Vaccinated	Controls
1	II	Texas GB strain	SPF chickens	4	4.0	Intramuscular	100	0
2	II	Texas GB strain	Commercial layers	19	4.0	Intramuscular	100	0
3	II	HB1 vacine strain	Commercial broilers	1 / 2 / 3 / 4 / 5	3.5	Ocular	0 / 10 / 60 / 90 / 100	0 / 0 / 0 / 0 / 0
4	II	HB1 vacine strain	Commercial turkeys	1 / 2 / 3 / 4 / 5	3.5	Intra-nasal	100 / 67 / 33 / 80 / 100	44 / 0 / 0 / 0 / 0
5	IV	Herts 33/56	SPF chickens	4	5.0	Intra-nasal	100	0
6	IV	Herts 33/56	Commercial broilers	3	5.0	Intramuscular	95	0
7	IV	JEL strian	SPF chickens	6	5.0	Ocular	100	0
8	V	APMV1/chicken/Mexico/D516/1/2008	SPF chickens	4	5.0	Intra-nasal	100	0
9	V	APMV1/chicken/Mexico/D516/1/2008	Commercial broilers	3 / 4 / 6	5.0	Oculo-nasal	81 / 95 / 100	0 / 0 / 0
10	V	Chimalhuacan strain	Commercial broilers	2 / 3 / 4 / 5	5.0	Oculo-nasal	0 / 84 / 100 / 100	0 / 0 / 0 / 0
11	V	Chimalhuacan strain	Commercial layers	5	5.0	Oculo-nasal	70	0
12	VII	Lopburi	Commercial layers	2, 3 ,4	5.0	Intramuscular	90 / 100 / 100	90 / 70 / 10
13	VIIa	D1598/1/11/PH	SPF chickens	4	5.0	Intra-nasal	100	0
14	VIIa	D1675/1/11/ID	Commercial layers	3 / 5 / 8 / 10	5.0	Intra-nasal	48 / 90 / 100 / 100	0 / 0 / 0 / 0
15	VIIb	D575/6/05/PE	SPF chickens	4	5.0	Intra-nasal	100	0
16	VIId	D1435/3/3/SA/10	SPF chickens	4	5.0	Intra-nasal	100	0
17	VIId	D1500/2/1/10/CN	SPF chickens	4	5.0	Intra-nasal	100	0
18	VIId	GPMV 171/06/ZA (Goose Paramyxovirus)	Commercial broilers	3 / 4 / 5	5.0	Choanal route	100 / 100 / 100	0 / 0 / 0
19	VIId	GPMV 171/06/ZA (Goose Paramyxovirus)	Commercial layers	72	5.0	Intra-nasal	100	0
20	VIIh	D1524/1/1,2/MY/10	SPF chickens	2 / 3 / 4 / 6 / 8	5.0	Intra-nasal	95 / 90 / 100 / 100 / 100	0 / 0 / 0 / 0 / 0
21	VIIh	D1524/1/1,2/MY/10	Commercial broilers	2 / 3 / 4 / 6	5.0	Intra-nasal	25 / 68 / 95 / 100	5 / 0 / 0 / 0
22	VIIh	D1524/1/1,2/MY/10	Commercial layers	3 / 4 / 6 / 10 / 15 / 25 / 34 / 40 / 55 / 72	5.0	Intra-nasal	74 / 95 / 100 / 100 /100 / 100 / 100 / 100 /100 / 100	20 / 0 / 0 / 0 /0 / 0 / 0 / 0 /0 / 0
23	VIII	RB Daagstan ND/01/ZA	SPF chickens	4	5.0	Intra-nasal	100	0

n.a. (not available)

Figure 1: Phylogenetic analysis of the NDV based on the 374 base pairs long (47-420 bp) nucleotide sequence of the F gene of NDV.



PATHOGENICITY OF 2015 NORTH AMERICAN H5N2 HIGHLY PATHOGENIC AVIAN INFLUENZA POULTRY ISOLATES IN CHICKENS AND MALLARDS

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INTRODUCTION

In late 2014 a reassortant H5N8 clade 2.3.4.4 highly pathogenic avian influenza (HPAI) virus spread by migratory waterfowl into Europe and North America further reassorting with North American low pathogenicity avian influenza viruses to produce a H5N2 HPAI virus (1, 2, 3). This virus subsequently spread through Midwestern U.S. causing severe outbreaks in poultry in 2015. In order to examine for changes in host adaptation, we evaluated the infectivity, transmissibility, and pathogenicity of H5N2 HPAI viruses isolated from the Midwest poultry outbreaks in 2015 in chickens and mallards and compared the results with those obtained with the index H5N2 wild bird isolate (A/Northern pintail/Washington/2014).

MATERIALS AND METHODS

Infectious dose, pathogenicity and transmission studies were conducted using the following H5N2 HPAI poultry isolates: A/Tk/MN/12582/2015, A/Tk/SD/12511/2015, A/Ck/IA/13388/2015, and A/Tk/AR/7791/2015 (obtained from the National Veterinary Services Laboratory, USDA, Ames, IA). Four week old SPF White Leghorn chickens and two week old commercial mallards (*Anas platyrhynchos*) were separated into control groups and virus-inoculated groups. Treatment groups contained 5-10 birds and were inoculated by the intranasal route (choanal cleft) with 10^2, 10^4 or 10^6 50% egg infectious doses (EID_{50}) per bird of each virus. Contact-exposure birds were added to the virus inoculated groups at one day post inoculation (DPI) to examine for virus transmission. Oropharyngeal and cloacal swabs were collected from directly inoculated and contact birds at one, two, three, and four DPI for chickens, and at 2, 4, 7, 11 and 14 DPI for ducks. Blood was collected from all surviving birds at 14 DPI for serology. Two birds from the groups inoculated with the 10^6 dose were necropsied at two DPI and tissues collected for microscopic evaluation and virus replication.

RESULTS AND DISCUSSION

Three of the H5N2 poultry isolates (Tk/MN/15, Tk/SD/15, and Ck/IA/15) required a lower dose of the virus to infect and kill chickens than the index H5N2 virus. The mean bird infectious dose (BID_{50}) for these viruses was between $10^{3.2}$ and $10^{3.6}$ EID_{50} compared to the wild bird index virus which was $10^{5.7}$. The fourth isolate (Tk/AR/15) had a similar BID_{50} ($10^{5.1}$) as the index virus, indicating that it was most likely a wild bird introduction. The mean death time for the chickens that died in all groups was between two and four days, similar to the mean death time for the index virus. Also, similarly to the index virus, the H5N2 poultry isolates transmitted poorly to contact exposed chickens, with the exception of the group that received the high dose of Tk/MN/15 in which the contacts became infected. All H5N2 poultry viruses replicated systemically with the less viral staining observed in tissues from the birds inoculated with Tk/AR/15.

All mallards inoculated with Tk/MN/15 or Ck/IA/15 became infected regardless of the dose administered ($BID_{50} \leq 10^2$), similar to what was observed with the H5N2 wild bird index virus. All contact ducks became infected, with the exception of two ducks from the group inoculated with the low dose of Ck/IA/2015, demonstrating the easy transmission of these H5N2 viruses among ducks. Mallards inoculated with Ck/IA/15 showed no clinical signs and birds shed lower amounts of virus and for a shorter period of time than mallards inoculated with Tk/MN/15 or the index virus, and also showed less virus replication in tissues. Interestingly, some ducks inoculated with Tk/MN/2015 presented neurological signs and two died, and virus was detected up to 14 DPI in oral and cloacal swabs, indicating that this virus was more virulent for ducks than the index wild bird virus.

CONCLUSION

The later H5N2 poultry-origin viruses showed increased infectivity for chickens compared to the earlier H5N2 wild bird-origin virus. In mallards, the poultry isolates were as infective as the index virus; however the Tk/MN/15 isolate was more virulent than the index virus causing disease and death in some ducks. The Ck/IA/15 virus, although also infectious for ducks, did not replicate as well as the index virus. In conclusion, the H5N2 poultry isolates, as expected, were more adapted to chickens than the index wild bird isolate, indicating adaptation after circulating in poultry. However, adaptation to poultry did not necessarily affect the ability of the virus to replicate in the mallards.

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MANAGEMENT IS KEY IN LIVE COCCIDIOSIS VACCINATION

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SUMMARY

Live coccidiosis vaccines are currently used to combat coccidiosis in Canadian “raised without antibiotics” poultry production. Since these vaccines use live parasites, environmental factors that impact parasite infectivity, ingestion and transmission apply during vaccination. Protection against coccidiosis is best accomplished with uniform vaccine administration and environmental conditions that promote parasite re-ingestion (“cycling”); vaccine administration is largely beyond the control of producers but management can certainly affect cycling. Several studies assessed variation in vaccine ingestion and explored environmental factors (e.g. housing, humidity) influencing vaccination and protection against challenge. The latter studies focused on conventional cage modifications (40% cage floor coverage with fibrous material) for replacement layer pullets to enhance cycling and vaccine efficacy. This research demonstrated that successful vaccination can be achieved through a minor environmental modification and maintaining environmental conditions that support parasite cycling, even if vaccination was non-uniform. Understanding the parasite life cycle and applying this knowledge can improve live vaccination success in poultry production, including layer pullets.

INTRODUCTION

Chicken intestinal health and performance can be affected in the commercial industry by a triad of factors: environment, management, and intestinal disease (e.g. coccidiosis). Coccidiosis, caused by *Eimeria* species parasites, costs producers through negative impact on flock performance and coccidiosis control programs (1). Anticoccidial drugs remain a common method of coccidiosis control; however, the use of alternative prevention methods are increasing driven by legislation restricting in-feed antimicrobials as well as retailer and consumer demands. A common alternative coccidiosis prevention method is live coccidiosis vaccination.

EIMERIA SPECIES

The typical eimerian lifecycle consists of an endogenous and an exogenous phase (2). The environmentally resistant infective stage of the parasite, the oocyst, is ingested to initiate the endogenous phase of the lifecycle. The mechanical action of the gizzard as well as the chemical action of the upper intestinal tract releases the infective components of the parasite (3, 4). The parasite then infects host intestinal epithelial cells and undergoes a predetermined number asexual cycles. Once asexual replication is completed, sexual replication will occur to form an uninfective oocyst that is shed in the feces. The shed oocyst only becomes infective during this exogenous phase of lifecycle after approximately 22 to 77 hours (5), depending on the species, with a supportive temperature (4-37°C; optimum ~29°C), relative humidity and oxygen access. Once infective, the oocyst is available for ingestion by a bird to initiate another endogenous cycle.

LIVE COCCIDIOSIS VACCINATION

Eimeria species are transmitted fecal-orally (“cycling”) and, unlike most pathogens, the number of parasites ingested by immunologically naïve birds controls the number of parasites shed and infection severity (6). Live coccidiosis vaccination exploits species-specific protective immunity (PI) that blocks parasite development; PI is acquired naturally from repeated infections with low numbers of *Eimeria* parasites cycling among birds (6, 7).

To achieve successful PI through vaccination two factors must be considered. The first factor is a vaccine administration that provides synchronous, uniform ingestion of infective parasites (8). Various vaccine administration methods are available for live coccidiosis vaccines (9); however, commonly used methods are through water or gel droplet application. This type of application relies on the chick directly ingesting the vaccine oocysts and, indirectly, preening the colored vaccine off of other chicks in the same chick tray. The indirect nature of this type of vaccine delivery suggests there is an inherent potential for variation in initial oocyst ingestion. Individual measurements of oocysts per gram of feces (OPG) at six days post vaccine inoculation,

when most vaccine oocyst progeny are shed, demonstrated that of the 59 samples assessed some chicks shed no detectable oocysts, some shed ≤ 10,000 OPG and some chicks shed >100,000 OPG (10). These results suggest a range of vaccine oocysts are ingested and achieving uniform live coccidiosis administration is challenging. The second factor to achieve successful coccidiosis vaccination is environmental control in the barn that should allow for controlled low-level vaccine progeny (oocysts) cycling to provide second and subsequent infectious oocyst exposures (11). To achieve the continuous and controlled cycling, the vaccine progeny oocysts must become infectious and remain viable for ingestion, preferably over an extended period of time, and be ingested to continue cycling among birds. Consequently, environment and management can influence the infectivity, survival and accessibility of oocysts required for successful cycling.

MANAGEMENT IN THE BARN

Environmental control in the barn can be separated into the atmospheric and the physical barn environments (12) and both contribute to the oocyst becoming infective and cycling.

The atmospheric barn environment consists of factors required for the oocyst to become infective, such as oxygen access, temperature and relative humidity. Relative humidity can vary during production, between barns and between seasons (10). Decreased relative humidity during periods critical for oocyst cycling can negatively impact live vaccination success. Price et al. (10) demonstrated that a decrease in relative humidity levels (13% to 19%) during the first two weeks dramatically reduced oocyst cycling and birds were not protected against a coccidial challenge. As a result, measurement of relative humidity in the barn after vaccine administration, regardless of the rearing environment, may be a tool to help predict the relative success of the live vaccination process (10).

The physical barn environment consists of the housing and management of poultry to permit low-level oocyst transmission. Each poultry barn has the potential to provide an environment suitable for the oocyst to become infective and the chickens to ingest infective oocysts. However, not all poultry barns provide equal availability and duration of availability of infective oocysts for the housed chickens.

A single infected bird may transmit *Eimeria* species to as many as 3.4 susceptible birds when housed on litter because this environment promotes fecal-oral transmission (6, 13-14). Nevertheless, time for sufficient oocyst cycling is needed for birds reared on litter. Fetterer et al. (15) demonstrated that broilers artificially administered uniform numbers of oocysts only developed protection against challenge infection after three weeks of age; protection was measured by oocyst output, body weight gains and plasma carotenoids. At two weeks of age, protection was variable and some birds were still susceptible to infection. These authors (15) also examined the impact that non-uniform vaccination (e.g. 75% of chicks were administered a single *Eimeria* species and reared with non-inoculated cohorts) had on protection. While protection against a challenge was elicited for all birds in the non-uniform dosed birds, the non-inoculated cohorts had lower levels of protection parameters compared to the vaccine-inoculated cohorts. These results suggest that even more time and oocyst cycling may be needed for birds missed during initial vaccination.

A common misconception is that the risk of coccidiosis outbreaks is reduced or eliminated in tiered cages used commonly for pullet and layer production (16). However, a tiered caging system reduces but does not eliminate oocyst cycling (17) and coccidiosis outbreaks, sometimes preceding or concurrent with necrotic enteritis, have been reported in stressed birds throughout pullet rearing (17) such as when pullets are moved from the pullet barn to the layer barn (18-19). For pullets and layers housed on mesh floors, achieving and maintaining low-level oocyst cycling following live coccidiosis vaccination is challenging (19; Dr. M. Petrik, personal obs.).

Several cage floor coverage modifications were tested for their ability to enhance within-cage oocyst cycling and improve vaccine efficacy (20-22). Firstly, pullets vaccinated by gavage were reared with 0%, 20%, 40% or 60% of the cage floors covered with biodegradable material (lasting ~5 weeks). Covering 40% of the cage floor was determined to be optimal by increasing both intensity and duration of within-cage oocyst cycling and significantly improving live vaccine efficacy (comparing post-challenge body weights, oocyst shedding and lesion scores) with negligible impact on animal welfare. Further experiments tested if 40% cage floor coverage could enhance cycling and vaccine efficacy with non-uniform vaccine dosing (by gavaging subsets of pullets or by using spray vaccination). Covering 40% of the cage floors increased within-cage oocyst cycling (as measured by oocyst shedding) and significantly enhanced vaccine efficacy (as measured by post-challenge body weights, oocyst shedding and lesion scores) even following non-uniform dosing. Collectively, this research demonstrated that live *Eimeria* vaccines can be used effectively with caged-reared pullets. Successful vaccination can be achieved by

introducing a minor environmental modification (40% cage floor coverage with folded chick paper) and maintaining barn environments (adequate humidity) that support parasite cycling among pullets, even if initial vaccination was non-uniform.

As consumers and retailers are publically scrutinizing conventional cage systems, especially for layer hens, some organizations are moving towards alternative systems such as enriched colony or aviary houses. Each of these systems provides new challenges for intestinal health, especially coccidiosis. Consequently, understanding the transmission and cycling dynamics for live coccidiosis vaccination must be further investigated to better prepare for these industry changes.

MANAGEMENT AND SUPPORTING INTESTINAL HEALTH

Cycling of vaccine oocysts is a fine balance between promoting sufficient cycling for PI to develop while restricting excessive cycling that could generate subclinical or clinical disease. Ensuring the intestinal health of the bird is supported may allow the bird to be better suited to respond to vaccination and achieve optimum performance (6, 23). As a result, maintaining intestinal health during live coccidiosis vaccination as well as management of oocyst cycling during production are important considerations.

CONCLUSION

Understanding the parasite lifecycle, transmission dynamics and oocyst cycling in different housing systems following live coccidiosis vaccination may help to encourage proactive management that will enhance live vaccine success. Regardless of the poultry production system being managed, the physical (e.g. housing of poultry) and atmospheric (e.g. relative humidity) environments, and intestinal health of the flock will impact vaccine success; thus, management during coccidiosis vaccination must become comprehensive to ensure success.

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QUORUM SENSING INHIBITORS TO ENHANCE THE CONTROL OF AVIAN PATHOGENIC *E. COLI*

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SUMMARY

Colibacillosis caused by avian pathogenic *E. coli* (APEC) is associated wide range of symptoms including, airsacculitis, peritonitis, synovitis, colisepticemia, etc., (2). In addition APEC also negatively affects weight gain, feed conversion ratio, and carcass condemnation leading to significant economic losses to the industry. Therefore, the control of APEC is crucial for sustainable poultry farming and food security. Currently APEC infection is controlled by managing other predisposing viral diseases combined with other biosecurity measures including the use of antibiotics. Although commercially available vaccine (Poulvac *E. coli*) has been shown to be efficient in reducing morbidity and mortality associated with APEC in poultry, the vaccine does not provide complete protection and mortality can be still significant in the vaccinated broilers that are commonly affected by APEC at four to nine weeks of age. Therefore, while the vaccine decreases mortality and clinical symptoms, the losses due carcass condemnation remain considerably high. Consequently, there is a need for a complimentary approach to Poulvac *E. coli* in order to effectively control APEC in chickens. We hypothesize that using novel small molecule inhibitors of APEC virulence in tandem with the Poulvac *E. coli* vaccine will enhance the APEC control in chickens. The use of small molecules is a highly practical approach because; first, they can be selected to inhibit a specific enzymatic reaction that does not exert selective pressure on the targeted bacterium, which is therefore less likely to develop resistance to these compounds; secondly, small size of small molecules allows them to diffuse readily into target cells, making them highly suitable for mass application, a prerequisite in a commercial poultry setting; and, lastly, these molecules can be modified to enhance qualities desired for specific applications, such as solubility and stability under adverse environmental conditions. Small molecules can be customized to target a pathogen's unique features without exerting toxic side effects on both the host and commensal bacteria.

In a previous study it was showed that that deletion of the *luxS* gene decreased the virulence of APEC by 31.5-fold, reduced the mutant's the ability for cell adherence and invasion by 50.0% and 40.7%, respectively, and negatively impacted survival *in vivo* (1). The deletion of the *luxS* gene also prevented the production of autoinducer-2 (AI-2), which regulates important functions and adaptations in bacteria, including quorum sensing. Here we identified novel compounds that inhibit APEC quorum sensing (QS). Using the bioluminescent indicator *Vibrio harveyi* BB170 (AI-1^{+}, AI-2^{-}), we screened a library of ~4,200 compounds to identify compounds that significantly inhibit QS auto-inducer (AI-2) activity of the APEC. To accomplish this, APEC 078 was grown in the presence of 200 µM of each compounds in a 96 well plate; culture free supernatant was used to assess the AI-2 activity by measuring the bioluminescence from the indicator bacteria. Culture supernatants from the *V. harveyi* BB120 (AI-1^{+}, AI-2^{+}) and *E. coli* DH5α were used as controls. The majority of the compounds did not impact the growth of APEC and only 1.5% (62/4,200 compounds) had 20-100% growth inhibition. Screening supernatants from the rest (that did not affect the growth) identified 10 compounds that significantly inhibited AI-2 activity. These 10 compounds were tested for cytotoxicity to Caco-2 cells; five compounds showed <30% cytotoxicity and the other five compounds showed 30-50% cytotoxicity. Further, most of the compounds (9/10) exhibited no hemolytic activity to sheep RBC. The selected compounds also significantly affected the survival of APEC O78 in HD-11 macrophage cells. In summary, we identified several novel compounds that inhibit APEC quorum sensing (QS). Further characterization of these potential leads would facilitate development of novel therapeutics to augment APEC control in poultry.

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POULTRY NUTRITION AND FUTURE OF THE INDUSTRY WITH CONSUMER DEMANDS

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INTRODUCTION

The poultry industry has advanced remarkably over the past 50 years. In particular, poultry meat production has undoubtedly been the most successful of any animal industry. Production standards of broilers and layers have continually improved over this period, with male broilers currently reaching a live weight of 2.5 kg at 33 to 35 d of age and white egg layers capable of producing 330 eggs in 52 weeks of lay. Genetic selection brought about by commercial breeding companies is responsible for bulk (85% to 90%) of the improvements in broiler growth and advances in nutritional management have provided 10-15% of the changes (4).

The need to achieve and sustain the improvements in genetic potential was the driving force behind the recent advances in poultry nutrition and, there had been continuous refinement in the nutrition and feeding of commercial poultry. Compiling an overview of the advances in nutrition over the past 50 years is a daunting task and beyond the scope of this presentation. In this paper, the key advances in poultry nutrition will be discussed. The overall target is feeding to lower costs and maximize economic efficiency.

Today, the challenges faced by the poultry industry are multiple and include expectations from the consumers and policy makers. The relevance of issues relating to consumer concerns will be discussed briefly and their importance recognised, as the society is becoming increasingly concerned about the environment, food safety, the type of raw materials used (e.g. in-feed antibiotics, genetically modified ingredients, meat and bone meal) and the welfare of the birds producing the food.

PAST ADVANCES IN POULTRY NUTRITION

Defining nutrient requirements. A major challenge in defining the nutrient needs is the fact that they are influenced by a number of factors and are subject to constant changes. Nutrient requirements are influenced by two main factors, namely bird-related factors (genetics, sex and, type and stage of production) and external factors (thermal environment, stress, husbandry conditions). Precision in defining requirements involves accuracy at both these levels. Requirements of major nutrients for various classes of poultry are now available and these developments are made possible largely because of increasing uniformity of genotypes, housing and husbandry practices in the poultry industry.

Historically, the industry has utilized the nutrient requirements recommended in the publication by National research Council (NRC). The most recent publication on poultry was in 1994 and now 22 years old, which is a long period given the genetic advances that have made in both broilers and layers over this period. Currently the recommendations suggested by commercial breeding companies provide guidelines that closely match the requirements of modern bird strains than those recommended by NRC (7).

Of all the dietary components, the most expensive and critical are essential amino acids and energy. Defining the requirements for the ten essential amino acids poses considerable degree of difficulty, but has been made easier by the acceptance of ideal protein concept. Like other nutrients, the requirements for amino acids are influenced by various factors, including genetics, sex, physiological status, environment and health status. However, most changes in amino acid requirements do not lead to changes in the relative proportion of the different amino acids. Thus the actual changes in amino acid requirements can be expressed in relation to a balanced protein or "ideal protein." The ideal protein concept uses lysine as the reference amino acid and the requirements for other essential amino acids are then set as a percentage (or ratio) of the lysine requirement.

The advantage of this system is once the lysine requirements under a variety of conditions are determined the needs of all other essential amino acids can be calculated. This approach has now become an accepted practice in the industry to set the amino acid specifications in feed formulations

Defining nutrient composition and ingredient quality. The principal role of feed ingredients is to provide the nutrients that can be digested and utilized for productive functions by the bird. Over the years,

enormous volume of data has been generated and compiled on the nutrient composition of raw materials. The variability that is inherent to each raw material is also recognized and such variability places pressure on precise feed formulations. Data on variation (or matrixes) are available for the main feed ingredients and applied in feed formulation packages to achieve better precision. A related development is the availability of rapid tests, such as the near infrared reflectance (NIR) analysis, to predict gross nutrient composition and to access the variability in ingredient supplies on an on-going basis.

However, not all of the nutrients in ingredients are available for production purposes and a portion of nutrients are excreted undigested or not utilized. With advances in feed evaluation techniques, data have been accumulating on the availability of nutrients, especially of amino acids and phosphorus, for poultry. In the case of amino acids, a recent development had been the wider use of digestible amino acid concentrations, rather than total amino acid concentrations, in feed formulations (1,8,9). The use of digestible amino acids is particularly relevant to situations where diet formulations consist of a range of poorly digestible ingredients. Formulating diets based on digestible amino acids makes it possible to increase the range and inclusion levels of alternative ingredients in poultry diets. In effect, this approach improves the precision of formulation, may lower feed cost and ensures more predictable bird performance.

The use of appropriate energy system is a critical issue because of the importance of energy to bird performance and diet cost. Despite its limitations, metabolizable energy (ME) has been the system of choice of describing available energy (NE) system, which is a refinement of the ME concept, has received attention from time to time. However, no real progress has been made in determining the NE of raw materials for poultry. In theory, NE will more closely describe the energy available in an ingredient for bird's metabolic functions and is more predictive of animal performance. It is, however, difficult to assay, costly and time consuming, and has limited use in the routine screening of ingredients. Also to be acceptable to the commercial industry, formulations based on NE values should demonstrate an economic advantage over the current system.

Better feed formulation. Once the nutritional needs are defined, the next step is to match these needs using combinations of ingredients and supplements. The object of formulation is to derive a balanced diet that will provide appropriate quantities of available nutrients at least cost. Given the range of possible ingredients and nutrients involved, a large number of arithmetical calculations are needed to produce a least-cost diet.

Over the years, feed formulation has evolved from a simple balancing of few feedstuffs for limited number of nutrients to computer-aided linear programming systems. Currently newer systems of stochastic non-linear program are becoming popular with the commercial availability of this formulation software. Because variability in ingredient composition is non-linear, stochastic programs address this issue in the most cost-effective manner possible.

A related development is the use of growth models to simulate feed intake and production parameters under a given husbandry condition. Such models are effective tools to compare actual versus potential performance, which can indicate the extent of management or health problems in the flock, and provide economic analysis of alternative feeding regimens. It must be noted, however, the models are only as good as the data sets used to develop them.

Feed additives. Feed additives are products used in animal nutrition for purposes of improving the quality of feed and the quality of food from animal origin, or to improve the animals' performance and health, e.g. providing enhanced digestibility of the feed materials.

In-feed antibiotics have been thus far the most effective and successful additive used by the poultry industry. One could say that in-feed antibiotics are partly responsible for the performance efficiency currently enjoyed by the industry. However, the recent mandatory or voluntary removal of in-feed antibiotics from poultry diets, spurred by reports of potential antibiotic resistance in humans, is creating a major challenge. As discussed later, a number of alternatives are being tested and researched, but yet to be broadly accepted by the commercial industry.

The growth in acceptance of other feed additives in poultry production over the last two decades has been an extraordinary development. Perhaps the most important additive to enter the animal feed market is exogenous feed enzymes, which have evolved from an undefined entity to a well-accepted tool to improve nutrient utilization. The availability of glycanases (xylanases and glucanases) in the 1990s has effectively overcome the anti-nutritive effects of non-starch polysaccharides (NSP) and enabled the increased use of viscous grains (wheat and barley) in poultry diets. Today, the use of these enzymes in wheat and barley-based poultry diets is routine. The use of another enzyme, microbial phytase, in poultry diets is on the increase, in response to concerns over phosphorus pollution from effluents from intensive animal operations. In recent years, carbohydrase enzymes such as

xylanases and amylases, as well as other exogenous enzymes such as proteases, have also gained commercial relevance.

The availability of crystalline/synthetic amino acids is another notable development, and this additive has enabled the nutritionists to more precisely meet the ideal amino acid profile and to improve the performance and yield of high-producing modern birds.

Among the other additives, mycotoxin binders need special mention. The negative effects of mycotoxins have been known for many years. But until the 1990s, the only avenue of control was the use of clays such as bentonite. During the past two decades, the availability of more effective/specific mycotoxin binders/deactivators has substantially lowered the risk of compromised bird productivity. With high-energy prices, there is recent interest in the use of emulsifiers to improve the utilization of fats by breaking up the fat into small, finely divided globules and increase the access for lipase action. In recent years, some effective emulsifiers, compared to the older versions (crude lecithins), have come into the market.

Feed processing. The progress in the technology of feed manufacture during the past 50 years represents a major and necessary development in improving bird performance. The technology has progressed from simple mixing of mash feed to pelleting, which involves various physical, chemical and thermal processing operations.

Currently, majority of the feed used in the production of broilers is fed in pelleted or crumbled form. Offering feed to poultry in pellet or crumbled form has improved the economics of production by improving feed efficiency and growth performance. These improvements are attributed to decreased feed wastage, higher nutrient density, reduced selective feeding, decreased time and energy spent for eating, destruction of pathogenic organisms, and thermal modification of starch and protein.

Phase feeding. Phase-feeding, a form precise-feeding, is another progress during the past two decades. This is a feeding system in which dietary amino acid levels are reduced steadily over time in an attempt to reduce costs associated with excess dietary protein or amino acids. Commercial phase feeding programs may include several phases to step down amino acids and other nutrients for broilers and layers. The number of phases to be implemented in production cycle is dictated by both economics and the practicability.

FUTURE DIRECTIONS IN POULTRY NUTRITION

Future directions in poultry nutrition will be driven by on-going changes in world animal agriculture and by societal issues. Sometime in the future, we may have to modify feed formulations to accommodate not only science-based needs but also the needs of the society. The impact of social issues (in-feed antibiotics, environment, welfare, traceability, use of meat and bone meal, GM ingredients, etc.) will influence the decision-making from farm level to retail distribution of poultry products (5).

In-feed antibiotics: a major consumer concern. The ban in the European Union and different degrees of voluntary withdrawal in other parts of the world on the use of in-feed antibiotics will put extra pressure on the gut health and general health of animals. Currently, there is increasing focus on alternatives to sustain good gut flora and gut health, and these include enzymes, probiotics, prebiotics, essential oils, botanicals, and organic acids. In the last 10 years, these products have been widely tested and the evaluation will continue in the future. While most of these products are reported to mimic the effects of in-feed antibiotics on gut microflora, it is obvious none on their own will be able to replace antibiotics in terms of sustaining animal performance. It is possible that these alternatives may be more effective in combination rather than individually. However, to be accepted by the commercial industry, effects of these products must be demonstrated by improvements in animal performance, similar to that achieved with in-feed antibiotics.

In addition to these antibiotic replacers, a combination of nutritional and management strategies are also needed to promote gut health and good gut flora and these may include:

1) Use of more digestible diets.
2) Use of lower dietary protein levels and better balance of amino acids.
3) Whole grain feeding to enhance gizzard development.
4) Maintenance of good litter quality.
5) stocking density, improved climate control, etc.

Importance of gut integrity. Gut integrity is a neglected aspect of gut health, but is equally important as good microflora balance to achieve bird’s genetic potential. Intestinal integrity for commercial poultry can be defined as the maintenance of intestinal health to enable the expression of the full genetic potential for growth and yield, and to fully utilize the dietary nutrients. Normal flora plays an important role in maintaining

gut structure, strengthening the gut mucosal barrier, and protein metabolism of the gut. In situations where the profiles are shifted by pathogenic flora (e.g. clostridium, coliforms), there is significant damage to the mucosal layer and the barrier function. Coccidiosis is a major cause of poor gut integrity and an effective anti-coccidial program must be in place. Raw material quality is another contributing factor for poor gut integrity. Substances (e.g. mycotoxins) or raw materials (e.g. fibrous feeds) that can irritate the gut must be closely monitored.

Environment and sustainability. With increasing public interest over environment, the reduction of nutrient excretion in effluents from intensive animal operations has now become a major issue. Not long ago, when feeds were formulated, the main objective was how to supply the nutrients (nutrient input). In the past, there was a tendency to over-formulate diets when doubts exist on the availability of critical nutrients or if the nutrient requirements were uncertain. This practice of over-formulation is no longer acceptable because this is not only wasteful, but also excess nutrients are excreted in the manure and ultimately a source of pollution.

Today there is much public concern about what comes out of the bird (nutrient output). Animal agriculture, including commercial poultry sector, clearly has a problem of releasing excess nutrients into the environment and it must assume ownership of its impact on environment, especially water quality. From the nutrition point of view, the most obvious strategy is to feed the bird to match the requirement and to improve the efficiency of nutrient utilization by the bird, which in turn will reduce nutrient load in the manure. Among the other possibilities to improve the nutrient utilization efficiency, the use of feed enzymes is most promising.

Feed resource base and sustainability. It is projected that the global demand for pig and poultry continue to increase over the next decade and such a growth will have a profound effect on demand for feed and raw materials. It is also becoming clear that the requirements for traditional raw materials, both energy and protein sources, cannot be met even with optimistic forecasts. The first strategy available to the industry is to expanding the feed resource base by evaluating potential new raw materials**.** Once these are characterized, the next step is to examine ways to maximize their value by judicious use of additives such as feed enzymes, supplemental crystalline amino acids, etc. Given that high fiber (and NSP) levels may be limiting nutrient availability in most of these ingredients, development of appropriate enzyme combinations targeting the fiber fraction (mannanases, cellulases) will be crucial.

Feed additives and sustainability. In the future, there will be more pressure to extract every kcal of energy and every unit of nutrients. A combination of strategies has to be employed and exogenous feed additives will have a key role to play in maximizing the release of nutrients. One can expect development of new additives, especially enzyme products that are effective in range of diet formulations. There is evidence suggesting that preparations with multiple enzyme activities may provide a competitive strategy to improve nutrient utilization in poultry diets (2,3,10). Such enzyme cocktails, rather pure single enzymes, represent the next generation of feed enzymes. This is because feed ingredients are structurally exceedingly complex. In the "native" stage, nutrients in raw materials are not isolated entities but exist as complexes with various linkages to protein, fat, fiber and other complex carbohydrates. Advances in enzyme technology will continue and one can expect that better forms of enzymes will be developed in the future. The "next-generation" enzymes will be close to being "perfect," with high specific catalytic activity (per unit of protein), good thermostability, high activity under wide ranges of gut pH, resistance to proteolysis, and good stability under ambient temperatures.

Commercial availability of crystalline amino acids has enabled the nutritionists to meet the high amino acid requirements of modern broilers. This feed additive will continue to assist the nutritionists to more precisely meet the ideal amino acid profile, to use digestible amino acids rather than total amino acids as the basis of feed formulations (6), and to reduce dietary crude proteins levels and meet the amino acid requirements more precisely. This will lead to greater efficiency of nitrogen utilization and protein accretion, eventually lowering the nitrogen output in the manure, and to develop phase-feeding programs. Currently three crystalline amino acids, namely methionine, lysine, and threonine, are available to the industry at competitive prices. Though somewhat expensive, tryptophan can also be purchased in feed-grade forms. Valine and isoleucine, the next limiting amino acids in practical diets, are expected to become available in the near future and may lead to further improvements in feed efficiency.

Need for research into barriers to optimum digestion. Though broilers and layers are highly efficient in converting feed to food products among farm animals, still they excrete significant amounts of unutilized nutrients. For example, broilers lose almost 25-30% of ingested dry matter, 20-25% of gross energy, 30-50% of nitrogen, and 45-55% of phosphorus intake in the manure. Thus there is

considerable room to improve the efficiency of conversion of feed to animal products. Much of the inefficiency results from the indigestibility of nutrients in the feed. For this reason, future nutritional research in poultry should focus on issues relating to identifying barriers to effective digestion and utilization of nutrients, and approaches to improve feed utilization and reduce nutrient excretion. In this endeavor, poultry nutritionists must combine their expertise with those of specializing in other biological sciences, including immunology, microbiology, histology, and molecular biology.

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REPEATED ELEVATED CONDEMNATIONS OF MEAT CHICKENS FOR CELLULITIS AND DERMATITIS AT AN ONTARIO PROVINCIAL SLAUGHTERPLANT

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Condemnations of chickens with skin lesions processed at Ontario provincially inspected slaughter plants are common but typically represent less than two percent. Repeated elevated condemnations of three month old, brown feathered chickens for dermatitis and cellulitis from two small flock producers from one region in Ontario were recently reported. Results of further investigations into underlying factors, including husbandry and flock diseases will be reported.

A NEW COMPUTER BASED COCCIDIOSIS PREVENTION PLANNER© DESIGNED TO HELP GUIDE AND RECORD MEDICATION AND OTHER DECISIONS ASSOCIATED WITH THE PREVENTION OF COCCIDIOSIS IN BROILER CHICKENS

S. Ritchie and G. Jacob

Canadian Poultry Consultants Ltd

SUMMARY

The prevention of coccidiosis and necrotic enteritis in all commercial broiler farming systems requires short, medium and long term planning strategies. These intestinal diseases are prevented through an understanding of the various interactions between the chicken, the environment, and the causative agents. Disciplined coccidiosis prevention strategies are very effective in minimizing the significant negative impact associated with coccidiosis and necrotic enteritis, not only on performance, but also on welfare, health, antibiotic resistance and food safety. Disciplined coccidiosis and necrotic enteritis strategies are also extremely important for the long term sustainability of the various additives currently permitted for use in the chickens feeds.

A new computer based Coccidiosis Prevention Planner© (CPP©) has been developed to provide easy access to a library of information relative to available coccidiostats and antibacterials, with an added provision to add "custom" ingredients, for example, a probiotic or a prebiotic. The CPP provides a guide for entering various inputs, including feed stages and down time for example. The CPP output allows for the integration of performance data parameters and includes a provision for the production of a prescription template. The CPP output also produces an annual "cocci wheel" allowing the user to efficiently share results in an easy to interpret multidimensional diagram.

The CPP is structured to be available in a secure format on-line and has been developed using the following program languages: PHP: Hypertext Preprocessor and Javascript. The server stores data in a MySQL database.

The Coccidiosis Prevention Planner will be introduced and described in this presentation.

Figures 1 and 2. Example of the cocci planner.

Coccidiosis Prevention Planner - Coccidiosis Prevention Planner http://www.platinumbrooding.com/cpp/?action[]=cpp&action[]=calendar&farmid=8&start=...

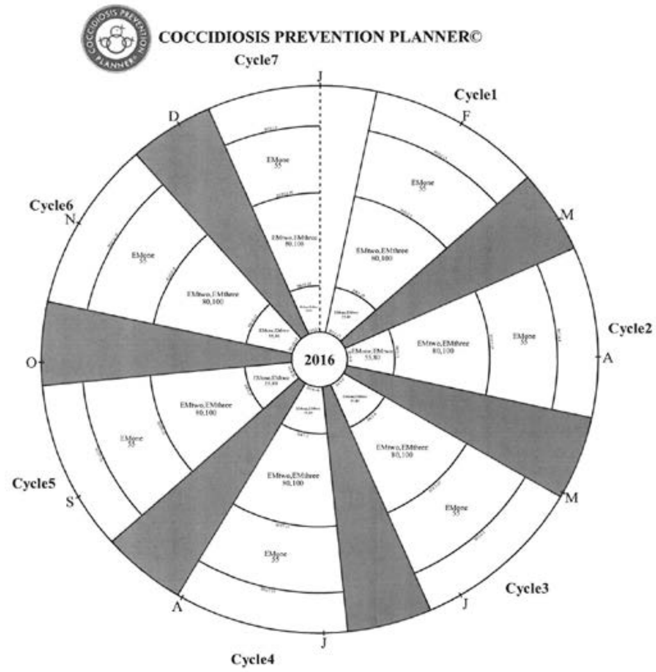

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Figure 2.

COCCIDIOSIS PREVENTION PLANNER©

Cycle	Placement Date	Cycle Length	Cycle End	Downtime	Flock Size	Feed Program
Cycle1	13 Jan 2016	37 days	18 Feb 2016	18 days	10000	N/A

Feed Stage Name	Start Date	Start Day	Length	End Day	End Date	Medication	Trade Name	% Conc	Dosage
Starter	13 Jan 2016	1	7	7	19 Jan 2016	z-ExampleMed1	z-ExampleMed1	100%	55
						z-ExampleMed2	z-ExampleMed2	100%	80
Grower	20 Jan 2016	8	14	21	2 Feb 2016	z-ExampleMed2	z-ExampleMed2	100%	80
						z-ExampleMed3	z-ExampleMed3	100%	100
Finisher	3 Feb 2016	22	10	31	12 Feb 2016	z-ExampleMed1	z-ExampleMed1	100%	55
Withdrawal	13 Feb 2016	32	6	37	18 Feb 2016				

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ENILCONAZOLE EFFECT ON BACTERIA AND FUNGI ISOLATED FROM POULTRY FACILITIES AND AVIAN HOSPITAL

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INTRODUCTION

Infectious diseases are among the main problems causing millions in losses in the poultry industry as well as in pet bird area. If a sick bird is not diagnosed, it could cause expensive and prolonged treatment or death of the animal, and in some cases, the transmission of some diseases to the owners. For these reasons, the contamination, either bacterial or fungal, represents a critical point in the development of infectious diseases. Accordingly, the cleaning and disinfection of the environment of birds becomes an essential part of management of any poultry house (3). Among the major pathogens in poultry, are bacteria like *Escherichia coli* and *Salmonella* spp., while the company and ornamental birds are susceptible to several types of bacteria such as *Escherichia coli, Aeromonas* spp., *Salmonella* spp., *Klebsiella* spp., and *Enterobacter* spp. (5). On the other hand, *Aspergillus fumigatus* is considered among the major diseases associated with respiratory tract in poultry, both production and pet birds (8).

Enilconazole also known as imazalil, is a broad-spectrum antifungal belonging to the group of imidazoles, which are able to inhibit cell wall synthesis. It is a product widely used in fruits, vegetables and some grains production, due to its activity against fungal pathogens (7). In veterinary medicine, has been widely use in the treatment of fungal diseases in dogs and horses both topically and in the form of smoke or spray (7). Furthermore, it has been suggested that enilconazole has an antibacterial effect, because some studies show that azoles show inhibition of gram-positive and gram-negative bacteria, being the main activity on gram-positive bacteria, as *Staphlylococcus aureus* and *Bacillus subtilis* (6).

Given the importance of bacterial and fungal contamination in poultry houses and hatcheries, as well as the hospital of pet birds, is relevant to determine the effect of this product on major importance fungi and bacteria in poultry since the antibacterial activity in the poultry area is scarce. Therefore, the objective of this study was to determine the effect of antifungal enilconazole in *Aspergillus* spp. and *Penicillium* spp. and the antibacterial effect on *Escherichia coli, Staphylococcus* spp. *Salmonella* spp. and *Pseudomonas* spp. isolated from poultry production facilities, and pet bird hospitals.

MATERIAL AND METHODS

Strains. The strains were isolated from the environment in different areas of the Centre for Education, Research and Extension in Poultry Production (CEIEPAv) of FMVZ UNAM from environmental exposure of Petri dishes containing Trypticase soy agar (TSA) MacConkey agar (MCC) and Sabouraud dextrose agar (SDA) for the isolation of gram-positive organisms, enterobacteria and fungi, respectively. The same procedure was performed at the Hospital of pet birds belonging to the Department of Poultry Medicine, FMVZ, UNAM.

Identification of microorganisms. After incubation, identification of the isolated microorganism was performed using biochemical tests according to Jang et al (2). The fungi were isolated from the SDA and identified by observing the macroscopic and microscopic morphology, according to Funder, S. (1). *Escherichia coli, Staphylococcus* spp., *Pseudomonas* spp. and *Aspergillus* spp. were selected from CEIEPAv and *Escherichia coli, Staphylococcus* spp. *Aspergillus* spp. and *Penicillium* spp. from the hospital. Also a *Salmonella* spp. strain from the collection of the Department was included.

For preparation of the inoculum, bacteria were seeded in brain heart infusion broth and the concentration was adjusted to obtain approximately 1 x 102 CFU / plate; likewise the control of dishes that would not be exposed to enilconazole were seeded. In the case of fungi, the concentration was adjusted in the same way as described for bacteria.

Enilconazole exposure. Petri dishes containing bacteria and fungi were exposed for 18 hr to a smoke generator enilconazole per 50m3, two groups were divided, one corresponding to bacteria isolated both CEIEPAv and hospital; and the other group of fungi. Similarly, TSA and SDA dishes that correspond to control groups were not exposed to smoke generator. After the exposure period, treated groups and control

groups were incubated for 24 hr at 37°C and then colonies were count to determine the percentage of reduction of the treated groups compared with control ones.

RESULTS

The results of the bacteria exposed to enilconazole are shown in Figure 1; it shows different levels of reduction for *E. coli*, depending on the area of isolation. Enilconazole had better inhibition effect on *E. coli* isolated from kitchen (hospital) (95.57%), compared with the same bacteria isolated from laying hens (64.11%), unlike that *E. coli* from broiler in which the enilconazole had no effect. In the case of *Salmonella* spp. enilconazole had not inhibition effect, whereas in *Pseudomonas* spp. there was a slight inhibition (11.19%); in the other hand, enilconazole showed a reduction of 99.61% for *Staphylococcus* spp. isolated from hospital.

In the case of fungi, enilconazole was able to eliminate 100% of the fungi tested, regardless of where they were isolated.

DISCUSSION

In this work, the disinfectant effect of enilconazole was evaluated using both fungi and bacteria isolated from poultry production and pet birds hospital. Results of exposure showed that enilconazole eliminated 100% of fungi regardless the origin, either hospital or farm, probably due to inhibitory effect on enilconazole in the ergosterol synthesis, a key component in the structure of fungi[8]. It can even be attributed to the absence of resistance of the strains used against enilconazole, consistent with previous studies where strains of *Aspergillus fumigatus* isolated from domestic geese in Poland, presented 100% of susceptibility to enilconazole and voriconazole unlike developed resistance against other antifungals such as clotrimazole, miconazole, amphotericin B plus (9). However, the effect was different in bacteria; probably because they have a different chemical structure. It has been suggested that the effect on gram-positive bacteria is better than on the negative ones, as it was observed in the present work, in which variable results were found not only depending on the genus and species, but also related to the place of isolation. The results obtained with the strain of *Staphylococcus* spp. from hospital, which showed a reduction of 100%, consistent with previously reported by Rostom, et.al. (6).

The inhibitory effect of enilconazole in *Escherichia coli* strains depended on the area in which was isolated. *E. coli* from Hospital presented a nearly 100% inhibition (95.57%), compared to that isolated in the laying hens and broiler houses which showed a lower percentage reduction (64.11% and 0%, respectively), this can be attributed to some resistance due to the selection of strains.

CONCLUSIONS

Results in this experiment can showed that the antifungal effect of enilconazole was excellent on all fungi tested (100% reduction). In the case of bacteria, the reducing effect on gram positive bacteria such as *Staphylococcus* spp. was good, but not in the case of gram negatives, which showed a variable resistance to enilconazole as in the case of *Pseudomonas* spp. and *Salmonella* spp; while in the case of *E. coli*, sensitivity to the antifungal varied depending on their origin. Apparently, strains isolated from poultry production are more resistant than its counterpart of pet-bird hospital; however, more studies are needed to verify if this observation is true.

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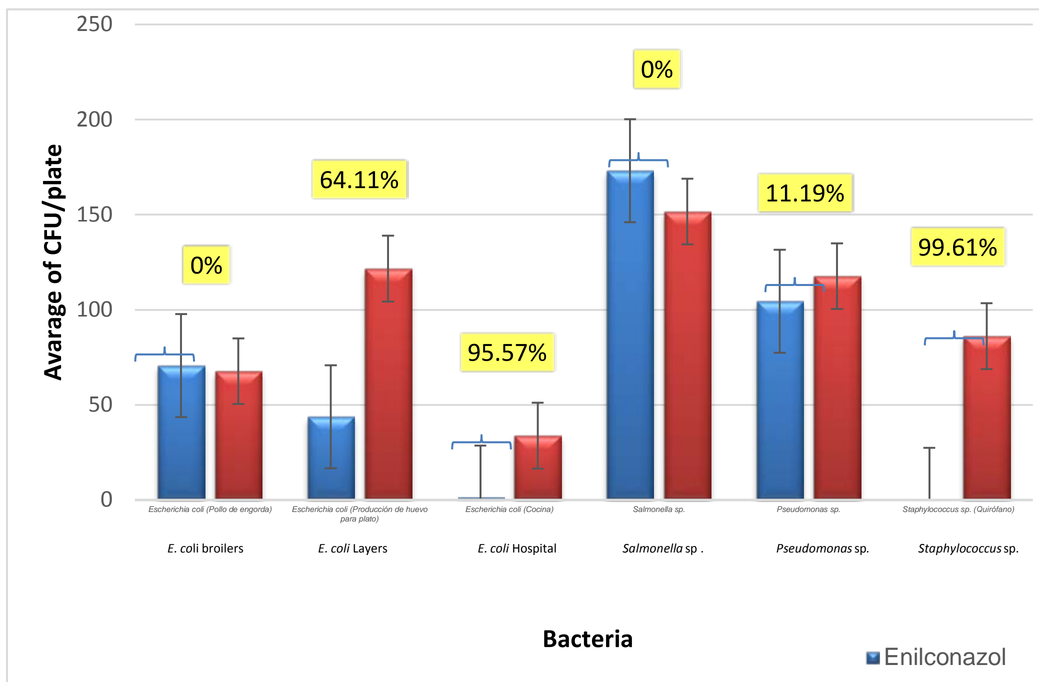
Figure 1. Results of growth reduction to enilconazole exposure on several bacteria isolated from poultry facilities and pet bird hospital. Numbers shown above the bars represent the percentage of reduction of the enilconazole exposure group compared with control group.



MANNOSE-RICH FRACTION EFFECTS ON THE IMMUNE SYSTEM AND GASTROINTESTINAL HEALTH OF POULTRY

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INTRODUCTION

Mannan oligosaccharides (MOS), components of the yeast cell wall, have recognized modes of action in animal health including the ability to effectively bind pathogens, to modulate the immune system response and to improve the integrity of the gastrointestinal tract. An original MOS product (Bio-Mos®; Alltech Inc., Nicholasville, KY) was developed in the early 1990s and after nearly 20 years of research and development, a 'second generation' mannose-rich fraction (MRF) product (Actigen®; Alltech Inc., Nicholasville, KY) was commercially launched in 2009 (10).

PATHOGEN BINDING

Pathogens, such as *Salmonella* spp. *and E. coli*, are known to bind to the gastrointestinal tract (GIT) via type-1-fimbriae. Attachment and colonization of the GIT allow the pathogenic bacterial population to flourish, increasing the chance of disease. Additionally, pathogen growth may result in decreased performance efficiency by directly competing for nutrients or indirectly by negatively impacting GIT health and the ability to uptake nutrients. Selected for attachment specificity, MRF binds pathogens as it passes through the GIT, thus eliminating colonization (10).

POULTRY RAISED WITHOUT ANTIBIOTICS

The efficacy in limiting pathogen colonization has established MRF as an effective solution for poultry raised without antibiotics. In a comparison of MRF versus bacitracin methylene disalicylate (BMD) for the reduction of necrotic enteritis caused by *Clostridium perfringens* in broiler chicks, there was no difference in feed conversion ratio between the treated groups and both exhibited better performance than the non-treated control (5). Similarly, MRF was compared with BMD when chicks were raised on built-up litter. In the first of two experiments, dietary inclusion of MRF or BMD increased body weight gain and feed efficiency over 42 days compared to the non-treated controls. In the second experiment, feed conversion, litter conditions and morphology of jejunum tissue, including villi height, villi height:crypt depth ratio and goblet cell numbers, were improved on day 42 by MRF or BMD treatments compared with the non-treated controls (1). Using an established necrotic enteritis challenge model, researchers at the University of New England in Australia observed that weight gain and livability of birds was improved comparably by zinc bacitracin, salinomycin and MRF compared to non-treated controls. Furthermore, MRF-fed birds displayed increased villus height, decreased crypt depth and increased villus height:crypt depth ratio (6).

GUT HEALTH

Importantly, MRF supports digestive function and enzyme activity while also enhancing the status of the immune system and controlling inflammation. The cecal microbiota has been shown to be consistently and reproducibly altered by MRF supplementation (2). This suggests an indirect mode of action – alteration of microbiota composition – is responsible for improvements in growth performance because MRF is not digestible (10).

At the University of Kentucky, chicks were fed diets without or with 400 g/T MRF over 21 days. Dietary inclusion of MRF tended to increase body weight. Histology measurements of jejunum tissue from 21 day old birds indicated thinner apical widths and smaller individual surface area of villi. However, as a result of more total villi, the total absorptive surface area of the GIT was increased (9).

IMMUNE MODULATION

In addition to support of GIT health, MRF has demonstrated activities in immune modulation. In conjunction with the University of Kentucky, one hundred and forty broiler chickens were administered lipopolysaccharide (LPS), a component of the outer cell wall of gram negative bacteria, as a mimic of a pathogenic bacterial challenge. Microbial components such as LPS initiate pro-inflammatory

pathways which coordinate the innate immune response. When MRF was present in the diet at the standard dose of 400 g/T, chicks had greater concentrations of nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and tended to have greater expression of cytokine interleukin 1β (IL1β) in serum compared to chicks fed diets without MRF. As a result, MRF-fed chicks had a more effective early immune response that reduced the negative impact on feed intake (4).

HEAT STRESS

The broiler chicken has the ability to regulate its body temperature within a narrow range under normal physiological conditions. However, ambient temperatures above the thermoneutral zone result in heat stress, causing changes in metabolism and in the immune system. Under these environmental conditions, birds can no longer regulate their body temperature, resulting in reduced performance due to morbidity or mortality (3). Researchers at North Carolina State University exposed birds to acute heat stress (41°C for 60 minutes) before ileum tissue samples were collected for transcriptome sequencing. As a result of MRF supplementation, the expression of inflammatory genes was reduced and the expression of nutrient transporter genes was increased in the GIT (8). Heat shock protein expression changes were observed in the liver, suggesting a signaling cascade traversing the GIT, possibly from alterations of the microbiota composition, due to MRF supplementation that reduced negative effects of heat stress (7).

CONCLUSION

Dietary MRF appears to have direct and indirect effects on GIT health and structure, promoting robustness, improved feed efficiency and growth performance. The ability of MRF to effectively bind pathogens and to modulate the immune system demonstrate the importance of MRF is an effective tool in modern poultry production.

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INHIBITORY EFFECT OF NITRIC OXIDE AGAINST LOW PATH AVIAN INFLUENZA VIRUS REPLICATION IN CHICKEN

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ABSTRACT

CpG DNA can be used for the stimulation of Toll-like receptor (TLR) 21 signaling pathway in chicken, that ultimately leads to up-regulation of gene transcription for pro-inflammatory molecules including nitric oxide (NO). NO plays a key role in innate immune system against viruses that infect mammals and avian species. The objective of the study is to determine the antiviral effect of NO, produced in response to *in ovo* delivery of CpG DNA, against low pathogenic avian influenza virus (H4N6) infection in the respiratory system of chickens. In our experiment, first we treated developing embryo *in ovo* with CpG DNA and PBS at embryo day (ED) 18 and subsequently challenged with H4N6 virus at ED19. We observed that *in ovo* delivery of CpG DNA significantly inhibited the replication H4N6 virus infection in the respiratory system of chickens.

INTRODUCTION

Recently, the number of avian influenza outbreaks increased dramatically across the world. Avian influenza infections in commercial poultry operations lead to high morbidity and mortality because of intensive nature of the poultry farming globally. Moreover, the control of transmission of avian influenza infection becomes difficult since the spread of the disease is closely associated with the migratory nature of the waterfowl depending on the season (16). Although the vaccination is considered as a key component of avian influenza control, the protection is unreliable because of continuous mutation of the virus *in vivo* due to selective pressure imposed by vaccine-mediated immune responses (12). Given the limitations of current control measures against avian influenza, it is a necessity that novel measures are developed leading to sustained long lasting immunity against avian influenza infections. Any development of novel control measure should be based on through understanding of host responses elicited against this infection.

The innate components of first line of host-protective mechanisms recognize highly conserved molecular patterns of invading microbes known as pathogen-associated molecular patterns (PAMPs). The detection of PAMPs by a host depends on a broad range of host receptors called pattern recognition receptors (PRRs)(14). TLRs are the well studied host receptors that are indispensable in recognizing PAMPs and eliciting appropriate host responses (10, 15). TLR21 in avian species and TLR9 in mammals are the only identified receptors that recognizes unmethylated microbial deoxy-ribonucleic acid (DNA) (5, 7). Synthetic ligand of TLR9 and TLR21, CpG DNA, has been investigated widely as a potent immunotherapeutic mean (6, 8, 9). Binding of CpG DNA results in conformational changes in the cytoplasmic signaling domain of TLR9 and TLR21 and consequently recruitment of adaptor molecules takes place to initiate downstream signaling (8, 11), that ultimately lead to production of pro-inflammatory active molecules including NO (2).

NO, a free radical molecule produced from L-arginine via inducible nitric oxide synthase (iNOS) enzyme particularly by macrophages, plays a vital role in innate host defense against invading infectious agents including viruses (1, 13). Recent studies indicated that CpG DNA enhances NO production in chickens (3, 4). However, the antiviral activity of NO, mediated by CpG DNA has not been studied against avian influenza virus infection in chickens. Therefore, our objective of this study is to determine the antiviral activity of NO elicited by *in ovo* delivery of CpG DNA against low pathogenic avian influenza virus (H4N6) infection.

MATERIALS AND METHODS

In this study, CpG DNA was delivered *in ovo* at ED 18 with a control group receiving PBS. At ED 19, the eggs were infected with H4N6 virus and subsequently the lungs were sampled at ED 20. The samples were homogenized and the supernatants were collected to quantify the live virus particles using standard plaque assay technique. Meanwhile, NO concentration in the homogenized lung

supernatants were determined using Griess assay reagent system.

RESULTS

We found that *in ovo* delivered CpG DNA at ED 18 reduces the avian influenza viral replication.

(This article will be submitted as a full length manuscript to a peer reviewed journal.)

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SAFETY AND EFFICACY OF A CHICKEN EMBRYO PROPAGATED FOWL POX VIRUS VACCINE ADMINISTERED SUBCUTANEOUSLY TO ONE-DAY-OLD BROILER CHICKS

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INTRODUCTION

Fowl pox is recognized as one of the economically important diseases in poultry industry. In pox endemic areas, fowl pox vaccination is often done as early as one-day-old. For vaccination of day-old chickens, cell-culture-origin fowl pox vaccines are commonly used and reported to be safe and efficacious (1, 2, 3). To our knowledge, no published information was yet available on the safety and efficacy of the commercially available chicken-embryo-origin fowl pox vaccines in day old commercial broilers. In this study, we evaluated safety and efficacy of a chicken-embryo-origin fowl pox vaccine in day old commercial broilers by administering the vaccine through subcutaneous route.

MATERIALS AND METHODS

One-day-old broiler chicks were vaccinated with a commercially available fowl pox vaccine manufactured by Hygieia Biological Laboratories, Woodland, CA. The vaccine was administered through subcutaneous route. Another group consisting of same number of chicks was kept as unvaccinated challenged controls.

All chickens (vaccinated and controls) were observed daily up to three weeks post vaccination for vaccine associated mortality, adverse reactions. At three weeks, all vaccinated and control chickens were challenged using a standard challenge strain of fowl pox virus to evaluate vaccine induced immunity. At six days post challenge, each chicken was checked for development of pox lesion at the site of challenge virus administration. All vaccinated and control chickens were also weighed on the day of vaccination, at 10 days, three weeks, and five and a half weeks of age to find out if day old vaccination has any negative effect on body weight gain.

RESULTS AND DISCUSSION

The vaccine proved to be safe for subcutaneous administration in one day old broiler chicks. None of the vaccinated chickens revealed any adverse reactions or mortality associated with the vaccine. No other abnormality was observed in the vaccinated birds in respect to their growth and health status. Although, vaccination stress caused a slight reduction in body weight in the beginning, it started to pick up weight quickly and soon became comparable to the control chickens. The vaccine induced excellent protection in the vaccinated broilers. All vaccinated chickens (100%) resisted challenge against the challenge virus. Whereas, none of the unvaccinated control chickens (0%) protected against challenge virus infection.

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ALLEVIATING THE IMPACT OF COCCIDIOSIS WITH MICRO-ENCAPSULATED ORGANIC ACIDS AND ESSENTIAL OILS

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SUMMARY

Clinical coccidiosis leads to important production losses in the poultry industry. The advancement of vaccines and the changing perspective on antimicrobial use is transforming the current approaches on coccidiosis prevention. Dietary supplementation of organic acids and essential oils has been suggested as alternatives to control coccidiosis. The objective of this study is to evaluate the anticoccidial efficacy of an in-feed micro-encapsulated combination of organic acids and essential oils or micro-encapsulated essential oils against a challenge of *Eimeria* spp. mixture given orally at 14 days of age. In a battery cage trial, a total of 320 birds were attributed either to the unchallenged control (UC), to the challenged control (CC), CC plus micro-encapsulated organic acids and essential oils (M(OA+EO)) or CC plus micro-encapsulated essential oil (M(EO)). Six days post-infection, coccidiosis lesion score, oocysts count per gram of fecal material, and performance parameters were recorded and compiled. Birds supplemented with M(OA+EO) and M(EO) obtained a statistically lower coccidiosis lesion score compared to CC at 14-28 days. Total oocysts count per gram of fecal material was also statistically lower for the M(OA+EO) and even more so for the M(EO) treatment compared to CC at 14-28 days. For zootechnical parameters, the M(EO) treatment had similar, but higher average body weight gain than M(OA+EO) (0.430 kg vs. 0.406 kg) and CC (0.430 kg vs. 0.355 kg), respectively. For the feed conversion, the M(EO) treated group had similar feed conversion as with M(OA+EO) birds (2.231 vs. 2.318) but better than the CC group (2.231 vs. 2.502) at 14-28 days. In conclusion, the dietary supplementation of micro-encapsulated organic acids and essential oils blend or microencapsulated essential oils alone alleviated the negative impact of coccidial challenge in broiler chickens. This reiterates the positioning of these micro-encapsulated organic acids and essential oils blend products to improve production performance and as aid in the reduction of coccidiosis.

INTRODUCTION

Coccidiosis is recognized as the major protozoan disease in the poultry industry worldwide. This parasite (*Eimeria* spp.) cycles in the birds' intestinal villi and results in feed conversion losses, lowers weight gain, and increases cost of production. Therefore, poultry producers turned to pharmaceutical interventions to maximize profits and lower husbandry costs. The main foci of pharmaceutical products or coccidiosis vaccines were to control or to prevent infection and its negative impact on performance.

However, with current modification of antimicrobial use by global authorities, the poultry industry needs to shift gears and start considering other products as options to maintain productivity. Different blends of essential oils have been previously identified having destructive properties on *Eimeria* oocysts *in vitro* (3). Organic acids alone or in combination to anticoccidial or probiotic have been investigated with positive results on the intestinal integrity but little to no performance enhancement when tested during coccidiosis challenge (1) (2). To our knowledge, there is very limited amount of studies testing organic acids and essential oils in a coccidial challenged birds.

The objective of this study is to measure lesion scores, oocysts excretion and zootechnical parameters in order to evaluate the anticoccidial efficacy of a micro-encapsulated combination of organic acids and essential oils or micro-encapsulated essential oils alone against a challenge of *Eimeria acervulina, E. maxima,* and *E. tenella*.

MATERIALS AND METHODS

A total of 320 off-sex male (byproduct from female line) chicks were distributed in each battery cages for this trial at the Southern Poultry Research facility. Birds received the routine vaccination at the hatchery and were provided with feed and water *ad libitum*. Cages were randomly assigned to one of the following treatments in eight replicates: an unchallenged control (UNC), a challenged control (CC), CC supplemented with micro-encapsulated

essential oils at 500g/MT of feed (M(EO)) or CC supplemented with micro-encapsulated organic acids and essential oils at 300g/MT of feed (M(OA+EO)). Cages were checked twice daily, unusual observations and mortality in addition to most probable cause of death was recorded. Birds and non-consumed feed at day 0, 14, 20 and 28 were weighed.

The challenge consisted of a mixture of three *Eimeria* strains: *E. acervulina, E. maxima* and *E. tenella* prepared at the Southern Poultry Research. It was administrated by oral gavage to individual bird at day 14 for all three challenged treatments, whereas the unchallenged control received 1 mL of distilled water. The coccidial challenge was aimed at producing a mild to moderate infection.

At day 20 (six days post-infection), mixed feces were collected from drop pens for each replicate and total oocysts count per gram of fecal material was measured by fecal flotation method. On the same day, four birds per cage were humanely euthanized and coccidiosis lesion scoring was performed on each segment of the birds' intestine following the Johnson and Reid (1970) method.

Data for performances parameters, mortality, intestinal lesions score and oocysts count per gram of fecal material was compiled and means were compared statistically with a p-value of <0.05.

RESULTS AND DISCUSSION

From the current study, the birds supplemented with M(OA+EO) and M(EO) obtained statistically lower coccidiosis lesion score compared to the CC for all three *Eimeria* strains at 14-28 days (Graph 1). Birds in the UNC were free of coccidiosis lesions, associated with a zero lesion score. In addition, total oocysts count per gram of fecal material at 14-28 days were also lowered in birds supplemented with M(OA+EO) and M(EO) at 63,533 and 43,014, respectively compared to the challenged control (164,477). The microencapsulated essential oils blends in this study have the greatest protection effect for coccidiosis, as similarly observed previously (4, 5).

Average body weight gain and feed conversion were shown in Table 1 at day 0 to 14 prior to the challenge and day 14 to 28 during the challenge period. Average body weight gain was higher with M(EO) at 0.430 kg than the challenged control but not statistically different from M(OA+EO) at 0.406 kg. Birds receiving M(EO) had the best feed conversion (2.231) followed by M(OA+EO) when compared to the challenged control (2.502) at 14-28 days. These results suggest that the lower lesion score and reduction in excretion of oocysts is able to give an edge to birds and translate in better performance especially with the M(EO) although not statistically different from the M(OA+EO). The industry is in great need of an effective anticoccidial, which should demonstrate a broad-spectrum of activity as well as enhance performances in broilers as pointed out by McDougald (6).

CONCLUSION

Different approaches to coccidiosis prevention were forced in commercial operations to answer new regimen on antimicrobial use. Blends of organic acids and essential oils were suggested as potential aid in coccidiosis control. Under controlled experimental conditions, the dietary supplementation of micro-encapsulated organic acids and essential oils blend or microencapsulated essential oils alone improved production performance and help alleviate the negative impact of coccidial challenge in broiler chickens. These additives should be studied further in commercial farms for their potential to be considered as a viable aid in reduction of coccidiosis.

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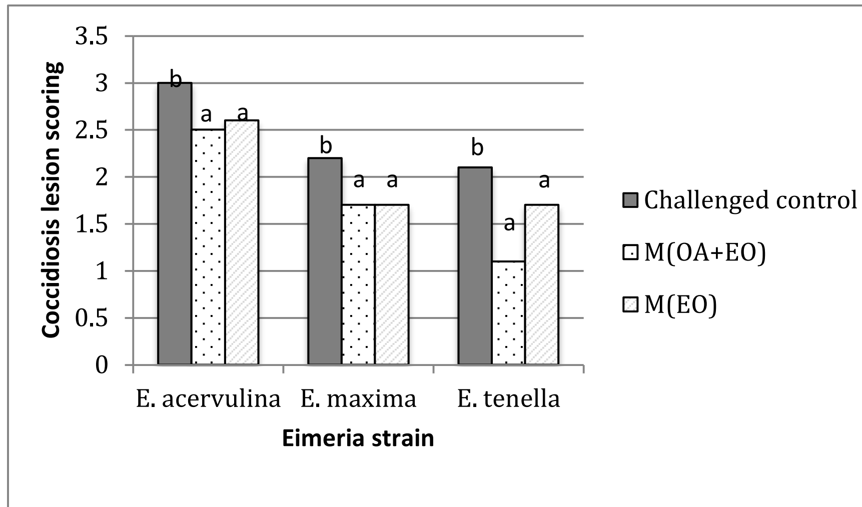
Table 1. Average body weight and feed conversion for each treatment prior to the challenge and during the challenge.

Treatments	Performance parameters			
	Average body weight gain (Kg)		Feed conversion	
	0-14 days	14-28 days	0-14 days	14-28 days
Unchallenged control	0.224	0.631[a]	1.378	1.665[a]
Challenged control	0.214	0.355[c]	1.393	2.502[c]
M(OA+EO)	0.221	0.406[bc]	1.401	2.318[bc]
M(EO)	0.226	0.430[b]	1.439	2.231[b]

[a,b,c] Means within column with no common superscript differ significantly

Graph 1. Coccidiosis lesion score[1] from birds' intestine for each *Eimeria* strain.



[1]Lesion score from 0 to 4 (4 representing severe lesions) was done on each intestine segment.
[a,b] Means within column per species with no common superscript differ significantly.

NECROTIC ENTERITIS AND FACTORS CRITICAL FOR SUCCESSFUL COCCIDIOSIS VACCINATION OUTCOMES

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SUMMARY

Necrotic enteritis (NE) remains an ongoing concern for broiler producers especially as coccidiosis vaccination becomes more commonplace. Three studies were undertaken to better understand the pathogenesis of NE in commercial broiler flocks and to quantify the performance benefits associated with improved vaccination strategies. The data suggest that coccidiosis vaccines are inadequately administered at the hatchery and missed or under-dosed chicks are predisposed to NE. Optimized administration, herein represented by a gavage treatment, not only significantly ($p<0.05$) reduced NE in commercial broiler houses, but it also produced tangible performance benefits and offers the potential for improving live production efficiencies.

INTRODUCTION

The use of coccidiosis vaccines in broiler production is increasing due to a number of factors including: a) the declining efficacy of anticoccidial chemicals and ionophores and the need to diversify rotational programs (3,6); b) the trend towards more antibiotic free production (2,4); and c) the complications associated with using ionophores where a high gangrenous dermatitis challenge exists (5). Concomitant with the increased use of coccidiosis vaccines, there is a heightened concern over NE. Vaccine cycling during early grow out is required to successfully immunize flocks against coccidiosis. When this cycling occurs under the right conditions in commercial broiler houses, necrotic enteritis is not uncommon (1). To better understand how necrotic enteritis can be mitigated in vaccinated broilers, a three part series of studies was undertaken to identify 1) which birds in a commercial flock are affected by NE; 2) why this subset is predisposed to infection whereas others are not; and 3) improved approaches to vaccination and quantifying the effects on bird performance

MATERIALS AND METHODS

Day of age commercial broilers were divided into seven groups according to how they were administered a coccidiosis vaccine at the hatchery: 1) non-vaccinated, 2) gavage, 3) *in ovo*, 4) posthatch spray, 5) posthatch gel, 6) .5X *in ovo* + .5X post-hatch spray and 7) .5X *in ovo* + .5X post-hatch gel. Chicks were placed into two commercial houses with a history of NE. Each treatment (T) was represented by 10% of the birds placed into each house except T6 which was represented by 40%. Daily mortality was monitored by treatment through day 18 of growout.

Also, on day of hatch, eight birds from each of T2-T7 were selected before transport to the farm and relocated to a facility with battery cages where each bird was placed into a separate cage. Feces were collected from these cages on days 4-8 and monitored for individual bird oocyst output.

In a third part to this series of studies, Mountaire Farms investigated the performance of flocks receiving gavage versus *in ovo* administered coccidiosis vaccine. Body weight and adjusted feed conversion were compared for a 58-day broiler grow out cycle.

RESULTS

In both commercial houses, compared to non-vaccinated controls, all vaccinated treatments had reduced mortality. Among T2-t7, gavage had significantly ($p<0.05$) less mortality compared to all other treatment groups, and in one house, actually had zero mortality on days 15, 16 and 17. Generally, with respect to mortality, two administrations were significantly ($p<0.05$) better than one, and gel was significantly ($p<0.05$) better than either spray or *in ovo* as a single administration. The exception was T5 versus T6 where only a numerical difference was observed.

The individual bird oocyst output data, as they relate to large oocysts (i.e., *E. maxima)* mirrored the mortality pattern observed in the commercial houses.

The gavage treatment demonstrated significantly ($p<0.05$) higher oocyst output than the other treatments. Of the eight birds per treatment, the number of birds showing *E. maxima* oocysts in their day 4 to 8 fecal collection sample was as follows: T2 (8/8), T3 (2/8), T4 (1/8), T5 (4/8), T6 (4/8) and T7 (6/8). Small and medium sized oocysts (i.e., *E. tenella and acervulina*) were not nearly as instructive. Gavage again had the highest counts, but the differences between treatments in the number of birds shedding oocysts were less obvious: T2 (8/8), T3 (8/8), T4 (7/8), T5 (7/8), T6 (8/8) and T7 (8/8)

In the performance trial, Mountaire demonstrated a 5.6 point benefit in adjusted feed conversion associated with gavage versus *in ovo* administration of the same vaccine.

DISCUSSION

NE in coccidiosis vaccinated commercial flocks is an easily recognized disease that typically occurs during the 3rd or 4th week of grow out. While mortality during this period in unaffected flocks is usually about 0.05%/day, in a flock experiencing NE, this number can easily rise more than fourfold (4,7). Prior to undertaking this investigation, it was unclear which birds in a flock were predisposed to NE and why mortality occurred in these individuals and not others. These data suggest that vaccine administration, and specifically whether *E. maxima* is applied adequately, is a major determinant. In the individual bird output study, 100% of the gavage birds (T2) were positive for large (i.e., *E. maxima*) oocysts in the day 4–8 fecal collection indicating that each bird had received vaccine at the hatchery. In the commercial broiler houses, T2 demonstrated the best protection against mortality during the critical 3rd week of grow out. The other treatment groups showed a lower incidence of birds positive for large oocysts in the day 4–8 collection and correspondingly higher mortality in the broiler house. The shortcomings in vaccine administration that were observed with *E. maxima* were not apparent with respect to small/medium oocysts (*E. acervulina and tenella*). The incidence of birds positive for small/medium oocysts in the day 4–8 fecal collection was high across all treatments and served to emphasize the importance of focusing on *E. maxima* exclusively when assessing the adequacy of coccidiosis vaccine administration.

These data suggest that birds insufficiently vaccinated at the hatchery are predisposed to NE during week three of grow out. The mechanism appears related to the prepatent period of *E. maxima* and the size of inoculum that chicks initially receive. Coccidiosis vaccines are formulated to deliver to each chick a small yet sufficient number of vaccine oocysts to stimulate immunity via a subclinical infection (i.e., coccidiasis). These vaccine oocysts amplify as they cycle through the bird during the first week of grow out resulting in a large number of oocysts being shed into the litter. Birds that received a dose of vaccine at the hatchery (T2) and encounter a sizeable inoculum of these amplified oocysts in the litter have sufficient immunity to limit the degree of oocyst cycling and minimize enterocyte damage. In these birds, a portal of entry is not available to *C. perfringens* and NE is mitigated. To varying degrees, all the other treatments contained a large number of birds immunologically naïve to *E. maxima*. Their first exposure to an *E. maxima* inoculum was on day 8 and onward when the amplified vaccine oocysts have sporulated. The size of this inoculum can be far greater than the typical vaccine dose. The combination of inoculum size, lack of prior immunity and the presence of a *C. perfringens* challenge leads to significant enterocyte disruption and NE in the flock.

With optimized administration as represented herein by gavage, immunity is established earlier and oocyst cycling is limited thereafter. Accordingly, performance benefits should ensue due to less energy wastage during the later stages of grow out (8). In this study, gavage delivered a 5.6 point benefit in adjusted feed conversion. These performance results confirm that optimized administration has effects beyond mitigating NE, and the potential exists, with improved vaccination strategies, to considerably reduce live production costs.

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WHITE CHICK SYNDROME IN ALBERTA

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INTRODUCTION

White chick syndrome (WCS) is a condition of broiler breeder flocks and their progeny that can occur with no warning resulting in a short term negative effect on day old chick production. Typical episodes occur in broiler breeder flocks that are 30-40 weeks of age (3, 5) and are characterized with a drop in egg production and increased numbers of small eggs. This is followed by a drop of 0.5% to 80% in hatchability and small, weak chicks that die shortly after hatching. Gross lesions include small chicks with white feathers and green livers in the late dead-in-shell embryos and affected chicks. After 1-3 weeks, normal egg production resumes and progeny chicks no longer have abnormalities.

WCS is believed to have an infectious cause. It has been hypothesized that the infection of naive breeding flocks with chicken astrovirus (CAstV) during lay is associated with WCS and that the replication of vertically transmitted CAstV impairs embryo development and reduces hatchability (4, 5). There can be spread between barns on the same farm with a lag time in between but the same flock is never affected a second time. Subsequent flocks into the same barn can become affected. Chicken astrovirus group B (CAstV GrpB) and chicken astrovirus group Aiii (CAstV GrpAiii) have been recognized as possible etiologies (3, 5). WCS has been seen intermittently in Canada, Europe (the UK, Finland and Poland) and the United States (2, 3, 5). Two cases of WCS that occurred from eggs sourced on the same farm approximately a year apart in Alberta, Canada are described in this report.

CASE HISTORIES

WCS occurred in two breeder flocks originating from the same farm in Alberta, Canada. In winter 2014 a 30 week old broiler breeder flock experienced a drop in production from 87% to 60%. The production returned to previous levels over a period of approximately 10 days. The number of small eggs (at or below 52g) increased by approximately 20 fold and returned to previous levels over the same time period, going from approximately 0.75% to 16% and back again of total eggs laid per day. No egg shell abnormalities were noted. Life of flock mortality was 9% (performance objectives are 4-5% in rearing period and 8% in the laying period (1)). Approximately 30 days later the hatching eggs from the flock collected at and around the time of the production drop experienced markedly depressed hatchability (as low as 19.7% hatchability for the worst hatch) and an increase in cull chicks (48% of chicks hatched from the worst hatch were culled) over several hatches. Production and hatchability from the affected breeder flock's eggs returned to normal levels over approximately five days.

Approximately one year later another incident of WCS originated from the same farm, with a flock placed in the same barn as the one affected previously. Another 30 week old broiler breeder flock was affected, but egg production was within normal limits for the farm with no obvious drop in production or increase in small sized eggs noted. The sudden drop in hatchability and increase in cull chicks the hatchery experienced was the only WCS manifestation. Drop in hatchability and increase in cull chicks was similar to the first case, with a resolution of problems approximately one week after they started. As in the first case affected chicks were very slow to hatch, and eggs that were held experimentally overnight to hatch beyond the normal hatch window produced chicks that were very white in color.

MATERIALS AND METHODS

Autopsies were performed on breeder birds from the first case collected at the time of the production drop, and cull embryos/chicks from the first and second case collected at the time of the decreased hatchability at the Poultry Health Centre of Excellence (PHCE) in Airdrie, Alberta. Serum samples from the first breeder flock were collected at 18, 25, 31, and 34 weeks of age for and tested for avian encephalomyelitis virus (AEV) and infectious bronchitis virus (IBV) antibodies at the PHCE using commercial ELISA kits. Brain, liver, and oviduct tissues from the first case breeder hens were used for tissue culture and egg inoculation for virus isolation

and tracheal and cloacal swabs from the hens were submitted for PCR testing for IBV and avian adenovirus (AAdV) at the Animal Health Centre (AHC) in Abbotsford, British Colombia. Feed samples from the first flock were submitted for the 3R + chloride testing package at Central Testing Lab (CTL), Winnipeg, Manitoba and for ionophore testing at Animal Feed Laboratory, University of Guelph. Tissue samples collected from cull chicks and cull embryos including kidney, liver, proventriculus, brain and heart were fixed in 10% neutral-buffered formalin, trimmed into cassettes and submitted to the Animal Health Laboratory, University of Guelph (AHL) for histologic evaluation. Livers of cull chicks were submitted to Animal Health Laboratory, University of Guelph for chicken astrovirus PCR testing as described previously (6) and from the first submission for virus isolation in cell culture (LMH/Leghorn male hepatoma cell line). Livers from the second submission were submitted to the Diagnostic Services Laboratory of the Poultry Diagnostic & Research Centre (PDRC) at the University of Georgia in Athens, GA, USA for Astrovirus isolation.

RESULTS

Autopsy findings of cull and healthy breeder hens from the first affected flock were unremarkable. AEV and IBV ELISA titers at all time points were within normal limits for flocks in this region on the same vaccination programs. Attempts at virus isolation from breeder birds from the first case did not isolate any viruses and PCR testing for AAdV and IBV was negative. Feed samples were within normal nutritional guideline limits for breeder diets in this region and no ionophore or nicarbazin residues were detected. Cull chicks/embryos were white in color with a greenish discoloration of the liver, with some chicks having brown wiry down. Normal chicks with no visible gross lesions were produced from the same hatches that produced white chicks. The livers of the cull chicks were PCR positive for CAstV and negative for avian nephritis virus. CAstV was isolated in cell culture in both the first and second cases from liver and no viruses were isolated from submitted brain samples. Histologically the livers and kidneys from cull chicks/embryos had lesions consistent with previous WCS case reports such as bile duct proliferation, bile stasis, peribiliary fibrosis and extramedullary hematopoiesis all observed in the liver tissue(5).

DISCUSSION

This is the first published case report of WCS in the province of Alberta. The two cases of WCS manifested differently at the breeder flock level, with an increase in small sized eggs that has not been previously reported (3, 5). Litter transfer from the affected flock to younger pullet flocks coming into production on the same farm was started after the first case to try and expose susceptible birds to CAstV GrpB before egg production began. Before litter transfer was considered all testing records were reviewed to ensure that the affected flock had tested negative for Salmonella in environmental and hatchery samples. Six 20 L pails of litter collected from the affected flock were dumped on each side of the lay barn in the scratch in a cone with the aim for the younger chickens to expose themselves to infective virus at 19 weeks of age. Exposure at this age was chosen partly due to convenience with a pullet flock needing immediate exposure and also to try and seed the lay barn with virus to ensure exposure. The litter transfer technique failed to prevent a reoccurrence of WCS on the same farm, however two flocks had normal production cycles in between the first and second case on the same farm.

CAstV infection appears to be common in broilers in Ontario (unpublished findings), but the prevalence and incidence of CAstV infection in Canadian breeder flocks is not known. The pathogenesis of WCS has not been fully confirmed or explored by experimental studies. Innovative diagnostic tools such as a novel ELISA serology test for CAstV GrpB have recently become available in Europe, but are not available in Canada at this time (4). Management techniques and interventions to prevent WCS need to be developed for breeder flocks. Efficacy and optimal techniques for exposure of naïve flocks to the feces of exposed flocks or for autogenous vaccination programs have yet to be determined. Large numbers of chicks may be affected by WCS, and broiler producers may be shorted on placements as hatcheries and breeder producers may have no forewarning of the condition before hatch day. When a smaller number of chicks are affected the lowered hatchability from these flocks could adversely affect the profitability and sustainability of broiler breeder producers and hatcheries over the long term. Further research is needed on the pathogenesis of CAstV associated “White Chick Syndrome,” on the prevalence of the condition and effective prevention strategies in the field.

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EVALUATION OF DIETARY ENTEROBAC™ IN BROILERS CHALLENGED WITH *EIMERIA MAXIMA* AND *CLOSTRIDIUM PERFRINGENS*

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INTRODUCTION

Necrotic enteritis (NE) is a pervasive disease in 14-28 day-old chickens which can often result in high levels of morbidity and mortality. The etiologic agent is typically *Clostridium perfringens*. However, *Eimeria maxima* infections are considered a significant predisposing factor for NE (2). There is a need for alternatives to therapeutic antibiotics in the control of NE as producers decrease antibiotic usage. Direct fed microbials, particularly Calsporin® (*Bacillus subtilis* C-3102) (Calpis Co., Ltd., Tokyo, Japan) as a feed additive, has shown an effect on reducing *C. perfringens* in fecal samples of broiler chickens (3,4). Recent studies indicate that swine fed Calsporin resulted in significantly less *C. perfringens* in fecal samples (4,5). EnteroBac™ (Quality Technology International, Inc., Elgin, IL) is a proprietary blend of plant derived products also containing the direct fed microbial, Calsporin. The product is incorporated into rations prior to feed pelleting. EnteroBac is resistant to pelleting temperatures up to 194°F. Previous studies with plant derived combination products have shown reductions in the effects of parasitic diseases in chickens (1). The purpose of this study was to examine the effect of EnteroBac in reducing NE. The EnteroBac treatment was compared to non-treated controls and to the conventional therapeutic antibiotic effectiveness of Stafac® (Phibro Animal Health Corporation, Teaneck, NJ), virginiamycin.

MATERIALS AND METHODS

A battery trial was conducted from 0—28 day of age, using Cobb male broiler chicks. Except for Treatment 1, all birds were challenged with ~5,000 oocysts of *Eimeria maxima* on day 13 and with $1x10^8$ cfu of *Clostridium perfringens* per bird at 18, 19, and 20 day of age. No anticoccidial drug was used in the feed and no coccidia vaccine was administered to birds. Each treatment consisted of eight cages (rep) of eight birds each per treatment with each bird having 0.62 ft^2/bird. Birds were randomly assigned to cages and given ad libitum access to feeders and water via nipple drinkers. Treatments were randomized by pen prior to the start of the study.

Birds were weighed by cage on day 0, 13 and 28. Feed was weighed on day 0 and remaining feed was weighed on day 13 and 28. The trial was terminated at 28 day of age.

The four treatments used in this study were:

1. Non-infected chicks, non-treated diets
2. Infected chicks, non-treated diets
3. Infected chicks, 20 g/ton Stafac
4. Infected chicks, EnteroBac 2 lb./ton diet

NE intestinal lesion scoring (0 to 3) was performed at day 20. Three birds from each cage were randomly selected, sacrificed, weighed, and examined for the degree of presence of NE lesions. If less than three birds were present at time of scoring, all remaining birds within the cage were scored. The scoring was based on a 0 to 3 score, with 0 identified as the absence of gross NE lesions and 3 identified as the presence of severe gross NE lesions.

RESULTS

An NE mortality of 26.6% and an average NE score of 0.88 were attained in the infected/non-treated (positive control) group. When comparing the body weight gains (BWG), the two groups receiving a feed additive were significantly different from the positive control, but not the non-infected/non-treated (negative control) group. However, as expected, those birds that were infected and receiving no feed additive had the lowest BWG at day 28. Similarly, the infected/non-treated control group had significantly inferior mortality-adjusted F/G (feed-to-gain, 0-28 d of age) compared to other treatments.

When comparing the two feed additive treatments, birds fed EnteroBac had similar BWG, F/G, and NE mortality percent to birds fed diets supplemented with Stafac at 20 g/t. The birds treated with EnteroBac had significantly lower NE scores compared to other NE-infected groups.

DISCUSSION

The NE challenge in this trial can be considered moderate based on mortality levels and lowered body weights of the infected/non-treated group compared to the non-infected/non-treated control group. The feed additive treatments provided significant reductions in mortality levels compared to the positive control. The reduction in NE related mortality with the feed additive treatments was similar and not statistically different between Stafac and EnteroBac. A significant reduction in NE lesion scores was achieved with the EnteroBac treatment when compared to both the infected/non-treated control group and the Stafac treated group. Both the EnteroBac and Stafac treatments resulted in a significant improvement in the 28-day body weight gains and feed/gain ratios, compared to the infected/non-treated control group.

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Table 1.

Treatment	*NE Mortality% 28 Days*	*NE Lesion Score 20 Days*	*Body wt. 0-28 Days lbs.*	*Feed/gain 0-28 Days**
Non-infected/Non-treated	0 a	0 a	1.61 a	1.596 a
Infected/Non-treated	26.6 b	0.88 b	1.49 b	1.858 b
Stafac® 20 g/ton	10.9 c	0.83 b	1.68 a	1.657 c
EnteroBac	10.9 c	0.54 c	1.68 a	1.674 c

[a, b, c] Within data set, values with different superscript letters, differ significantly $P<0.05$.

* Feed/gain calculations are mortality adjusted

A FIELD SURVEY OF *STAPHYLOCOCCUS* SPP. AND *ENTEROCOCCUS* SPP. ENUMERATIONS FROM COMMERCIAL BROILER BREEDER FECAL SAMPLES

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SUMMARY

Staphylococcus spp. are known pathogens of chickens that can result in several diseases, most notably musculoskeletal and articulation diseases. Recently *Enterococcus* spp., particularly *Enterococcus cecorum*, has been associated with musculoskeletal infections in broiler chickens. A survey of fecal samples from 42 broiler breeder flocks throughout the Southeast United States was conducted. Multiple representative fecal samples were collected from each commercial pullet and broiler breeder chicken farm for statistical analysis. Bird age ranged from 7 to 65 weeks-of-age for pullets and broiler breeders. A range of average enumerations for *Staphylococcus* spp. was from 5.97 $\log_{10}$ in a flock of 52 week-old chickens to 7.92 $\log_{10}$ in a flock of 18 week-old chickens. Enumerations of *Enterococci* spp. ranged from 5.96 $\log_{10}$ in a flock of 55 week-old chickens to 7.76 $\log_{10}$ in a flock of 10 week-old chickens. A significant linear regression with age was associated with *Enterococcus* spp. enumerations (P=0.016; increased with age) in pullets, but not in breeders (P=0.851). A similar analysis of *Staphylococcus* spp. enumerations also showed a linear regression with age (P=0.059; decreased with age) in pullets, but not in breeders (P=0.614). Although the sampling size is small, these data indicate a possible age relationship with fecal levels of *Staphylococcus* and *Enterococcus* bacteria. These bacteria are associated with musculoskeletal diseases in broilers. A potential source of these pathogens is vertical transmission from the breeder hen. The age-related presence of either of these bacteria may be an indication of possible risk-factors associated with vertical transmission.

INTRODUCTION

Staphylococcus and *Enterococcus* are gram-positive cocci. *Staphylococcus* spp. are often identified as causative pathogens for musculoskeletal infections, particularly osteomyelitis of the femur, tibiotarsus, and spine (7). Both *Staphylococcus* spp. and *Enterococcus* spp. are generally considered to comprise large populations of commensal bacteria in the chicken intestinal microflora (3).

Recently *Enterococcus cecorum* has been identified as an emerging disease associated with vertebral osteomyelitis and spondylolisthesis in broilers and broiler parent stock (2,6,7). A survey was conducted using 16S rRNA analysis of bacterial populations associated with bacterial chondronecrosis with osteomyelitis (BCO) in broilers (4). In this study, *Staphylococcus* spp. was the most overrepresented genera identified in association with BCO lesions. Further work reported that an opportunistic *Stapylococcus agnetis* associated with BCO contained genetically distinct virulence determinants (1). The transmission of Staphylococcus and Enterococcus is generally considered to be horizontal (7,8). However, the large populations of *Staphylococcus* spp. and *Enterococcus* spp. identified in intestinal samplings of broilers, gives concern as to the source of the populations. A previous study reproduced BCO through a raised wire flooring model (9). The proposed pathogenesis is that opportunistic bacteria translocate through compromised intestinal epithelia and spread hematogenously to microfractures in skeletal areas experiencing high levels of stress. The purpose of this study is to retrospectively assess levels of *Staphylococcus* spp. and *Enterococcus* spp. from fecal samples obtained from broiler breeder flocks of various ages. The samplings were conducted over a large geographic range and from commercial farms that are representative of the US broiler industry. Bacterial enumerations of fecal samples were performed to determine shifts in bacterial populations, which could serve as risk factor indicators of high levels of *Staphylococcus* spp. and *Enterococcus* spp.

MATERIALS AND METHODS

A total of 42 fecal sample sets were collected over a diverse geographical base representing seven different states. The predominant origin of the samples was the Southeastern US and was designed to be representative of the US broiler population. A fecal sample set is comprised of six representative homogenates of three-to-five fecal droppings. The fecal droppings are collected sanitarily from random areas in the poultry housing facility. Each fecal sample is to be a fresh dropping, with minimal environmental contamination. The fecal sample consistencies were representative of healthy animals, where the contents did not contain excessive amounts of cecal material (cecal flush). The collections are conducted to minimize the chance of obtaining two samples from the same animal.

In all samplings, fecal droppings are sanitarily placed in sterile transport bags. The entire collection was performed within approximately one hour per facility. The collection from each geographic location was shipped in cooled packaging to the Calpis America, Inc. analytical laboratory. Samples were received within 24 hours of collection and bacterial enumeration procedures began immediately.

Bacteriological enumerations are performed by blinded laboratory technicians. Only the geographic origins of the samples were disclosed. Typical bacterial enumeration procedures were performed using dilution techniques and selective media for *Enterococcus* spp. and *Staphylococcus* spp.

RESULTS

Of the 42 samples, all were quantified for levels of *Enterococcus* spp. and 39 were quantified for levels of *Staphylococcus* spp. The total sample set was further segregated to 15 samples obtained from pullet flocks, < 18 weeks-of-age, and originating from pullet facilities. Twenty-seven sample sets were obtained from mature breeder flocks, >18 weeks-of-age, and originating from breeder facilities. All results are reported in $\log_{10}$ CFU/g feces.

The bird age ranged from 7-65 weeks-of-age for the entire data pool. The range of average enumerations for *Staphylococcus* spp. was from5.97 $\log_{10}$ /g feces, from a flock of 52 week-old chickens to 7.92 $\log_{10}$ /g feces from a flock of 18 week-old chickens. Enumerations of *Enterococcus* spp. ranged from 5.96 $\log_{10}$ /g feces from a flock of 55 week-old chickens to 7.76 $\log_{10}$ /g feces from a flock of 10 week-old chickens. A significant linear regression with age was associated with *Enterococcus* spp. enumerations (P=0.016; increased with age) in pullets, but not in breeders (P=0.851). A similar analysis of *Staphylococcus* spp. enumerations also showed a linear regression with age (P=0.059; decreased with age) in pullets, but not in breeders (P=0.614).

DISCUSSION

The linear regressions correlated with age for the enumerations of *Enterococcus* spp. and *Staphylococcus* spp. in pullets is relevant. The enumerations of *Enterococcus* spp. significantly increased with age during the pullet phase, 7-18 weeks-of-age. The enumerations of *Staphylococcus* spp. decreased with age during the pullet phase, but these data were not statistically significant ($P< 0.05$). For both genera, the relative average populations were similar. The average range was from approximately 5.90 $\log_{10}$ to7.80 $\log_{10}$ /g feces. This indicates that there is a relative 2 $\log_{10}$ variance, on a per/g of feces basis, in levels of these bacteria in normal fecal samples of broiler breeder chickens.

The lack of an age correlation in mature broiler breeder chickens is of interest. No significant linear regression was noted when an age relationship was analyzed. It has been suggested that mature chickens experience little variance in bacterial microflora populations (5). These data support that finding.

The small scope of this study precludes it from forming a conclusion as to possible risk factors associated with the vertical transmission of pathogens related to BCO in broilers. However, the increasing populations of *Enterococcus* spp. in pullets indicates that the highest level of fecal shedding of this potential pathogen occurs in the early breeding phase; most probably from 22-29 weeks-of-age.

It is clear that high populations of *Staphylococcus* spp. and *Enterococcus* spp. are resident in the feces of both sexually immature broiler chickens as well as in breeding stock. This finding supports data from a previous work (3). Previous reports have not correlated the vertical transmission of *Enterococcus cecorum* (7). However, recent data indicate that diverse genera are associated with BCO infections (4). It has been proposed that opportunistic *Staphylococcus* spp. are vertically transmitted, which can ultimately result in the establishment of BCO in broilers (1). Continuing research is warranted to determine if the high populations of either *Enterococcus* spp. or *Staphylococcus* spp. in feces of breeder chickens serves as a risk factor to the presentation of BCO in progeny broilers.

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POULTRY WELFARE: THE IMPACT OF CHANGING MANAGEMENT SYSTEMS FROM A SCIENTIFIC POINT OF VIEW

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INTRODUCTION

Poultry producers are facing changes in the methodology of managing their birds. The pressure for these changes comes from a number of sectors, including the food industry and animal humane groups. Do the changes ensure an improvement of the welfare of the birds? Are there benefits AND costs for the birds?

One of the most public of these changes is the move to cage-free egg systems for table egg producing hens. The list of restaurant chains and food suppliers moving to purchasing eggs from this system alone seems to be increasing daily. Recently, a large group of scientists studied many aspects of hen housing, including bird welfare, and have supported the conclusion that the situation is more complicated than some think, and that consideration of costs and benefits for the birds should be involved in this decision (1). This presentation will involve a summary of some of the findings with respect to bird welfare, of three housing systems, including conventional and enriched cages, and free-run systems.

CAGE SYSTEMS

Conventional cage systems. Conventional cages were introduced into commercial production in the 1920s, and for many years, it was believed that the cage system provided many advantages, including economics and, in some respects, bird welfare (2). The cage itself is a small, wired cage system, with a sloping wire floor that allows eggs to roll away from the hens. The cage also has access to a feeder and a drinker, and equipment to remove manure from the system.

Benefits to the birds: There are a number of benefits to the birds of being housed in cages. With regards to behavior, group sizes are small (usually six to ten birds per cage), which allows a stable social hierarchy that, once established, keeps aggression, fear, and feather pecking to levels lower than in non-cage systems. Other benefits are noted in the health of birds. The cages allow fecal matter to be removed from bird access, and the incidence of disease in a flock is generally lower than in more extensive systems. The removal of fecal matter also helps to keep air quality (dust and ammonia) to acceptable levels. Parasites such as mites are generally less of an issue in caged housing than in extensive systems. Bone breakage can occur, but the majority of those occur at the end of the flock, so birds are not living with broken bones throughout the production cycle as can occur in cage-free facilities (3).

Costs to the birds: Space is severely limited in conventional cage systems. Because of that issue, the birds do not have space to perform most behaviors, including comfort behaviors such as wing stretching or feather ruffling (4). Exercise (walking, running, and flying) is restricted, which not only is an issue for the mental status of the bird, but there is also a health component resulting in weaker bones (5) and an increased incidence of osteoporosis (6). As a result, bone breakage occurs at depopulation and during transport (3, 7). Equipment for behavioral enactment is also deficient, and of particular importance is the lack of nests, which hens clearly find frustrating, and perching, which again relates to behavior and health. Toe hyperkeratosis can occur in birds housed in conventional cages to levels higher than in either enriched or non-cage systems (8), and toe nail length may be excessive (5), leading to increased scratches. Feather damage may occur in cage facilities depending on design of the cage (9).

Furnished or enriched cage systems. A furnished or enriched cage is still a cage system, but there are some important alterations to the cage system. These include an increased vertical and horizontal space allowance, and the presence of nesting areas, perches, and in some cases scratch pads and/or dustbathing areas.

Benefits to the birds: Because enriched cages are still cage systems, most of the advantages realized with conventional cages carry through. Group sizes can vary, but if appropriate, can result in a stable social structure, and aggression and feather pecking can be significantly less prevalent than in non-cage systems. . Manure is removed from the system at a regular schedule, so disease and parasites are limited as in conventional cages, and air quality can be better than in litter systems (10). The addition of perches allows birds to express a behavior innate in jungle fowl, and also improve bone strength, which reduces

the impact of osteoporosis. The opportunity to nest has been shown to reduce frustration in hens. Dustbathing opportunities can provide a positive affective state for birds (15) and improve feather health (important for temperature regulation and as a protector of the skin), and scratch pads shorten and blunt toe nails. A number of studies have shown that mortality is lowest in furnished cages as compared to other systems (11).

Costs to the birds: While space per bird is certainly greater than in conventional cages, it is still limited. Behavioral expression is not as extensive as it is in cage-free facilities. Feather pecking, which can be impacted by increasing group sizes (12) can occur at levels above what might be seen in conventional cages.

Cage-Free systems - Free-run. *Benefits to the birds*: Free-run allow birds the freedom to move throughout their housing barn at will, so meets the "natural living" criteria of animal welfare more so than do the cage systems. The benefits for this housing system is that space is not a limiting factor, and as a result, birds move up and down to perches and fly between perches, increasing their ability for behavioral expression and improving bone density. The result of this latter point is less occurrences of osteoporosis. Birds have nest boxes for the expression of the pre-egg laying behavior, and can scratch and dustbathe in the litter area at will.

Costs to the birds: Flock sizes in free-run systems can vary, but generally are in the thousands per barn. The social stability of the group will be influenced by the actual numbers and distribution of hens in the system (13). Feather pecking and/or cannibalism behaviors may not occur in each flock, but occur more often in cage-free systems, with larger consequences in mortality levels when an incident begins. Birds have access to fecal matter, and disease levels are more prevalent as well, again leading to possible higher mortality and morbidity levels (10, 14). Parasites are more difficult to control, hence are more of a problem in these systems. Injuries can occur to the birds that are flying in free-run systems, and if it results in a broken bone, are much more difficult for managers to identify than in cage systems. Therefore, birds live with broken bones for a longer period of time (3, 12). Keel fractures also occur at a higher incidence than occurs in cage systems (16).

CONCLUSIONS

Each of the housing systems described above has advantages and disadvantages. The quality of management and attention to detail of the specific designs can improve the outcome of each. In general, enriched caged systems provide health benefits to birds, with a reduced ability for behavioral expression as compared to cage-free systems, but more than conventional systems. Cage-free systems, on the other hand, provide ample opportunity for behavioral enactment, but the cost of providing this space is reduced health and higher mortality for birds. Currently, educated concerned consumers have the ability to choose eggs at the grocery store from the housing system they believe provides better welfare for birds, but pressure to move to one housing system will remove that choice.

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CLINICO-PATHOLOGICAL CHARACTERIZATION OF RECENTLY ISOLATED NEWCASTLE DISEASE VIRUS STRAINS IN QUAIL

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SUMMARY

Newcastle disease (ND) is caused by virulent strains of Newcastle disease virus (NDV). In quail, the pathogenesis and the potential for transmission of NDV is poorly understood. To characterize the ability of virulent NDV strains to cause disease in quail, groups of 14, three-week-old Japanese quail (*Coturnix japonica*) were experimentally inoculated with four highly virulent NDV strains. At day twp post-infection (PI), 14 non-infected quail were mixed to each infection group (contact) to assess the efficacy of NDV transmission. In inoculated birds, NDV strains showed mild to moderate virulence, with highest mortality being 29% for N2 strain. Microscopic lesions consisted of non-suppurative encephalitis. Contact birds showed no clinical signs or lesions. Virus replication was minimal to absent in inoculated birds, as observed by immunohistochemistry. This study shows that virulent NDV strains can only minimally replicate and be transmitted between Japanese quail, and that this species has a certain degree of natural resistance to NDV infection.

INTRODUCTION

ND is a devastating disease of poultry worldwide. ND is caused by virulent strains of NDV, an enveloped virus within the *Avulavirus* genus, with a single stranded RNA genome of approximately 15.2kb (1,2). Based on the severity of clinical signs elicited in infected birds, NDV strains are divided (from least to most virulent) into asymptomatic enteric (avirulent), lentogenic, mesogenic, and velogenic (1,2). The intracerebral pathogenicity index (ICPI) in day-old chicks is the internationally accepted method to classify NDV virulence. ICPI scores range from 0.00 to 2.00, and NDV strains with score ≥ 1.5 are considered highly virulent (velogenic) (6). Although chickens and turkeys appear to be most severely affected, NDV strains can infect over 200 species of birds (1,2). The pathogenesis of NDV in quail is poorly understood. Few case-reports indicate that virulent NDV strains can cause severe lesions and induce high mortality rates in domestic quail, however other studies showed that experimental inoculation resulted in low mortality rates (3,5). The aim of the present study is to describe the clinical signs, lesions, and extent of virus replication (through immunohistochemistry) in groups of Japanese quail experimentally infected with four highly virulent NDV strains.

MATERIALS AND METHODS

Viruses. All viruses were propagated in embryonated specific pathogen free chicken eggs. Target dose of inoculum was 10^8.1 embryo infectious dose 50% (EID50) / bird. The following viruses were used for the study:

1). APMV-1/861-Quail (Coturnix Japonica) /Nigeria/VRD17(ND2)/2004, referred here as *N2* strain, ICPI = 1.96. This strain was isolated from quail in Nigeria in 2004.

2). APMV-1/Nigeria 23 870- Quail (Coturnix Japonica)/Nigeria/VRD17(ND2)/2004, referred here as *N23* strain, ICPI = 1.76. This strain was isolated from quail in Nigeria in 2004.

3). APMV-1/Pakistan 556-Chicken/SPVC /Karachi/NDV/33/2007, referred here as *Pakistan* strain, ICPI = 1.85. This strain was isolated from chickens in Pakistan in 2007.

4). APMV-1/Israel 826-Broiler-Breeders/Israel (Kvuzat-Yavne)/2013, referred here as *Israel* strain, ICPI = 1.85. This strain was isolated from chicken in Israel in 2013.

Animal experiment. Four groups of 14, three-week-old, Japanese quails were inoculated in the conjunctival sac with the viruses or brain-heart infusion (BHI) as controls. At two days PI, 14 non-infected quail were mixed to each infection group (contact) to assess transmission. Two inoculated and contact birds from each group were euthanized and necropsied at four, seven, and 10 days PI. Birds in extremis were sampled regardless of schedule.

Organs were fixed and processed for routine histology and immunohistochemistry (IHC). All quail used in this experiment were serologically negative against NDV antigen (hemagglutination inhibition test).

Immunohistochemistry. IHC was performed using a mouse monoclonal primary antibody against NDV LaSota strain (Novus Biologicals) at 1:7500 dilution. Heat-induced epitope retrieval was performed in sodium citrate. Detection system was a 3,3' - diaminobenzidine (DAB)-based polymer against the primary antibody (Leica Biosystem, UK).

RESULTS

Animal experiment. In inoculated animals, highest mortality was 29% for N2 strain, and below 10% for the others. Clinical signs consisted of head tremors and ataxia in N2-inoculated birds and eyelid hemorrhages in N23-inoculated birds. No significant clinical signs or gross lesions were observed in the contact or control birds.

Histopathology and immunohistochemistry. Microscopic lesions were present in the nervous tissues of birds infected with all virus strains (except N23) at 7 and 10 days PI. Lesions consisted of randomly distributed areas of non-suppurative encephalitis characterized by perivascular mononuclear cuffing, gliosis, and vacuolation of the white matter. Lesions appeared more severe in N2- and Israel-inoculated birds (Table 1). No microscopic lesions were observed in contact or control birds. IHC for NDV had a cytoplasmic and finely granular signal, and was attempted only in inoculated birds. Overall, IHC signal for NDV was minimal. Scattered immunolabelling for NDV was only observed in the spleen of N23- inoculated quails (three birds at four, seven, and 10 days PI); in the spleen and turbinates of one Israel-inoculated bird at seven days PI; and in the thymus of a N2-inoculated bird at 10 dpi.

DISCUSSION

The tested NDV strains showed mild to moderate virulence for quail, with highest mortality being 29% among N2-inoculated birds, and remaining below 10% for others. Microscopic lesions were observed only in the brain, and consisted of non-suppurative encephalitis, a lesion commonly observed in avian species that are less susceptible to NDV infection (2). No lesions in the lymphoid organs, commonly targeted by virulent NDV strains in chickens (2), were present in infected quail. Highest mortality rate, clinical signs and lesions were observed with the N2 strain, which is a quail-derived isolate. This suggests that the N2 strain may be more host-adapted and therefore slightly more pathogenic for quail than chicken-derived isolates. The other quail-derived isolate (N23) caused no lesions; perhaps due to the relatively low ICPI score (1.76). Minimal immunolabeling for NDV in the tissues of inoculated quails reflects low magnitude of NDV replication, and is consistent with the low mortality and overall mild lesions observed throughout the experiment. No lesions were observed in contact quails, and this finding underscores the low replicative capability of NDV in this species. In the context of nervous lesions, lack of IHC labeling for NDV is most likely due to the onset of encephalitis at later time points, when the virus was no longer replicating in tissues. In agreement with these results, NDV-induced encephalitis is a later event in chickens, and is commonly observed after five days PI (4).

In conclusion, this study shows that virulent strains of NDV can only partially induce clinical signs and lesions in quail, and suggests a certain degree of natural resistance of this species to NDV infection.

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Table 1. Severity of lesions in the brain of N2-, N23-, Israel-, and Pakistan-inoculated birds.

N2 strain		**N23 strain**		**Israel strain**		**Pakistan strain**	
HE		HE		HE		HE	
7 dpi	10 dpi	7 dpi	10 dpi	7 dpi	10 dpi	7 dpi	10 dpi
++	+++	-	-	-	++	-	-
-	++	-	-	+	+	-	+
N/A	-	N/A	-	N/A	+++	N/A	-

Legend: - = no lesions present; + = lesions present in < 25% of brain tissue; ++ = lesions present in 25% to 50% of brain tissue; +++ = lesions present in > 50% of brain tissue; N/A = no bird sampled.

CHLAMYDIOSIS OF NASAL GLANDS IN COMMERCIAL ORGANIC TURKEYS: PIGEON TRANSMITTED?

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CASE REPORT

In October 2015, chlamydiosis causing nasal gland adenitis was diagnosed in a 13 week old, commercial, free range organic turkey flock of 36,000 birds. This case was unrelated to a similar case diagnosed in turkeys in the Central Valley of California in 2012. The only lesions involved were either unilateral or bilateral adenitis of the nasal glands and cellulitis in the upper periocular area. Chlamydia antigens were detected by both fluorescent antibody (FA) and immunohistochemistry in nasal glands, but not in the conjunctiva, spleen, and liver of the affected turkeys. Pigeons located at a feed mill located within a mile of the farm were positive for chlamydia by FA on conjunctival smears. Gross and microscopic pathology, FA, and immunohistochemistry in the turkeys and pigeons affected, control measures, and discussion on probable factors involved with this outbreak will be presented.

SAFETY AND EFFICACY OF A VECTOR MAREK'S/NEWCASTLE VACCINE (*R*HVT-F) APPLIED TO BROILERS REARED IN NON-VACCINATING AND VELOGENIC NEWCASTLE DISEASE VIRUS (NDV)-FREE POULTRY PRODUCTION SYSTEMS

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INTRODUCTION

Velogenic Newcastle disease (ND), a dreadful poultry disease caused by a paramyxovirus type-I Newcastle disease virus (NDV), continues to be an important threat to the modern poultry industry all over the world (4). An effective control of ND consists primarily of good biosecurity practices, preventive vaccination of flocks, and the culling of infected birds and birds at risk of being infected. Vaccination programs are designed to suit the prevailing ND epidemiological situation and take into account other factors such as maternal immunity, additional vaccination programs, other pathogen circulation, types of flock, available labor, climatic conditions, inferred cost and type of vaccine used (1,4,8).

The main goal of this work was to investigate the field safety and efficacy of vaccinating against ND broilers reared in non-vaccinating and velogenic ND-free production systems. Companies involved in the field trial are located in the southeastern and southern region of Brazil and have not vaccinated their commercial broilers against ND for more than 20 years.

MATERIALS AND METHODS

Testing vaccine. Commercial broilers were vaccinated with a recombinant turkey herpesvirus-based vector vaccine (*r*HVT) expressing a key protective antigen (F glycoprotein) of the NDV (Vectormune® ND - Ceva Animal Health - Lenexa, Kansas, USA – hereinafter referred to as *r*HVT-F).

Experimental groups. The data to be presented are from two large poultry companies (integrated production systems) located in the southern region of Brazil.

Company 1. This company had not vaccinated its commercial broilers against ND for more than 20 years. A large number of broilers (943,000) were vaccinated (day one/SQ) against ND with the vector vaccine *r*HVT-F and their seroconversion and growth and clinical performance compared with that of contemporary broilers not vaccinated against ND (902,000).

The objective of the use of the vector Marek's/Newcastle (*r*HVT-F) vaccine was to assess its field safety and induced seroconversion in a totally ND-free epidemiological situation.

Company 2. This company not vaccinated their commercial broilers against ND for more than 20 years as well. The growth and clinical performance and broilers (298,000) not vaccinated against ND was compared with those of contemporary broilers (285,000) vaccinated (*in ovo*) against ND with the vector vaccine *r*HVT-F.

The objective of the use of the vector vaccine *r*HVT-F was twofold: a) to assess the vector vaccine's field safety and induced seroconversion and, b) to control ND seroconversion and NDV circulation in some of the company's integrated farms due to lateral contamination of those farms with the NDV vaccine strain HB1 and/or La Sota (5) most probably originated from either/both broiler breeder or/and commercial layer farms located in the same states. No overt clinical disease (high mortality, neurological signs) and none ND typical organ lesions (e.g., hemorrhages and necrosis in the gastro-intestinal and reproductive tracts, thymus and bursa of fabricius) were ever expressed by these NDV vaccine strains laterally-infected non-ND vaccinated broiler flocks. However, some slight increase in late grow out period mortality by bacterial secondary infections and a slight increase in carcass condemnation also by bacterial lesions (airsacculitis, septicemia) were correlated with those flocks seroconverting to ND. This is a typical clinical situation seen in broiler flocks vaccinated with live ND vaccines (HB1/La Sota).

In both, Companies 1 and 2 above, all broiler flocks had absolutely the same vaccination program (except for Newcastle), management procedures, nutrition levels, type of houses and disease challenges in general.

Sampling for laboratory analysis. Blood sampling for Elisa serology ((Idexx® NDV Ab Test) was carried out in 10 different broiler farms at

different broiler ages during the grow put period at Company 1 and in eight broilers farms at the slaughter age in Company 2.

Productivity data. When available, some key productivity parameters (daily weight gain, slaughter weight, feed conversion, final mortality and European productivity index) were per-flock collected and statistically analyzed at both companies for the different experimental groups (vector rHVT-F vaccinated broilers and not ND vaccinated broilers).

Statistical analysis. When appropriate, clinical, productivity and laboratory results were statistically analyzed by completely random analysis of variance and means compared by Tukey HSD All-Pairwise test at $p<0.05$ level. (Statistix 9.0 software – www.statistix.com)

RESULTS

Company 1. Figure 1 depicts the ND ELISA titers throughout the grow out period for broilers not vaccinated against ND and broilers vaccinated with a vector *r*HVT-F vaccine. At all sampling ages the titers were not different between both experimental groups. In addition, the clinical and growth performance data presented in Figure 2 equally indicate no difference between ND vaccinated and not vaccinated broilers.

Company 2. In Figure 3, ND ELISA titers for eight different broilers farms (integrated small farms) are shown subsequently for broilers not vaccinated against ND (previous flock laterally infected with the NDV live vaccine strain HB1), and the immediately next flock vaccinated with the vector *r*HVT-F vaccine via *in ovo* application. Vaccinated broilers presented very lower ND ELISA titers at slaughter age, in fact, in most of the farms the flocks were either negative or barely positive on the ND ELISA.

A tendency for a slightly numerically better (not different at $p<0.05$) clinical and productivity performance was detected in flocks vaccinated with the vector *r*HVT-F (Figure 4) when compared with immediately previous flocks not ND vaccinated and laterally contaminated by the live NDV vaccine strain HB1.The total % of carcass condemnation rate at slaughter was statistically lower (at $p<0.10$) for broilers vaccinated with the *r*HVT-F vaccine.

DISCUSSION AND CONCLUSIONS

The effectiveness of the *r*HVT-F ND vaccine in protecting broilers and commercial layers against experimental and field challenges from very virulent velogenic NDV has been recently and clearly confirmed in several instances (2,3,6,7,8,9). However, due to some specific ND epidemiological status and/or international market goals, some countries choose not to vaccinate against ND or to selectively vaccinate certain types of industrial birds only. This is the case of Brazil where ND vaccination is optional and many small, medium and large broiler integrator companies, located in southeastern and southern regions, have chosen not to vaccinate commercial broilers (only broiler breeders and commercial layers) since more than 20 years ago. The reasons for such decision were: a) avoid economic losses induced by post vaccination reaction induced by live commercial vaccines and, b) be able to effectively monitor any eventual NDV circulation in the companies through very a simple and cheap slaughter-age ELISA or HI serology.

Serology, clinical and growth performance of broilers vaccinated with the *r*HVT-F vaccine in companies 1 and 2, clearly indicate that it was able to effectively control NDV vaccine strain circulating in broilers flocks (Company 2) as well as inducing very low to negative antibody and very uniform titers as measured by a conventional ND ELISA (Companies 1 and 2).

In conclusion, the *r*HVT-F ND vaccine tested in this field trial was able to completely control NDV circulation in broilers while still allowing the production systems to maintain a cheap, quick and affective tool for monitoring NDV infections in their broiler flocks (slaughter-age serology). Therefore, the *r*HVT-F vaccine has provided de companies with an effective immunological tool that will protect the flocks against ND without interfering with the NDV-free status of the region.

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Figure 1. Elisa serology (Idexx® NDV Ab Test) titers (GMT) throughout the grow out cycle from broilers not vaccinated against Newcastle (black bars) and broilers vaccinated against Newcastle with a vector Marek's/Newcastle vaccine (*r*HVT-F; grey bars). Titers were not different (p>0.05) between vaccinated and non-vaccinated flocks at each age (average % CVs at all ages ranged between 24-44).

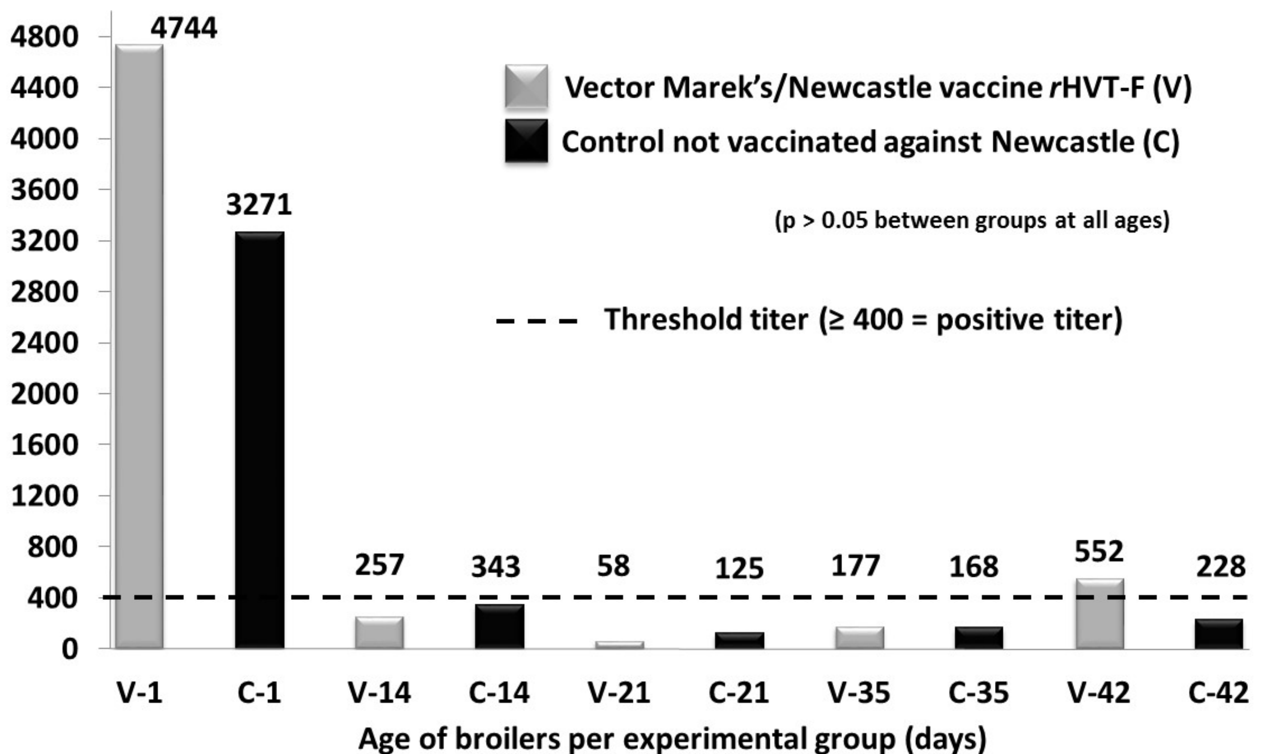


Figure 2. Clinical and growth performance of broilers not vaccinated against ND (Control) and broilers vaccinated against ND with a vector Marek's/Newcastle vaccine (*r*HVT-F). All parameters' values depicted in the graphs were not different between groups (p>0.05).

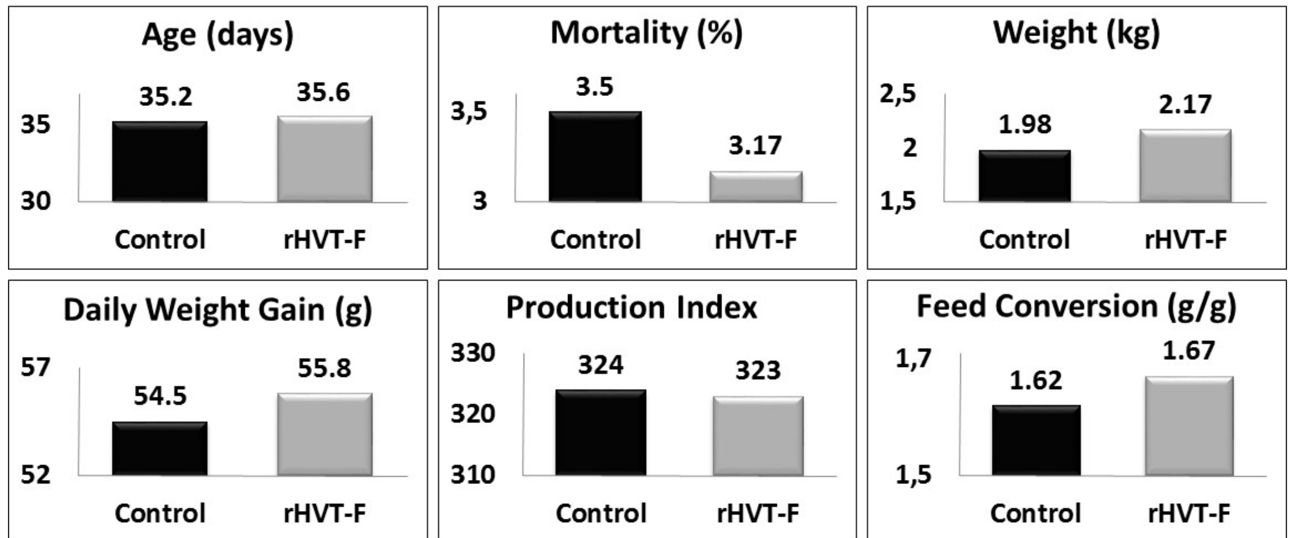


Figure 3. Elisa serology (Idexx® NDV Ab Test) titers (GMT) at slaughter age (41-43 days of age) at eight (1-8) different broilers farms. **Black bars** = titers from flocks not vaccinated against Newcastle but laterally infected with a ND live vaccine strain. **Grey bars** = titers from the first grow out cycle of flocks vaccinated against Newcastle with a vector Marek's/Newcastle vaccine (*r*HVT-F) at the same farms. Titers of non-vaccinated and vaccinated flocks at each farm are different ($p<0.05$; average % CVs ranged between 29-41 for *r*HVT-F vaccinated flocks and between 67-118 for not vaccinated flocks).

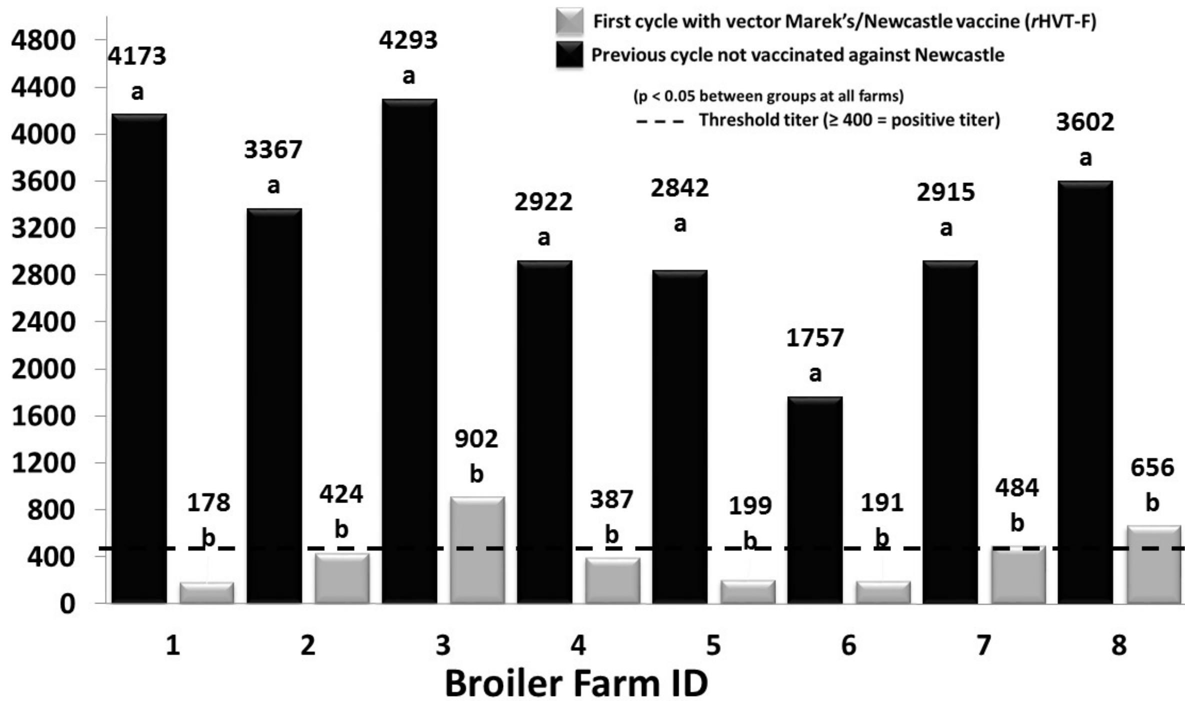
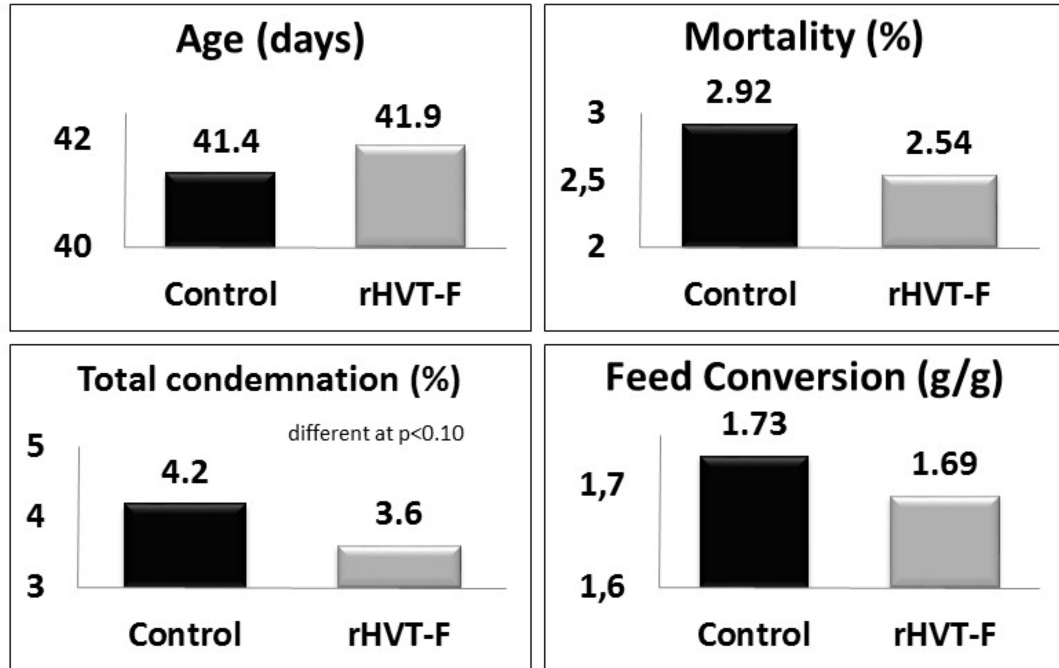


Figure 4. Clinical and growth performance of broilers not vaccinated against ND (Control) and broilers vaccinated against ND with a vector Marek's/Newcastle vaccine (*r*HVT-F). All parameters' values depicted in the graphs were not different between groups ($p>0.05$)



MULTI-STRAIN INFECTION BY INFECTIOUS BRONCHITIS VARIANT VIRUSES IN BROILER AND BREEDER FLOCKS IN LATIN AMERICA

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INTRODUCTION

Infectious bronchitis virus (IBV) causes a highly contagious respiratory tract disease in chickens, Infectious Bronchitis (IB), and some strains are nephropathogenic. It is possibly the most economically important viral respiratory disease of chickens in regions where there is no highly pathogenic avian influenza virus or velogenic Newcastle disease (vND) virus and is found everywhere that broilers are commercially produced (5). And even in some countries endemic for vND (e.g., Peru and Colombia), IB is regarded as having similar or even higher economic impact than vND (12; present report). The virus can also cause significant egg production losses and mortality in commercial layers and breeders (17).

Coronaviruses, particularly IBV, are RNA viruses that present high genetic mutation rates. When those mutations occur in the spike gene they can result in the emergence of antigenic variant strain viruses or new serotypes (11, 14). These variant strains are often partially or not controlled at all by live and inactivated IBV Massachusetts strains-based vaccines.

During the last approximately eleven years, several countries in Latin America have reported an increasing incidence of clinical and subclinical outbreaks of IB caused by variant strains of the IBV (4, 7, 12, 13, 17).

This short article reports the findings in three broiler companies in Colombia (companies A, B and C; Midwest and northeast regions) which had several broiler breeders (five flocks investigated in Company A) and broiler flocks (several flocks in Companies B and C) presenting overt respiratory signs, nephritis, late mortality, decreased egg production and decreased fertile egg quality, investigated for IBV infection. Results from ELISA serology and a molecular diagnostic survey confirmed that flocks had been infected by either one or concomitantly by more than one of the following IBV variant strains:

1) Q1 originally isolated in China (20).

2) YE/L 2865/05 original molecular detection in swabs taken from industrial poultry in Yemen (19).

3) K46/10 originally isolated in South Korea (10) and, d) PT/L 898/04 original molecular detection in swabs taken from industrial poultry in Portugal (19).

Details of the clinical and diagnostic investigation and control attempts will be reported.

MATERIAL AND METHODS

IBV molecular detection and genetic characterization and GD Animal Health. All molecular detections and genetic characterizations were carried out at the poultry diseases diagnostic laboratory GD Animal Health (Deventer, Holland). Samples were comprised by clinical tissues imprints from trachea, lungs, kidneys, cecal tonsils and oviduct on FTA card (GE Healthcare Bio-Sciences Corporation, Piscataway, NJ, USA). The FTA card material was processed for IBV molecular detection (RT-PCR) and sequencing of PCR products as described elsewhere (16). In short, nine sample discs from the dried spot on each of the four FTA card circles were collected using a 2 mm diameter Harris micro punch and placed in a 1.5 mL tube. 270 µL of RNA Rapid Extraction solution (Ambion) was added to the tube and incubated for five minutes after mixing. Subsequently 200 µL of solution for RNA extraction (MagMAX system) was added. The RT-PCR was performed as described earlier (16). A fragment of about 350 base pairs of the S1 gene was amplified with the primers $XCE1^+$ and $XCE3^-$ (3). The S1 amplicons were separated on a 1% agarose gel, and visualized with ethidium bromide staining and an ultraviolet light transilluminator. The purified amplicon was sequenced (BaseClear, Leiden, The Netherlands) using both $XC1^+$ and $XCE3^-$ primers. The sequence data were aligned using computer software (Bioedit, Ibis Biosciences, Carlsbad, USA).

IBV serology. When used, IBV serology was carried out locally (laboratories in Colombia) in commercial ELISA kits (Idexx® IBV Ab Test, Idexx

laboratories, Westbrook, Maine, USA).

Field data. Clinical signs, macroscopic lesions and flocks' clinical and productivity data were collected when appropriate or possible for those flocks suspect of undergoing an Infectious Bronchitis outbreak.

RESULTS

Table 1 presents the variant IBV strains diagnosed in the three poultry companies in Colombia. Four different IBV variant strains were detected (Q1, YE/L, K46/10 and PT/L). The diagnosis of the Q1 and K46/10 strains was carried out in few broiler flocks in Companies B and C respectively. Broiler flocks' sampling and samples submission to molecular diagnostic were done at different occasions. Likewise, the detection of strains Q1, YE/L and PT/L in Company A was done in five different breeder flocks sampled at different ages and sampling dates and samples submitted to the laboratory at a different occasions. Percentage nucleotide homology of the detected partial portion of the S1 spike gene in the variant IBVs on the FTA cards when compared with the originally GenBank-deposited sequences were 96.7-98% for the Q1 (accession numbers: AF286302 = original Q1 and HM446006 = Q1 from Chile) and, 96.3 % for YE/L (accession number = EF006524), 97% for the K46/L (accession number = JF804679) and 93% for the PT/L strain (accession number = EF066521). Further genetic comparison of the variant IBVs detected with the only IBV live vaccine strain (Massachusetts H120) available in Colombia indicated a quite small S1 protein gene homology between them (Table 2).

ELISA serology titers at slaughter in broiler flocks affected with the Q1 strain were quite high at Company C (maximum titer as high as 6000 and GMTs around 2500; vaccination scheme = 1 dose H120 spray at day one and one dose of Ma5 spray between two to three weeks of age) similar to what has been reported elsewhere (15). For Company B, infected with the K46/10 strain, IB titers were at medium to normal levels (maximum GMT = 2769; vaccination scheme = 1 dose H120 spray at day one; average slaughter age = 36 days). In all investigated flocks' serology for other respiratory disease (e.g., Newcastle, metapneumovirus infection) were not indicative of field infection.

Clinical signs in broilers were quite diverse in Company B and C and a more severe incidence of clinical disease was observed in those flocks infected by the K46/10 strain (Table 3). Late mortality (>4 weeks of age), renal and respiratory signs as well as septicemia due to secondary bacterial infection were quite common in affected flocks. In Company B affected by the K46/10 strain many infected flocks presented average mortality of up to 15% at slaughter age, most of it occurring during the last week of the grow out period (fifth week).

The investigated five breeder flocks in Company A were all clinically affected in a similar manner (Table 3). Severity of clinical signs varied from flock to flock but was always present and easily identifiable. Flocks with diagnosed infection by either the Q1 or the YE/L or the PT/L strain presented clinical disease at the ages of 27, 28, 30, 44, and 65 weeks (Table 1).

DISCUSSION

This is a quite unusual report of a multi-strain variant IBVs infection clinically and simultaneously occurring in a given country in Latin America. The Q1 strain has been causing severe losses to the broiler industry of several countries of South America during the last seven to eight years including Colombia (2,12,17).

For the first time in Latin America the Q1 strain was not only detected but also correlated with clinical disease in a breeder flock. In addition, three other variant strains never previously reported in Latin America (K46/10, YE/L and PT/L) were also detected and correlated with clinical disease and macroscopic lesions in broilers and breeders.

Company A, where Q1 and YE/L and PT/L variant strains were detected, has been seen recurrent clinical outbreaks in their breeder flocks, particularly during first 15-20 weeks of production although, in the present investigation, a 65-week flock was also found clinically affected by the YE/L strain. Of particular clinical severity (Table 3 for main clinical signs and lesions) was the infection of two young breeder flocks (27 and 28 wk of age) by the strain PRT/L. The general perception in the company is that this clinical situation from IBV infection in breeders is increasing in severity during the last several months even after each flock had been vaccinated with four live and four inactivated doses of IB vaccines during the rearing period (Mass strains only). Live Mass revaccination during production seems to slightly alleviate the clinical condition but never completely prevent/control it.

A very similar situation is occurring in broilers in Companies B and C which have had their broilers flocks challenged by the K46/10 and Q1 strains, respectively. During the mid-part of 2015 (July), an epidemiologically critical period in Company B, mortality of K46/10 affected broiler flocks averaged 14%. Broilers in that are vaccinated via spray at day one only with the H120 strain. The efforts for improving biosecurity and decreasing the

environmental contamination plus a strong work for lessening mycoplasma vertical transmission to the broiler progeny in the company have improved considerably the IBV epidemiological situation in the company. By the end of 2015 the company had gone back to almost a normal clinical situation in the broiler farms by decreasing total mortality to an average of 5.5%. Clinical IB outbreaks in broilers caused by the Q1 strain in Company C are not new for this production system since the Q1 strain has been diagnosed in the company in the past. Since the first diagnosis the company has been vaccinating broilers against IB at day one and in the field (two to three weeks of age) using two commercial Mass vaccine strains (H120 and Ma5). Nonetheless, the vaccination program has never been totally effective in controlling the Q1 strain. The same situation has been seen in recent past years in Chile, Argentina and Peru (12). A total control of the Q1 strain, at the level of both controlled protectotype trials and in the field, has only been accomplished when broilers where vaccinated with an association of a Mass vaccine strain with a 793/B-type vaccine strain (12,13,18). Such vaccination program cannot be used in Colombia at the moment since there is no 793/B-type live vaccine registered in the country. As for the K46/10 and YE/L strains there is no published information from protectotype trials indicating either the protection afforded by the Mass H120 strain alone or in combination with a 793/B-type live vaccine. From the S1 gene nucleotide homology between the K46/10, YE/L and PT/L strains with the Mass H120 strain depicted in Table 2, it can be concluded that the protection afforded by the Mass strains alone will most probably be quite low against these new variants circulating in the country just like it is against the Q1 strain (9,15). Some few years ago, Alvarado et al. (1,2) were able to carry out the first molecular detection of four unique IBV genotypes variants in Colombia although no subsequent work (pathotype and protectotype trials) has been done with those indigenous genotypes isolated from commercial layers and broilers.

Finally, at the moment, there is no epidemiological evidence/clue as to where from and how these IBV variants where able to arrive in Colombia and establish a new and quite economically damaging clinical picture in some broiler and breeder flocks of three different poultry companies. The same questioning has very often occurred in many parts of the world where new IBV variants have appeared that are genetically very similar to strains originally isolated/detected in another continent several thousands of kilometers away (7,17).

CONCLUSIONS

Such an unusual IB epidemiological situation in Colombia should be thoroughly investigated and the dissemination of the four IBV variants in the Colombian poultry industry clearly and quickly established. In addition, the K46/10, YE/L and PT/L strains should be isolated, pathotyped, protectotype trials carried out, and eventually, new and effective vaccination programs made available for the Colombian poultry industry.

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Table 1. Molecular detection of different IBV variants in Colombia in 2015.

Poultry Companies	IBV variant strain detected and flocks' age (weeks) at detection				
	Q1 broilers	**K46/10** broilers	**Q1** breeders	**YE/L** breeders	**PT/L** breeders
A			30 wk	30, 44 and 65 wk	27 and 28 wk
B		4-6 wk			
C	>4 wk				

Table 2. Percentage (%) nucleotide homology of a portion of the S1 spike gene in the variant IBVs on the FTA cards and the Massachusetts (Mass) H120 live vaccine strain used in all three companies (10,19,20; Dr. Remco Dijkman – GD Animal Health, Deventer, The Netherlands – personal communication).

	Q1	**YE/L**	**K46/10**	**PT/L**	**Mass H120**
Q1	**100**				
YE/L	83.9	**100**			
K46/10	61.5	62.9	**100**		
PT/L	81.3	85.4	62.8	**100**	
Mass H120	74-80	82.4	<58.7-63	82.7	**100**

Table 3. Relative incidence of clinical signs in broilers and breeder flocks infected with different strains of variant IBVs (incidence score: + = low / ++ = low to medium / +++ = medium / ++++ = high / +++++ = very high).

<table>
<tr><th colspan="3">Company A - breeders (in production)</th></tr>
<tr><th colspan="3">Q1 & YE/L & PT/L strains</th></tr>
<tr><th colspan="3">Clinical signs, lesions</th></tr>
<tr><td colspan="3">Increased weekly mortality in production with and without overt clinical disease
(ranging from 0.11% before to 0.35% during IB challenge in females and from 0.44% before to 2.37% during IB challenge in males [challenge period of 2-3 weeks duration])</td></tr>
<tr><td colspan="3">Decreased egg production
(from 1-4% above down to 2-3% below standard during IB challenge [challenge period of 2-3 weeks duration])</td></tr>
<tr><td colspan="3">Higher incidence of colorless egg shells</td></tr>
<tr><td colspan="3">Salpingitis, congested ovary, nephritis, renal hypertrophy</td></tr>
<tr><th rowspan="2">Clinical signs, lesions</th><th>Company B
broilers</th><th>Company C
broilers</th></tr>
<tr><th>K46/10 strain</th><th>Q1 strain</th></tr>
<tr><td>**High late mortality (> 4sem)**</td><td>++++</td><td>++</td></tr>
<tr><td>**Severe septicemia**</td><td>++++</td><td>++</td></tr>
<tr><td>**Airsacculitis**</td><td>++++</td><td>++</td></tr>
<tr><td>**Nephritis / Urolithiasis**</td><td>+++</td><td>++</td></tr>
<tr><td>**Swollen head**</td><td>++++</td><td>++</td></tr>
<tr><td>**Respiratory distress**</td><td>++++</td><td>++</td></tr>
<tr><td>**Tracheitis**</td><td>++</td><td>++</td></tr>
</table>

GPS (GLOBAL PROTECTION STRATEGY): AN ORGANIZED, SIMPLE, OBJECTIVE AND FLEXIBLE POULTRY DISEASES SURVEILLANCE/DIAGNOSTIC AND VACCINATION MONITORING SYSTEM FOR A SUSTAINED AND PROFITABLE POULTRY MEAT AND EGG PRODUCTION

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INTRODUCTION

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

Active disease surveillance is a key tool for combating infectious disease because it shapes the control strategy to be applied to an individual poultry company and/or to an entire region/country, and provides the ongoing information needed to adapt our response to disease challenges. Disease surveillance belongs on the front burner of the poultry industry because an epidemic is a regional issue and not only the concern of the few farms that may be affected initially (3,7,8,9). An active surveillance system can help break the chain of infection by providing much needed information on the status of birds at risk, on the infectious agents in the area of interest, and on the reservoirs where these agents survive (7).

It is imperative to have a practical, objective and not too expensive survey system for disease surveillance/diagnostic and chiefly for flocks' vaccination monitoring. In addition, the survey results and their correct technical/scientific interpretation must be presented to the producers and poultry veterinary professionals in a very concise/practical although meaningful way. The GPS (Global Protection Strategy) concept intends to fill up the gap of the need for a more uniform, organized and effective way of preventing both acute and sub-clinical losses from disease in modern poultry production systems.

MATERIAL AND METHODS

Key field concepts and observations/realities. There exist many different protocols for poultry disease monitoring and control at production companies' level. However, they are very rarely used in a routine/continuous way and, more importantly, the results obtained are hardly used to build an organized databank for quick reference and effective decision making processes.

One of the main concerns of poultry companies worldwide is the escalating cost of poultry production, mostly because of the steady rise in costs and lower availability of poultry feed raw materials and costs associated with labor at poultry farms and broiler processing units. As well as important hidden costs associated with poultry welfare and environment sustainability (2,5,6). Because of these higher production costs, poultry companies tend to reduce production expenses in all areas and of the most common one is exactly the biosecurity area (4), particularly, the subjects of disease prevention, monitoring and control. Therefore, in general, poultry companies go through a series of highs and lows on disease monitoring and diagnostic activities, mostly, driven by the current disease challenges momentarily occurring in the area/country where there are located. Such a policy will invariable lead to higher expenses and economic losses than when a company is effectively and consistently producing epidemiological information.

Vaccination monitoring. It is crucial for the poultry veterinarians to be able to monitor vaccine "take" from many key commercial vaccines (live and inactivated) routinely applied to poultry flocks. The poultry industry has available today a large repertoire of modern immunological tools (vector/recombinant vaccines) for the prevention of several key poultry diseases (1) which require special sampling protocols and laboratory tests for an appropriate monitoring of vaccination "take".

Disease diagnostic. For an effective and rapid poultry disease diagnostic work it is necessary for the poultry companies to have a previously designed and agreed protocol which can quickly implemented when necessary. The protocols should be developed

individually for the different diseases thought to be circulating in a given company/zone/country.

GPS (Global Protection Strategy). The GPS concept takes into account the considerations listed above and offers to the poultry companies an objective alternative for a more effective and organized vaccination monitoring, disease diagnostic and control and epidemiological trends over time.

RESULTS AND DISCUSSION

Vaccination monitoring and Disease diagnostic. Tables 1 and 2 present the suggested guidelines for vaccination monitoring and disease diagnostic. These guidelines must be previously discussed and, if necessary, adjusted/adapted to the particular epidemiological circumstances of each company and each geographical region where the GPS concept will be implemented. Once implemented, the GPS protocols will remain unchanged for the duration of the usage of any particular vaccine.

Laboratory results interpretation and recommendations. All results from the laboratory tests carried out must be evaluated/interpreted by the veterinary professionals in charge of the GPS concept in any company in a given region/country. These professionals will be responsible statistically analyzing the laboratory/clinical/performance data and developing a concise, graphical/illustrative and very objective final report to be presented to the company where the GPS concept is being carried out. A specific report format must be followed and a set of specific technical recommendations must be the report's final segment.

Presentation and discussion of GPS results. The most crucial part of the GPS concept is the final meeting for presentation, discussion and recommendations from the GPS results obtained in the last sampling session carried out in the company either for vaccine monitoring and/or disease surveillance and diagnosis.

At this meeting, the veterinary professional responsible for the GPS will present and discuss the report and justify the final recommendations to the poultry company's key representatives (veterinary services, production and administration).

CONCLUSIONS

The use of the GPS concept has been of great success in several medium to large size broiler companies in Latin America (Brasil, Colombia and Argentina). At the moment, many other companies are under the GPS guidelines in Mexico and Peru.

Both administrative, veterinary and production personnel of those companies have clearly indicated their satisfaction with the way of GPS has been designed. It has been of great support in regard to the internal decision-making processes for fine tuning veterinary expenses and yet having a very effective and rapid epidemiological data (vaccination monitoring and disease diagnostic).

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Table 1. GPS for vaccine monitoring – general guidelines.

VACCINE	Age at sampling	Type of samples	# of samples	Lab tests	Recommended laboratory	Frequency
- All *r*HVT vector vaccines (any type of birds)	3 wk	Either wing feathers or spleen (preferably)	- 5 feathers/bird or - 5 spleens - 5 birds/flock - 5 flocks or farms/GPS sampling	PCR for detection of the specific *r*HVT	Poultry diagnostic lab formally validated for the test to be used	3-4 times /year
- Newcastle - *r*HVT-F$_{protein}$ of NDV - Vectormune ND	6 wk	Blood serum	- 20/flock - 5 flocks or farms/GPS sampling	- Conventional or specific ND Elisa - HI with specific control serum, antigen and protocol		
- Gumboro - *r*HVT-VP2$_{protein}$ of IBDV - Vectormune IBD	6 wk	Blood serum	- 20 /flock - 5 flocks or farms/GPS sampling	Conventional Elisa		
	6 wk	Bursa of Fabricius - BF	- 5 BF/flock - 5 flocks or farms/GPS sampling	- IBDV RT-PCR/sequencing - Histopathology		
- Mycoplasmosis / Laryngotracheitis - *r*FP vector vaccines (long living birds) - Vectormune FP MG or FP LT	6-7 days after Wing Web application		500 birds (uniformly distributed in the house)	Visual quantification of vaccine FP virus “take” on the wing web (% of birds with wing web lesion – skin swelling/scab)		
- IBD Immune complex vaccine / - Transmune	5 wk	Bursa of Fabricius - BF	- 5 BF/flock - 5 flocks or farms/GPS sampling	- IBDV RT-PCR/sequencing - Histopathology		
	6 wk	Blood serum	- 20/flock - 5 flocks or farms/GPS sampling	Conventional Elisa		
- IBV vaccines (day 1 - spray) - IBird	5-7 days	Trachea	- 10 birds/flock - 3 flocks or farms/GPS sampling	IBV RT-PCR/sequencing		
	6 wk	Blood serum	- 20/flock - 5 flocks or farms/GPS sampling	Conventional Elisa		

Table 2. GPS for disease diagnostic purposes – general guidelines

DISEASE	Age at sampling	Type of samples	# of samples	Lab tests	Recommended laboratory	Frequency
IBD or Gumboro Disease	When suspect clinical signs/ lesions occur	Bursa of Fabricius - BF (from affected birds)	5 BF/flock	- Virus isolation - IBDV RT-PCR/sequencing - Histopathology	Poultry diagnostic lab formally validated for the test to be used	Two rearing cycles – same suspect farms and close by farms
	Slaughter age (broilers)	Blood serum	20/flock	Conventional IBD Elisa		
ND or Newcastle Disease	When suspect clinical signs/ lesions occur	- Spleen - Intestinal tract - Trachea - Lungs (from affected birds)	5 birds/flock	- Virus isolation - NDV RT-PCR/sequencing		
	Slaughter age (broilers)	Blood serum	20/flock	Conventional ND Elisa		
ILT or Infectious Laryngotraqueitis	When suspect clinical signs/ lesions occur	- Inferior eyelids - Trachea (from affected birds)	5 birds/flock	- Virus isolation - ILTV RT-PCR/sequencing - Histopathology		
	Slaughter age (broilers)	Blood serum	20/flock	Conventional ILT Elisa (if flock not vaccinated)		
IB or Infectious Bronchitis	When suspect clinical signs/ lesions occur	- Trachea - Kidneys Cecal tonsils (from affected birds)	5 birds/flock	- Virus isolation - IBV RT-PCR/sequencing		
	Slaughter age (broilers)	Blood serum	20/flock	Conventional IB Elisa		
MD or Marek's Disease	When suspect clinical signs/ lesions occur	- Peripheral nerves - Tumors (skin and organs) (from affected birds)	5 birds/flock	- qRealTime PCR (for serotype 1) - Histopathology		

AN OVERVIEW OF OUTBREAKS OF LPAI AND HPAI H5N8 IN COMMERCIAL POULTRY IN CALIFORNIA

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Avian influenza (AI) viruses infect various species of birds and mammals including humans. AI viruses are divided in to various subtypes based on two surface glycoproteins; hemagglutinin (H) and neuraminidase (N). There are 16 H (H1- H16) and 9N (N1- N9) subtypes with a potential for 144 combinations. They are further divided in to low pathogenic AI (LPAI) and high pathogenic AI (HPAI) strains depending on their pathogenecity and LPAI strains can change to HPAI on some occasions. AI viruses of subtypes H5 and H7 are considered to be pathogenic to poultry species or have the potential to become HPAI. Waterfowl such as ducks, geese and swans and shore birds such as gulls, terns, plovers, etc. can act as reservoirs for AI viruses. The following is a summary of AI H5N8 outbreaks in California in quail, turkeys, chickens, and ducks during the years 2014 and 2015.

In April of 2014, LPAI H5 N8 was diagnosed in 10 to 15-week-old Coturnix quail (*Coturnix C. Japonica*) from a flock of 25,000 birds located in the Stanislaus County, CA. Except for increased mortality in the flock there were no significant clinical signs reported. Necropsy of 20 birds revealed confluent pale foci of necrosis and hemorrhages in the pancreas in most of the birds and pale foci of necrosis in the liver of a few birds. Histopathology revealed acute multifocal to locally extensive severe to massive coagulative necrosis of acinar cells with little or no inflammation in the pancreas. Livers had mild to moderate coagulative necrosis of hepatocytes with mild infiltration of lymphocytes. Immunohistochemistry (IHC) for AI revealed nucleoprotein in the nucleus and cytoplasm of pancreatic acinar cells of most birds and in the hepatocytes, monocular cells of the spleen and cells in the interstitium of lungs in a few birds. Oropharyngeal and cloacal swabs from the quail tested positive for AI by RT-PCR. AI virus was isolated, sequenced and determined to be LPAI H5N8 of the North American lineage. Birds in the affected premises were depopulated, the premises were cleaned and disinfected and surveillance of birds in the 10 to 20 km zone did not reveal any positive birds for AI.

In January of 2015, HPAI H5N8 was diagnosed in 14-week-old turkeys with a history of increased mortality of eight, 75 and 500 per day in the last three days from a flock of 10,000 turkeys in Stanislaus County, CA. There were a total of 150,000 turkeys on the ranch.

In February of 2015, HPAI H5N8 was diagnosed in 12-week-old brown chickens with a history of mortality of 72, 123, 110, 140 and 170 per day in the last five days from a flock of 26,500 chickens in Kings County, CA. There were a total of 100,000 chickens housed in four different houses on the ranch.

Necropsy of five turkeys and 11 chickens revealed similar lesions except for a few differences. These included enlarged and mottled pale spleens, pale patchy or red areas in the pancreas, hemorrhage in the cecal tonsils of turkeys and increased mucus in the trachea and small and pale spleen in the chickens. Histopathology in turkeys and chickens were similar and included encephalitis, pancreatitis, splenitis tracheitis, pneumonia, myocarditis and IHC revealed nucleoprotein in the cells of these organs.

The ranch which housed chickens also housed about 36,000 Pekin ducks ranging in age from two to four weeks in three different houses. One of the houses that housed four week old ducks in a house of 16,000 experienced decreased feed consumption, increased mortality that ranged from normal five to seven per day to 22, 24, 18, 28, 33, and 65 per day in the last six days. About 2% of the ducks in the flock were experiencing ataxia, torticollis and opisthotonus. Necropsy of six live ducks revealed mild cloudy air sacs in three birds and pale foci of necrosis in the liver and pale patchy myocardium in one bird each.

Histopathology in ducks was similar to turkeys and chickens and included encephalitis, pancreatitis, splenitis, tracheitis, pneumonia, myocarditis and also hepatitis. IHC revealed nucleoprotein in the cells of these organs. Similar to the quail, the oropharyngeal and cloacal swabs from the ducks and oropharyngeal swabs from turkeys and chickens tested positive for AI by RT-PCR. AI virus was isolated, sequenced and determined to be HPAI H5N8 of the Eurasian lineage. Birds in the two affected premises were

humanely euthanized by foam and composted in-house, pressure cleaned and disinfected. Swabs taken periodically from the houses were negative for AI by RT-PCR. Surveillance of birds in the 10 to 20 km zone did not reveal any positive birds for AI.

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AN OUTBREAK OF TS65 (WRY NECK) IN A FLOCK OF HERITAGE TURKEYS

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Turkey Syndrome 65, also called Wry Neck, was reported in turkeys in Great Britain in 1965, hence the name TS65. The disease is characterized by poor feathering, stunting, perosis, tibiotarsal deformities as well as deformities of the cervical vertebrae and airsacculitis in poults over three weeks of age. TS65 has been associated with *Mycoplasma meleagridis* (MM) but the role of MM or its pathogenesis with or without wry neck is not known. Genetics has also been proposed as the cause of this syndrome.

TS65 was diagnosed in three-week-old tom turkey poults from a flock of 210 and in seven-week-old tom turkeys from a flock of 316 heritage turkeys. History included difficulty walking, twisted tibias, "cowboy hocks," crooked necks, and increased mortality of 78/210 and 201/316 placed in the two flocks respectively. Clinically most of the six tom turkeys submitted for laboratory evaluation had trouble walking, swollen hock joints with slipped gastrocnemius tendon and crooked necks in the form of 'S' shape. Necropsy revealed misaligned cervical vertebrae, slipped gastrocnemius tendon and mild cloudy air sacs. Histopathology confirmed airsacculitis, and also sinusitis, tracheitis, pneumonia and synovitis. MM was isolated from the air sac and trachea of both groups. Serologically older birds were positive for MM and all the birds were negative for MG, MS, AI and NDV.

AN OUTBREAK OF CHICKEN INFECTIOUS ANEMIA VIRUS INFECTION IN A FLOCK OF 15-DAY-OLD BROWN CHICKENS

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Chicken infectious anemia is a disease generally of young two to four week-old chickens caused by a virus classified in the genus Gyrovirus of the family *Circoviridae.* The disease is characterized by anemia, lymphoid atrophy especially of the thymus, muscular hemorrhages and immunosuppression. Clinical signs include depression, decreased weight gain, anemia where the hematocrit values can range from 6 %-27% (normal 29%-35%). Often birds infected with the virus are predisposed to secondary bacterial, mycotic, parasitic and viral infections. Gangrenous dermatitis affecting primarily the wings is common. Diagnosis of the disease can be based on clinical signs, isolation of the virus in cell culture, serology and PCR. The disease can be controlled or prevented by vaccinating breeder flocks prior to the onset of egg production preferably no closer to egg production than four weeks.

Approximately 1% of birds from a flock of 13,000 fifteen-day-old brown chicks experienced depression, loss of feathers, gangrenous dermatitis and increased mortality; 30/day to 210/day in three days. More than 500 birds with clinical signs and gangrenous dermatitis were culled. Necropsy of eight live and six dead chicks confirmed severe gangrenous dermatitis primarily on the wings and some on the keel and thigh. In addtion most of the birds had severely atrophied thymuses and mildly atrophied bursa of Fabricius, pale bone marrow, pale blood, delayed clotting time and decreased packed cell volume ranging from 15%-20%. Histopathology of various organs revealed severe lymphoid depletion in the thymus, bone marrow hypoplasia of erythroid, myeloid and thrombocyte series. Six of eight birds were positive for chicken infectious anemia virus (CIAV) by serology. These lesions are consistent with CIAV infection resulting in immunosuppression and predisposing the birds for secondary gangrenous dermatitis. Lack of maternal immunity in the chicks due to failure to vaccinate the breeders resulted in CIAV infection.

IMPACT OF EMERGENCE OF AVIAN INFLUENZA IN NORTH AMERICA AND PREVENTATIVE MEASURES

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SUMMARY

Since 1959, the world has experienced 39 highly pathogenic avian influenza (HPAI) epizootics with the largest beginning in 1996 in China that spread to affect 70 countries in Asia, Europe and Africa, and recently North America. Eurasian H5N8 and reassortant H5N2 HPAI viruses were identified in USA. These H5 HPAI viruses spread through the Pacific Flyway and to the Midwest USA. The infected premises in Western and initial premises in Midwestern USA were point source introductions from wild birds, while most cases in the Midwest had secondary spread from common sources because the initial viruses were waterfowl adapted but later were adapted to gallinaceous poultry.

INTRODUCTION

The first cases of HPAI, previously termed fowl plague, were reported in 1878 in northern Italy, followed by widely dispersed geographic outbreaks throughout the late 1800's to 1950's in Europe, Asia, Africa, and North and South America. In general since 1959, HPAI outbreaks have been more geographically restricted with 39 distinct HPAI epizootics. Such HPAI viruses have arisen from H5 or H7 low pathogenicity avian influenza (LPAI), which the latter are non-pathogenic flora in some migratory waterfowl and shorebirds. These LPAI viruses after exposure, adaptation and circulation in gallinaceous poultry developed specific mutations in the hemagglutinin protein that conferred phenotypic traits of high pathogenicity. Most of these epizootic HPAI viruses were geographically limited, involved farm-to-farm spread and were eradicated from poultry by stamping-out programs; i.e. the HPAI viruses did not circulate in wild birds. However, an H5N1 HPAI virus emerged in 1996 in a goose in Guangdong China (Gs/GD lineage), and unlike the viruses in the other 38 HPAI outbreaks, has caused deaths in wild birds, poultry and humans, and spread to over 70 countries in Asia, Europe, Africa and North America; drastically changing the perspective on HPAI biology. The severity, size and broad geographic distribution of Gs/GD epizootic merits the term panzootic.

HPAI NORTH AMERICA, 2014-2015

Between December 2014 and June 2015, Canada and USA experienced an unpreceded outbreak of H5 Gs/GD-lineage HPAI as the first intercontinental spread of a Eurasian HPAI virus to North America. Initially, a reassortant H5N2 clade 2.3.4.4 HPAI virus was identified in British Columbia, Canada, and within a few days the original Eurasian H5N8 and a Eurasian-North American reassortant and variant H5N2 HPAI viruses were isolated from a gyrfalcon and a wild duck in Washington State, respectively. This H5N8 HPAI virus was spread by migratory waterfowl from Eastern Asia to Russia, Europe and North America (2,4). In North America, the Asian H5N8 HPAI virus reassorted with North America LPAI virus genes to produce H5N2 and H5N1 HPAI viruses (1,3). Subsequently, the H5 HPAI viruses spread through the Pacific Flyway and to the Midwest USA.

Molecular analysis indicated that the infected premises (backyard and commercial) in Western USA and initial premises in Midwestern USA were point source introductions from wild birds, while most cases in the Midwest had secondary spread from common sources; i.e. linkages between Midwest farms. In total, the H5 HPAI outbreaks has affected 21 states, with detections in four captive wild birds, 75 wild birds, 21 backyard flocks and 211 commercial flocks, totaling over 48 million birds. In the USA, the eradication effort cost more than $1 billion and the negative economic impact was over $3.2 billion.

TRADE ISSUES

HPAI can be used as a non-tariff trade barrier to prevent introduction into an HPAI-free country, but the risk of introduction through trade varies with commodity. Importation of live birds is the highest risk while importation of cooked products being the lowest risk for HPAI virus introduction. The surveillance data and risk analysis used to ensure

freedom from HPAI, and thus low risk for trade, must take into account several variables:

1) the extent of coverage such as whole country, a defined geographic zone or a management compartment.
2) the number of samples and number of farms/premises tested for a specific prevalence rate.
3) sensitivity and specificity of the surveillance test methodology.
4) stage of infection as either active viral infections or antibodies indicating a history of infections which may be over.

All results should be reported transparently to the World Organization for Animal Health and trading partners.

PREVENTATIVE MEASURES

Improvements have been made to HPAI response in USA based on lessons learned from the 2014-2015 outbreak and include redevelopment of an H5 vaccine bank, commitment to a timeline of 24 h from diagnosis to depopulation, and refocusing to rapid disposal methods with composting preferred and landfill as secondary method.

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BIOSECURITY SELF-ASSESSMENT

K. Takeshita

SUMMARY

During 2015 HPAI/LPAI Outbreaks, the need for improved biosecurity became apparent. Numerous Federal and State programs try to address biosecurity including NPIP, CEQAP, FDA egg rule and SEFS. To enable the producer/owner to assess/improve their own biosecurity, a Biosecurity Self-Assessment Guide was developed in CA. The Biosecurity Self Assess is divided sections (Figure 1.).

Each section is in tabular form divided into multiple rows representing Risk Categories and 3 columns representing Low, Moderate and High Risk. In each column for each category, an explanation of what a Low, Moderate and High Risk is described for that row (Figure 2.). Simply go down the list and determine the criteria that best fits your current biosecurity practices. Each response is rated as Minimal Biosecurity Risk, Medium Biosecurity Risk, or High Biosecurity Risk.

Minimal Biosecurity Risk. Based on current knowledge, these biosecurity practices are outstanding and you have reduced the risk of introducing infectious disease into your flock. Efforts should be directed toward improving the biosecurity practices that score in the previous categories to meet this level.

Medium Biosecurity Risk. Based on current knowledge, your farm has moderate biosecurity practices in place to prevent introduction of disease. However, there is room for improvement and you may consider consultation with your poultry veterinarian to review these practices and assess the value of making changes to further safeguard your flocks.

High Biosecurity Risk. Based on current knowledge, this biosecurity practice (or lack thereof) puts your flock at an extremely high risk of disease introduction. Consultation with your poultry veterinarian is recommended to determine if your biosecurity protocols in these areas should be or can be changed to better protect your flock and the rest of the industry.

Routine Biosecurity Risk Assessment. This Biosecurity Assessment was distribute at industry meeting and posted on CDFA and industry Web Sites since the middle of 2016. This Guide allows the producer or farm manager the opportunity to assess their current level of on-farm biosecurity. Their answers will provide them with an idea of where there are areas of weakness that require attention or practices that fall below current industry standards.

Biosecurity Risk Assessment During an Outbreak. Using the Biosecurity Self-Assessment, trained assessors will visit sites with birds within 3 kilometer around an infected premise. At the site they will identify any "High Risk" items and record them on "High Risk" Record Sheet (Figure 3) noting the category (row #) of "High Risk" in first column, description of "High Risk" 2nd column and if photos were taken in 3rd column. If "High Risk" items are observed, Surveillance and Epidemiology would be contacted and enhanced surveillance may be initiate till "High Risk" is mitigated. Biosecurity Risk Self-Assessment Guide can be found at https://www.cdfa.ca.gov/ahfss/Animal_Health/BioSpecies/CommercialPoultryBiosecurity.html

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Figure 1. Biosecurity Self Assess is divided sections.

General

1. Location ....p. 1
2. Premise Entry/Security ....p. 2
3. People Entry/Personnel Biosecurity ....p. 3
4. Employees & Visitors Exposure to Birds....................................P. 4-6
5. Poultry Houses ....p. 7
6. Pest, Wildlife, and Domestic Animals ....p. 8-9
7. Truck Traffic ....p. 10-11
8. Tools and Equipment ....p. 12
9. Cleaning and Disinfectant ....p. 13
10. Carcass/Manure/Garbage Storage ....p. 14
11. Flock Health ....p. 15-16
12. Biosecurity Assessment ....p. 11

Egg-Layer Biosecurity ....p. 18-19

Meat Type Biosecurity

1. Hatchery Specific ....p. 20-21
2. Breeder Specific ....p. 22
3. Breeder and Commercial Turkeys ....p. 23
4. Broiler Biosecurity ....p. 24-25

Location		Minimal Biosecurity Risk	Medium Biosecurity Risk	High Biosecurity Risk	
2	**Proximity to nearest unrelated commercial poultry operation**	Greater than 2 miles	1/2 mile to 2 mile	Less than 1/2 mile	i, ii
4	**Proximity to nearest backyard poultry**	Greater than 2 miles	1/2 mile to 2 mile	Less than 1/2 mile	ii
Premises Entry/Security					
10	**Perimeter Fencing And Gates**	Complete Perimeter Fence present. Driveway is gated and always locked or guarded	Perimeter fencing and gate present, but not always locked or guarded. Or fence not complete	There is no perimeter fencing or gate	i
13	**Vehicle Entry and Disinfection**	Freshly stocked vehicle disinfection station with high pressure sprayer at the gate for all vehicles	Inadequate vehicle disinfection station	No vehicle disinfection station or not used	ii
People Entry/Personnel					
34	**Sharing of personnel**	Personnel are dedicated to work on this farm only and not shared with any other farm or off-site facility	Personnel are shared between this farm and other farms of this same company, but not with any off-site facility	Personnel are shared between this farm and a farm of another company, or shared with an off-site facility	ii, iii
35	**Personal Protection Policy**	Shower in policy with disposable or dedicated clothing and footwear before entry	Disposable or dedicated clothing and footwear and required washing/disinfecting of hands before entry (no shower-in policy)	Personnel lack disposable or dedicated clothing and footwear and/or do not practice washing/disinfecting of hands before entry	i
42	**Fairs (bird exhibit areas)**	No contact with other birds within 72 hours prior to entry	No contact with other birds within 48 hours prior to entry	No restrictions on contact with other birds	i, ii
66	**Standing Water, Ponds, or other water bodies on Farm**	Water not allowed to pool or stand for more than 48 hours	Water not allowed to pool or stand for more than 72 hours	Allow standing water and/or ponds on the farm	iv

Figure 3. "High Risk" Record Sheet.

Row	**Description of "High Risk" Deficiency**	**Photo**
66	**There is a drainage ditch with standing about 10' from house 16. Ducks were observed in other areas of standing water**	Yes/No
#		Yes/No
#		Yes/No
#		Yes/No
#		Yes/No
#		Yes/No
#		Yes/No
#		Yes/No
#		Yes/No
#		Yes/No
#		Yes/No
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#		Yes/No
#		Yes/No

HEMORRHAGIC LIVER SYNDROME IN BROILER BREEDERS: CASE REPORT

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Hemorrahagic liver syndrome is mainly a disease of commercial layers. Usually a percentage of hens in the flock are overweight, and a drop of egg production is observed. This same situation was observed in a broiler breeder flock with around 30% of affected hens. This syndrome was observed in hens that appear to be healthy and during peak of egg production

Excess fat accumulation can only be caused by an excess of energy relative to needs for production and maintenance. Low protein and high energy diets and diets where there is an amino acid imbalance or deficiency can be major contributors to a fatty liver condition. Genetics also can play a part to in this syndrome.

Different etiologies can be mentioned, like chelated trace minerals, molds, rapeseed and canola meals. It is mentioned that high fiber diets or skip a day program can develop big appetite becoming more susceptible to this condition.

Egg production causes changes in the lipid metabolism due to the demand of yolk formation. Different authors mention the ability of birds to storage high quantities of energy as triglycerides mainly in liver. Lipogenesis take place primarily in the liver of birds, involving a series of reactions. The citric acid cycle, glycolysis, and the fatty acid synthesis, are some of the reactions that occur in the hen. Alteration in function due to nutritional, genetic or environmental factors can develop fatty liver. The condition is characterized by sudden death due to hepatic rupture and hemorrhage.

This condition in broiler breeders during production had an economic impact; therefore the diet plays a very important role in the presentation of this syndrome. Drop in egg production and high mortality were observed in a broiler breeder farm. Twenty broiler breeders were chosen randomly to do post-mortem. Enlargement of the liver was the most common finding. It was friable, some were pale, and in others we could observe hemorrhages.

Liver samples were taken for histopathology. Fibrosis and presence of vacuoles in the liver were the most common findings. Lesions were similar to those of fatty liver hemorrhagic syndrome. Samples from the meal were also taken to rule out mycotoxins.

It is important in this case to use a hepatic protector, as well as antioxidants in order to modify the hepatic cellular membrane. This could prevent the entrance of toxins in the liver and neutralize free radicals that are a result of metabolic/physiologic processes during point of lay. A product with Silymarin (milk thistle) was used as well as mycotoxin products in the feed.

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DISASTER FLOCKS: WHEN GOOD INTENTIONS GO BAD

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INTRODUCTION

Small specialty poultry flocks are exploding in popularity driven by public desire to have local fresh eggs and meat. Unfortunately, many of the farmers are new to poultry farming, and do not have training in the basics of biosecurity and disease containment. In addition, public demands for organic products limit the types of intervention that can be used when disease issues do occur on the farms. We will be presenting an ongoing case study on one of these farms, a client of the Purdue University Poultry Diagnostic Service.

CASE HISTORY

In October, 2015, the managers of the farm contacted our service for investigation of increasing mortality in broiler chickens. The farm consists of a single house in Central Indiana that was formerly used as a commercial turkey house. The flock of approximately 5,000 birds of various ages was held in single age pens of 600 birds. The farm had been in operation for a year with mortality steadily increasing from 7-8% during the first six months of operation until 25-30% in the last month. No treatment had been instituted as the farm was organic. The chicks were purchased from at least three different small Midwest hatcheries and were unvaccinated.

CASE REPORT

On the first visit, the following observations were made. The broiler pens occupied ¾ of the house while adult, mixed breed layers occupied ¼. There was no air separation and only a half wall separating the broilers and layers. Although the pens originally contained same age broilers within each pen, it was difficult to determine the age parity due to extreme lack of uniformity of the birds, and the ability of the birds to jump the barriers into other pens. One pen had recently contained growing turkeys and turkey feathers were observed both within the pen and in other pens throughout the house. The litter was not changed in between runs and some litter had been present for at least six months. New hatchlings had just been placed in the pen previously occupied by the turkeys. Ventilation was adequate but heat was less than adequate in three of the pens containing the youngest broilers. Numerous dead and moribund broilers were observed in each of the pens. Snicking, lameness, diarrhea, stunting, pendulous crops, ataxia, depression and feather loss were seen in the broiler pens. Recent total mortality at slaughter was 50-60%. No health issues were observed in the layers. Both moribund and dead broilers were brought to the laboratory for necropsy. Diagnoses included Reovirus tenosynovitis, IBD, IBV, MD and salmonellosis. The owner refused to depopulate and selected to clean out litter from the vacated pens and source MD vaccinated birds. A second visit one month later showed marginally improved mortality in the recently acquired broilers. A second submission of broilers approximately two months after the initial visit resulted in a diagnosis of Aspergillosis.

About one month after the initial visit, mortality was observed in the layers. The birds were coughing, had swollen sinuses and a drop in egg production. Diagnoses based on necropsy and serology was ILT and MG. The owner again resisted depopulation of the whole layer flock, and only depopulated birds on one side of the building that were given to him by an unnamed source.

DISCUSSION

We are continuing to follow the flock through both farm visits and laboratory examination. Although mortality in the broilers and layers has had some improvement, the owner is continuing to see total mortality in the broilers approaching 40%. The resistance to depopulation, cleaning, disinfection and single age broiler restocking has made it difficult to find ways to improve the health of the birds.

NECROTIC ENTERITIS IN YOUNG BROILER CHICKS

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SUMMARY

Necrotic enteritis (NE) is primarily a disease of broiler chickens between two and five weeks of age and is often seen in association with coccidiosis. In this case lesions consistent with NE were seen in seven of 37 broiler chicks less than 12 days of age housed together in one room of a research institute. Gizzard erosions without signs of NE were present in an additional seven of 10 birds examined at 14 days of age. An adjacent room housing 37 chicks of the same age and from the same source had no cases of NE and no apparent gizzard erosions. Feed, water and housing conditions were identical in both rooms, and no experimental procedures had been performed on any of the birds. The cause of the gizzard erosion and necrotic enteritis is not known.

INTRODUCTION

A larger than expected number of mortalities was experienced in five to 12-day-old broiler chicks housed at a research institute. Gross and microscopic examination revealed lesions consistent with NE in over half of the dead birds. Gizzard erosions without signs of NE were present in an additional seven of 10 birds euthanized at 14 days of age. An adjacent room housing chicks of the same age and from the same source had no cases of NE and no apparent gizzard erosions. Housing and husbandry conditions were identical in both rooms, and no experimental procedures had yet been performed on any of the birds. The cause of the gizzard erosions and necrotic enteritis could not be determined.

MATERIALS AND METHODS

Seventy-four Ross broiler chicks were picked up from a local hatchery on day of hatch (Day 0) for use in a research trial. Routine treatment at the hatchery included *in ovo* vaccination for Marek's and infectious bursal disease, and spray vaccination for infectious bronchitis virus at hatch. Chicks were examined, neck tagged, and divided between two biocontainment rooms (Rooms A and B) in a dedicated poultry building. The rooms had recently been painted and had new rubber flooring installed. In preparation for chick arrival, floors, walls, feeders and waterers had been decontaminated with 1% Virkon® (Vetoquinol N.-A inc, Lavaltrie, Québec), rinsed, and allowed to dry thoroughly. Heavy plastic sheeting was then placed over the entire floor surface and extended approximately two feet up the walls, where it was taped in place. The floor was bedded with about two inches of a soft cellulose substrate (BioFresh™ Comfort Bedding, Absorption Corp, Ferndale, Washington), and supplemental heat was provided with suspended heat lamps. A chick starter ration containing amprolium (Co-op Feeds, Saskatoon, Saskatchewan) was provided in plastic tray-style feeders. Municipal water was supplied in free-standing poultry waterers. Animal care staff donned dedicated coveralls, rubber boots, caps, masks, and gloves in an anteroom prior to entering the animal room.

On day four, one chick was found dead in Room A and was diagnosed with sudden death syndrome on gross examination. The next day a chick was found dead in Room B. Gross post mortem examination revealed a dark red colon, and both ceca and part of the colon had intussuscepted into the rectum. A diagnosis of intestinal accident was made and no further diagnostic tests were performed. On days six and seven two additional chicks were discovered dead in Room B. A cursory gross examination revealed the presence of food in the crops and gizzards and no obvious lesions to explain the cause of death. On days eight and nine, two more chicks were found dead acutely in Room B. A more complete gross post mortem examination revealed fibrino-necrotic enteritis. A sample of affected intestine from each chick was placed in formalin. One chick found dead in Room A was discarded before it could be examined.

Four more chicks were found dead acutely in Room B the next day. One was diagnosed with sudden death syndrome, and the other three had intestinal lesions. One chick had white plaques with depressed centers visible through the serosa throughout the small intestine, and fibrino-necrotic

debris was found associated with these areas. The second chick had a walled-off area of peritonitis surrounding an obvious intestinal rupture, and pericarditis. The third chick displayed a reddened cranial jejunum lined with a fibrino-necrotic membrane. A sample of affected intestine was placed in formalin and, along with the samples collected from day eight and nine, submitted to a diagnostic laboratory for microscopic examination.

On day 11, one more chick was found dead in Room B. Food was present in the gizzard, however there were urate crystals in the ureters indicating possible dehyration. The intestinal mucosa from the jejunum to the cecae was coated with a fibrino-necrotic membrane.

At this point results consistent with NE were received from the diagnostic laboratory. Microscopic examination of tissues from all three chicks showed lesions of extensive coagulation necrosis of the villi which were lined by rod shaped bacterial. Larger numbers of short rod bacteria were also present in the deeper portion of the necrotic area. In some locations, the inflammation was transmural.

On day 12, two more birds were found dead in Room B, but had lesions more typical of colibacillosis, with cellulitis, arthritis, pericarditis and perihepatitis, with chunks of caseous material throughout the body cavity. No further testing was done on these two birds. One additional runted bird was euthanized.

On day 14 a decision was made to euthanize the remaining 24 birds in Room B since we could not be confident they would be useful for the intended research. Fourteen of these birds were examined in-house for signs of NE; no apparent lesions were found. The remaining 10 birds were sent to a diagnostic laboratory for complete post mortem examination. No signs of NE were seen; however, multifocal erosive and ulcerative gizzard lesions of varying severity were seen in seven of the 10 birds. The lesions were described as focal superficial erosions in the koilin layer in some sections and focal ulcers extending deep into the mucosa in others. Infiltrates of numerous heterophils and bacteria were seen at the ulcerated surfaces. Periodic acid-Schiff (PAS) staining was negative for fungal organisms. No other significant gross abnormalities were observed. A summary of post mortem findings is presented in Table 1.

Representative feed samples from each room screened for 12 different mycotoxins revealed small amounts of deoxynivalenol and 3-acetyl-deoxynivalenol in each sample, and HT-2 toxin in feed from Room A. Results are shown in Table 2.

The remaining 35 chicks in Room A remained apparently healthy throughout the trial. No gross lesions were seen in any of the gizzards and intestines examined at the end of the trial (14 birds at 18 days of age and 21 birds at 28 days).

DISCUSSION

This report describes a case of NE and gizzard erosions in young chicks housed in a research institute. Necrotic enteritis primarily affects two to six week-old broiler chickens raised on litter although outbreaks in older birds have also been reported (1, 2). The causative agent of NE is *C. perfringens*, which is a normal inhabitant of the intestines of healthy chickens (1). The necrotic lesions result from toxins produced when the organism undergoes rapid multiplication due to a disturbance in the gut. Predisposing factors are thought to include a drastic change in intestinal microflora, high dietary levels of fishmeal or grains, or any condition that results in damage to the intestinal mucosa, such as coccidiosis or mycotoxicosis (1, 2, 3). Coccidiosis is probably the best-known predisposing factor of NE (2, 4). Colonization of the intestinal epithelium by *Eimeria* spp. results in damage to the gut lining providing an entry point for *C. perfringens* (3, 4).

Gizzard erosions have been linked to a number of causes, such as starvation of newly hatched chicks, feed particle size, nutritional deficiencies, adenoviral infections, and toxic substances such as mycotoxins and gizzersosine from heated fish meal (5). Gizzard erosions have also been associated with high numbers of *C. perfringens* (5, 6). In a case dating back to 1981, an increased frequency of gizzard erosions seen in one to five week-old white leghorns in Sweden was associated with large numbers of *C. perfringens* isolated from the koilin layer of the gizzard. The bacterial infection appeared to be the main cause of mortality; however, they suspected the gizzard lesions preceded infection (6).

The case presented here is unusual in many ways including: the age of the chicks affected by NE; the absence of coccidiosis or any other obvious predisposing factor; the presence of gizzard erosions and ulcers; and the occurrence of these lesions in only one of two essentially identical rooms in the well-controlled environment of a research institute. In retrospect, the cecal intussusception that occurred in the five-day-old chick may have been related to the disease process. The intestinal perforation seen on day 10 was also likely related either to gizzard erosions, NE, or both. Unfortunately in this bird and in the two birds that died on days six and seven, the gizzard and intestines were not fully examined, so lesions of NE and gizzard erosions might have been missed. Similarly, in the chicks that had obvious NE, and in the fourteen apparently normal chicks

euthanized on day 14, the gizzard lining was not fully examined, so it is not know if ulcers or erosions were present in these birds. Significantly, of the ten 14-day-old chicks submitted to the diagnostic laboratory, seven had gizzard lesions.

It seems reasonable to conclude that some predisposing factor present only in Room B led to gizzard and intestinal damage and subsequent exuberant multiplication of *C. perfringens*. Possibilities might include accidental exposure of chicks to high levels of Virkon from inadequate rinsing of feed or water containers, or exposure to mycotoxins from wet, moldy feed. The Material Safety Data Sheet for Virkon states that ingestion in people may cause gastritis, possibly progressing to necrosis or hemorrhage with gross overexposure (7). However it is difficult to imagine that contamination of feed or water with small amounts of 1% Virkon could result in the gizzard erosions and ulcers seen in this case. High levels of mycotoxins such as T-2 toxin, HT-2 toxin, nivalenol, moniliformin, pure fumonisins B_1 and B_2, and cyclopiazonic acid have been associated with gizzard lesions (5). In the present case, HT-2 toxin was only found in the feed sample from the unaffected room, and at levels unlikely to have caused gizzard erosions (5). However mycotoxins are not uniformly distributed in feed and more extensive contamination may have occurred at an earlier time point. Perhaps feed became wet and moldy in Room B resulting in levels of mycotoxins high enough to cause gastrointestinal damage in the young chicks.

More complete in-house post mortem examination of chicks may have led to an earlier diagnosis of the gizzard erosions; however, it is unlikely to have changed the outcome in this case. Despite this, it is important to remember to perform a complete post-mortem examination of all body systems even in young chicks, so lesions are not missed. The cause of the gizzard erosions and necrotic enteritis in Room B remains a mystery.

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Table 1. Summary of lesions seen in broiler chicks from Room A and Room B.

Days of Age	Lesions Seen in Room A	Lesions Seen in Room B
4	sudden death syndrome	
5		cecae intussuscepted into rectum
6		unknown - not fully examined
7		unknown - not fully examined
8	did not examine	necrotic enteritis
9		necrotic enteritis
10		necrotic enteritis (2); intestinal perforation (1); sudden death syndrome (1)
11		necrotic enteritis
12		colibaccillosis (2); runt (1)
14		gizzard erosions/ulcers (7)
Summary	Sudden Death Syndrome 1/37 Unknown 1/37 No Apparent Lesions 35/37	Gizzard Erosion 7/37 Necrotic Enteritis 5/37 Intestinal Accident 2/37 Unknown 2/37 Colibacillosis 2/37 Sudden Death Syndrome 1/37 Runt 1/37 No Apparent Lesions (gizzard not examined in 14 of these birds) 17/37

Table 2. Summary of feed mycotoxin analysis.

Mycotoxin	Amount Detected in Room A Feed	Amount Detected in Room B Feed
3-acetyl-deoxynivalenol	29.6 ppb	49.3
deoxynivalenol	70.0 ppb	91.5
HT-2 Toxin	103.2 ppb	not detected

ACUTE LEFT LIMB LAMENESS IN A ROLLER PIGEON: NOT YOUR USUAL CAUSE

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ABSTRACT

A three- year-old adult female roller pigeon used as part of a breeding pair for an ongoing research study was presented with acute left limb lameness. Palpation of the leg revealed a large lump near the hip joint. The bird was able to ambulate in the cage, but would not brood her hatchling. The bird was humanely euthanized and necropsy was performed. Grossly, multiple large white to pale tan nodules were noted in the pancreas, lung, ribcage, intestines and unilaterally in the left kidney. Microscopic examination of the various organs revealed neoplastic proliferation of round cells consistent with lymphoblasts. Immunohistochemistry was performed using CD3, CD79a, CD 20 and CD21 to attempt to characterize these neoplastic round cells.

Lymphoid neoplasia is one of the most common forms of hemolymphatic neoplasms in avian species, especially poultry species with viral induced lymphosarcomas (2). In pet birds, canaries, budgerigars, and other psittacine species all have reports of lymphoid neoplasms. Neoplasms in pigeons are uncommon in spite of longevity in some breeds (1). However, multiple types have been reported and include lymphomas, cutaneous neoplasms, reproductive (both male and female), alimentary, pulmonary, urinary, and endocrine systems (5). This report involved a research pigeon.

CASE HISTORY AND CLINICAL SIGNS

A three-year-old adult female roller pigeon used as a part of a breeding pair for an ongoing research study was housed with her mate at the Poultry Diagnostic and Research Center animal facilities. The housing consisted of a large wire cage with perches, water and feed. A cardboard nesting box was present for laying eggs and brooding hatchlings. Lighting was kept on a 24 hr on cycle to encourage mating and supplemental heat and cooling was provided to keep study animals comfortable at around 25°C. The breeding pair had produced two eggs laid a couple of days apart (Aug 26 and 28, 2015) and were brooding the eggs. Just after hatching (Sept 15, 2015 for both eggs), the adult female was presented with acute left limb lameness. She was able to ambulate in the cage but would not brood her recent hatchlings, which subsequently died. Palpation of the leg revealed a large lump that felt near the hip joint. She was unable to grasp with her foot on the affected leg. The bird was humanely euthanized.

Gross and microscopic lesions. Numerous large white to pale tan nodules were noted in the pancreas, lung, ribcage, intestines and unilaterally in the left kidney. Sections of affected organs were fixed in 10% buffered formalin, routinely processed, sectioned and stained with hematoxylin and eosin. Microscopic examination of the various organs revealed a neoplastic proliferation of round cells consistent with lymphoblasts. Immunohistochemical (IHC) staining was performed using CD3, CD 79a, CD 20 and CD 21on various organs to attempt characterization of the neoplastic round cells.

IHC results. IHC staining had scattered positive staining throughout the nodule for CD3, a T cell marker. CD79a (B cell), CD20 (B cell) and CD21 (dendritic cell) did not stain the neoplastic cells.

DISCUSSION

Acute limb lameness in birds is typically associated with dietary imbalance, such a vitamin deficiencies, or trauma (4). Vitamin D, calcium or phosphorus deficiencies lead to rickets in young growing birds (4). Osteopenia or osteoporosis is more likely to occur in adult birds and results in a reduction in bone mass. Typical causes include starvation, calcium, copper, phosphorus or vitamin D deficiencies (4). Females in production that lack sufficient dietary calcium can experience bone resorption due to a high calcium requirement during shell formation, resulting in a calcium deficiency. Acute trauma to the leg or foot is also associated with lameness. Common examples of trauma include foot entrapment in caging material, flying into objects, improper handling etc. In the present clinical case, we perceived that the bird had caught her foot in the

wire caging and dislocated the femur from the hip joint while struggling to get free. Nutritional diseases were ruled out as the diet supplied was balanced for egg production. This assumption proved to be incorrect.

Lymphoid neoplasia is not a common tumor found in pigeons. A previous study reported several types of tumors in a pigeon research colony at Perdue University (5). Eighty-three birds were submitted for necropsy and 28 (33.7%) had neoplasms or proliferative disorders. Seminoma and thyroid adenoma were the most common tumors diagnosed over an 11 year period. Lymphosarcoma was documented in four birds and grossly had multiple nodules in multiple organs. Kidney, spleen intestine with mesentery and pancreas were most commonly affected and the age range was 6-12 years old. In our clinical case, with the exception of the spleen similar organs were affected in a 3 year-old bird. Two other reports detected lymphosarcoma in the conjunctiva and multiple other organs including the kidney, intestines and pancreas (1, 3). Neither of these reports documented leg lameness as part of the clinical signs. Both studies reported excessive lacrimation with swelling of the conjunctiva and surrounding periorbital tissues. After treatment failure for suspected respiratory infections, euthanasia was selected and multicentric lymphosarcoma was diagnosed. In our clinical case, the neoplasia in the left kidney was most likely applying pressure to the sciatic nerve, resulting in lameness. The mass that was palpated before euthanasia was determined to be lymphosarcoma, which surrounded the ribs and infiltrated the skeletal muscle of the coelomic wall. The femur and hip were not dislocated, nor affected grossly by the mass.

Immunohistochemistry is typically performed on lymphosarcoma-suspected tissue to determine the neoplastic cell origin (T or B cell). For humans and veterinary patients, this diagnosis can help guide the choice of chemotherapeutic regimes. Interestingly, none of the previously cited reports characterized the cell origin of the lymphosarcoma. The results of IHC in our case identified CD3+ cells in a large percentage of the neoplastic tissue. Since not all of the neoplastic cells were positive for CD3, a pure T cell lymphosarcoma would appear unlikely. One possibility is that some of neoplastic cells were immature or anaplastic and thus, not able to exhibit the CD markers. While treatment in human and veterinary patients is largely dependent on the staging and pathological grade of the tumor, none of the these diagnostic parameters exist nor have any chemotherapeutic agents or protocols been approved in avian species (2).

(A case report manuscript will be submitted to *Avian Diseases* or *Journal of Veterinary Diagnostic Investigation*).

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SALMONELLA DIAGNOSTIC TOOLS

W. Witbeck

IDEXX

OVERVIEW

Bacteria in the *Salmonella* genus are very common commensal organisms of the GI tract of many species, including poultry. Many of these salmonellas do not pose any risk to human health and are not of significant health concern for the host animal. Although primarily intestinal bacteria, salmonellae are widespread in the environment and commonly found in farm effluents, human sewage, and in any material subject to fecal contamination. *Salmonella* organisms can be etiological agents of gastrointestinal and systemic infections in humans, most commonly as secondary contaminants of food originating from animals and the environment, usually as a consequence of subclinical infection in food animals leading to contamination of meat, eggs, and milk or secondary contamination of fruits and vegetables that have been fertilized or irrigated by fecal wastes.

Human salmonellosis is one of the most common and economically important zoonotic diseases. *Salmonella* organisms may also be found in animal feedstuffs, causing subclinical gastro-intestinal carriage or infectious disease in animals, particularly poultry and swine. The poultry industry works vigilantly to minimize and prevent the introduction of these pathogenic strains from entering the food chain. One key component in the effective prevention of salmonellosis is detection and identification for the presence of the bacterium, as done by various methods of diagnostic testing. The purpose of this talk is to identify and discuss some available detection methods.

Salmonella is a gram negative, rod shaped bacterium of the family Enterobacteriaceae. The species of interest to poultry and human health is *Salmonella* Enterica. This species is further subdivided into over 2000 serovars.

Poultry and swine can get infected without showing clinical illness. This factor can play an important role in relation to the spread of infection between flocks and herds and as causes of human food poisoning. In the latter case, this can occur when these animals enter the food chain thus producing contaminated food products.

In poultry, *Salmonella* infection has been a topic of interest and regulation for over 75 years. In 1935, the National Poultry Improvement Plan (NPIP) was first introduced, with the intent to provide guidelines in the control of *Salmonella* Pullorum in chickens. This has since expanded to include other pathogens of interest to the industry including additional *Salmonella* serovars (Gallisepticum and Enteritidis), pathogenic mycoplasmas, and avian influenza.

Various biochemical, serological and molecular tests can be applied to the pure culture to provide a definitive confirmation of an isolated strain. Salmonellae possess antigens designated somatic (O), flagellar (H) and virulence (Vi), which may be identified by specific typing sera, and the serovar may be determined by reference to the antigenic formulae in the Kauffman–White scheme. Some laboratories may need to send isolates to a reference laboratory to confirm the full serological identity and to determine the phage type and genotype of the strain, where applicable.

Many of the diagnostic testing methodologies used are outlined in the NPIP CFR guidelines. These include Culture, ELISA testing, agglutination testing, antigen capture and PCR. An important initial factor to consider in the determination of the diagnostic method of choice is whether the goal is to detect antigen presence (the bacteria is present) or antibody detection (evidence of previous exposure or infection to the bacteria). Of the above-mentioned methods, culture, antigen capture and PCR fall into the former category. One should then further consider the feasibility of the organism, to understand expected results for this testing. Culture requires live organism to provide a positive result, whereas PCR and antigen detection immunoassays may give positive results for a period of time after infection has passed and do not differentiate between the presence of live and dead bacteria.

Antibody detecting tests, such as Ab ELISA testing, or tube agglutination on the other hand, provide evidence that the host animal has had exposure to the bacteria in question, resulting in an immune response, and the generation of pathogen specific antibodies. It is important to know the history of vaccination to understand what results are expected with these modalities. Antibody ELISA is best interpreted in the context of regular testing, so that changes in trending can be observed and noted. Titer values can vary greatly with different

environments and management practices, so comparison of titers should be done with birds in similar management conditions whenever possible.

For detailed epidemiological investigations, strain identification is necessary and such investigations have traditionally relied on biochemical and serological methods, phage typing of some serovars, and antibiograms. Genotypic analysis of the organism by use of real time-polymerase chain reaction (RT-PCR) and molecular fingerprinting of DNA has been successfully studied and implemented.

Best practices for diagnosis and subsequent treatment of *Salmonella* positive animals relies on a complete clinical picture and the accurate identification of the infectious agents present and the immune response to the animal. This often is best done through combination of diagnostic methodologies, such as ELISA or tube agglutination for screening of presumed healthy flocks, followed by an antigen detection method, such as PCR to confirm the presence of the bacteria in question. A sample, which is antibody positive but PCR negative, may have been vaccinated or have cleared the infection previously. A bird, which is antibody negative, but PCR positive may be in very early stages of infection and have not yet developed an antibody immune response, or may have an immunocompromise which inhibits appropriate antibody generation. Finally, samples with test both antibody and antigen positive likely confirm both the presence of the pathogen in question and its ability to infect the host animals. Having a clear idea of expected results and understanding these interactions can help to apply testing results and treatment in a more accurate and effective manner.

The frequency of sampling and the type of samples obtained will depend largely on the objectives of the testing protocol (including any statutory requirements), clinical findings, level of detection or precision of prevalence estimates required, cost and availability of sampling resources, and laboratory facilities.

HELMINTH LEVELS IN "NATURAL" PASTURED LAYING HENS

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ABSTRACT

Organic, natural, and pastured poultry and egg production enterprises comprise a growing agricultural endeavor in Arkansas, the USA, and world-wide. In several respects, these sorts of production enterprises are a "throw-back" to when chemo-therapeutics were not available for poultry; products that have allowed for the high-density, housed production systems of today. In the study reported herein, randomly selected birds were obtained from three organic, pasture operations and then randomly subdivided at the research facility into control, levamisole (Prohibit™), fenbendazole (Safeguard™) and piperazine (Wazine™) treatment groups, resulting in eight to nine birds per farm X treatment group. All treatments were given on one calendar day. Animal acclimation and post-treatment periods were three and seven days, respectively. At seven days post-treatment, all birds were humanely euthanized and their intestines processed for helminth burden quantifications. Helminth incidences as seen in the control birds from the three sites (Table 1) were 55-100% (*Ascaridia galli* [roundworm]), 78-100% (*Heterakis gallinarum* [cecal worm]) and 33-73% (*Raillietina cesticillus* [tapeworm]). *Choanataenia infundibulum* (tapeworm) was found in only one bird. *Capillaria obsignata* ("wireworm"), an extremely common nematode seen in older commercial birds raised on litter, was not found in any of the study birds. Compared to parasite infections seen in "typical" commercial birds, the current infections were seen as less extensive in incidence and magnitude.

As mentioned above, anthelmintic treatment groups were established for all birds from the three organic farms; this in an attempt to find helminth isolates that are susceptible to current anthelmintics, and would therefore prove useful in future anthelmintic evaluations. All treatments were administered individually, orally, on the same calendar day and at the volumetric rate of 0.6 mL/Kg BW (birds individually weighed immediately prior to dosing). The actual mg/Kg BW dosage rates were 100, 12 and 5 for piperazine, levamisole and fenbendazole, respectively. These treatments were consistent with standard procedures at our lab, but not consistent with label indications. Both piperazine and levamisole are indicated for use in the drinking water over a day's period of time (approximately). Fenbendazole (AquaSol™), which is not cleared for use in adult birds, is cleared for broilers and replacement broiler breeders and for drinking water treatment over a five day period of time. Given the above, anthelmintic efficacies based on helminth numbers seen in this study might be lower than those seen after a more "real life" treatment regime, but can be used as approximations. Additionally, cestode populations are not within the spectrum of activity for piperazine or levamisole, and "treatable" with fenbendazole only at higher dose rates and as multiple-day administrations.

Helminth, arithmetic means (and ranges) by farm and treatment group are presented in Table 2. Helminth populations per bird ranged from 0 to 170, 0 to 1220, and 0 to 280 for *A. galli*, *H. gallinarum* and *R. cesticillus*, respectively. Generally, helminth levels were the highest in the control birds from each farm, and with efficacy ranges of 68 to 100 % for *A. galli*, and 0 to 100 % for *H. gallinarum*. Further work is planned at these organic farms and others, as we continue to establish incidence, magnitudes and epidemiology for the helminths (and arthropods) that parasitize this growing body of animals.

Table 1. Helminth incidence in control birds by organic/free-range farm.

Farm	N	*Ascaridia galli*	*Heterakis gallinarum*	*Raillietina cesticillus*
1	9	55	78	33
2	9	78	100	78
3	9	100	100	66

Table 2. Helminth, arithmetic means (ranges) by farm and treatment group.

		Helminth		
Farm	Trt Grp	*Ascaridia galli*	*Heterakis gallinarum*	*Raillietina cesticillus*
1	Control	6.9(0-25)	76.7(0-220)	21.1(0-160)
	Fenbendazole	0.0	80.0(0-560)	75.0(0-280)
	Levamisole	0.2(0-1)	16.0(0-90)	14.4(0-60)
	Piperazine	0.0	175.6(0-740)	12.8(0-90)
2	Control	24.0(0-170)	218.9(10-790)	18.0(0-90)
	Fenbendazole	5.5(0-30)	15.5(0-120)	10.9(0-70)
	Levamisole	3.9(0-30)	0.0	9.5(0-40)
	Piperazine	6.7(0-50)	43.3(0-240)	18.4(0-120)
3	Control	34.4(7-114)	274.4(10-1220)	14.4(0-80)
	Fenbendazole	2.2(0-10)	5.5(0-30)	2.2(0-20)
	Levamisole	3.3(0-9)	32.2(0-130)	4.6(0-20)
	Piperazine	11.0(0-90)	86.0(10-370)	2.2(0-20)