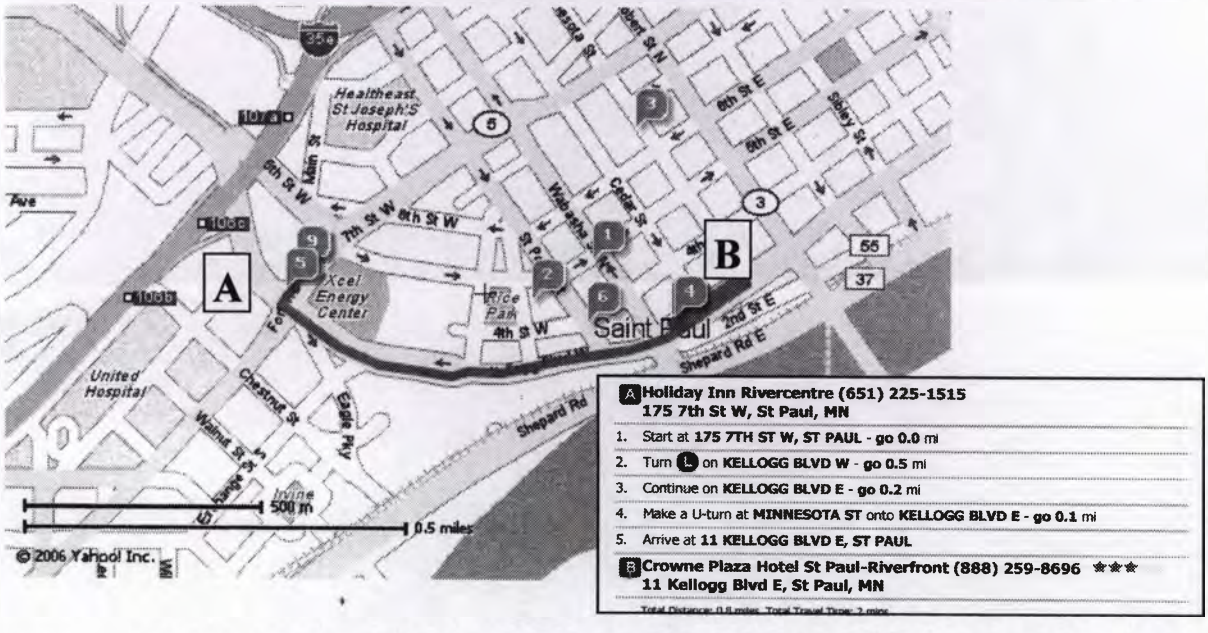
Proceedings

58th North Central Avian Disease Conference and Symposium on Lessons Learned from AI Preparation

58th North Central Avian Disease Conference
and
Symposium on
"Lessons Learned from AI Preparation"

March 11-13, 2007

Sunday, March 11	**St. Paul River Centre, Room 4**	
1-2 pm	On-site Registration	
2-5:40 pm	Scientific presentations	
6-8 pm	**Welcome Dinner at St. Paul River Centre, Room 5**	
	— Sponsored by Maple Leaf Farms	
Monday, March 12	**St. Paul River Centre, Rooms 4 and 5**	
7-8 am	Continental Breakfast	
8-11:40 am	Scientific Presentations	
1-4 pm	Symposium	
4:25 pm	Y. M. Saif	NCADC Disease Round Table
5:00 pm	Ching Ching Wu	NCADC Business Meeting
6-9 pm	**Award Dinner at Crown Plaza Hotel, Kellogg Room**	
	— Sponsored by Alltech	
Tuesday, March 13	**St. Paul River Centre, Rooms 4 and 5**	
7-7:30 am	Continental breakfast	
7:30-11 am	Symposium, continued	



Scientific Presentations

Sunday, March 11, 2007 ***St. Paul River Centre, Room 4***

Moderators: Mick Fulton, Tom Bryan

2:00	L. Long	Avian Influenza Virus Surveillance in Ohio Zoological Institutions
2:20	R. Slemons	Temporal and Spatial Comparisons of the AI Virus Combinations Recovered from Ducks Sampled at nearby Locations in different states over 2 years
2:40	J. Nolting	Phenotypic variations in H1N1 low pathogenic avian influenza viruses over time and by location
3:00	H. Yassine	Interspecies Transmission of H3N2 Influenza A viruses between Swine and Turkeys
3:20	M.K. Hsieh	A prime-boost approach to enhance DNA vaccination-mediated protection against infectious bursal disease
3:40-4:00	**BREAK**	
4:00	D. Karunakaran	New findings in turkey cellulitis
4:20	M. C. Kumar	Prevention of turkey cellulitis
4:40	A. Thachil	Development of a turkey Clostridium cellulitis inactivated vaccine
5:00	R.M. Hulet	Effect of NuPro® and Growth Performance of Turkey Hens Grown to Market Age
5:20	A.E. Sefton	Comparison of Bio-Mos® and Flavomycin® as Growth Promoters
5:40	R. Porter	Production losses associated with ionophore toxicosis in white leghorn pullets

Scientific Presentations, continued

Monday, March 12, 2007 *St. Paul River Centre, Rooms 4 and 5*

Moderators: Rob Porter, Tsang Long Lin

8:00a	D. Lauer	The Minnesota Avian Pneumovirus Eradication Project
8:20	S. Noll	Avian Pneumovirus Vaccine Evaluation Project
8:40	B. Velayudhan	The attachment glycoprotein gene variation among avian metapneumovirus subtype C strains isolated from 1996-2003 in the United States
9:00	Y.N. Chen	Detection and quantitation of turkey coronavirus in turkey tissues by a specific real-time reverse transcription-polymerase chain reaction
9:20	Irene Lesley	Prevalence of *Campylobacter* and *Salmonella* in the Turkey Brooder House
9:40-10:00	**BREAK**	
10:00	C. Gustafson	Efficacy Comparison of Three Commercial MG Vaccines
10:20	T.M. Huang	A Three-Year analysis of antibacterial susceptibility and resistance in chicken *Escherichia coli*, *Salmonella* spp. and *Pasteurella multocida* isolates
10:40	A. Yiannikouris	Counteracting mycotoxin contamination: the effectiveness of *Saccharomyces cerevisiae* cell wall glucans for sequestering mycotoxins
11:00	R. Hauck	The Effect of Natustat® for Prevention of Histomoniasis in Turkeys
11:20	S. Taylor/C. Schmelik	Poultry Vaccines Labeling and Adverse Events

Symposium

Monday, March 12 ***St. Paul River Centre, Rooms 4 and 5***

Moderators: K.V. Nagavaja, Susan Williams

1:00 pm	Welcome	Y. M. Saif
1:05 pm	Dennis Senne	Avian Influenza and Lessons Learned on AI Diagnosis
1:35 pm	Andy Rhorer	NPIP Program, State Preparedness and Containment of Low Path AI
2:05 pm	Randall Levings	High Path AI Responses and Update of Veterinary Stockpile
2:35 pm	BREAK	
2:45 pm	Chang-Won Lee	AI Vaccine—A New Approach
3:15 pm	Dave Halvorson	Lessons Learned on AI Prevention and Control
3:45 pm	T.J. Meyers	The Use of Vaccines and Biologics in Controlling AI and the Concept of Compartmentalization and Regionalization
4:15 pm	BREAK	(Symposium will continue Tuesday morning)

4:25pm	Y. M. Saif	NCADC Disease Roundtable
5:00pm	Ching Ching Wu	NCADC Business meeting

6:00pm Award Dinner at Crown Plaza Hotel *Sponsored by Alltech*

Symposium, *continued*

Tuesday, March 13

Moderators: Dave Halvorson, Jose Linares

7:30 am	Alice Black	Dealing with Media When You Are In Charge of Disease Issues
8:00 am	Jose Linares	AI Preparedness—the Texas Experience
8:30 am	Susan Williams	Actions and Lessons Learned in Preparing the Broiler and Broiler Breeder Industry for AI
9:00 am	BREAK	
9:15 am	Mick Fulton	The North Central Experience
9:45 am	Eric Gingerich	Pennsylvania Experience: LBM and Backyard Birds
10:15 am	Will Hueston	Business Continuity in the face of an AI Outbreak
10:45 am	Richard Slemons	Conclusion
11:00 am		**NCADC Conference ends and Midwest Poultry Consortium Research Summit begins**

Scientific

Presentations

Avian Influenza Virus Surveillance in Ohio Zoological Institutions

Lindsey J. Long, Patricia H. Dennis, Jacqueline M. Nolting, Dennis A. Senne, William Saville and Richard D. Slemons, Department of Veterinary Preventive Medicine, the Ohio State University 1920 Coffey Road, Columbus, OH 43210

Avian collections in zoological institutions accredited by the Association of Zoos and Aquariums (AZA) serve as unique sentinels for emerging infectious diseases in North America. Found in urban and rural settings across the United States, AZA institutions quarantine all animals entering their collections, house individually identified animals which receive regular health examinations, and veterinarians perform complete necropsies on collection animals that die. During 2005, AZA avian collections in Ohio were incorporated into our ongoing avian influenza virus (AIV) surveillance efforts. The rationale was that the outdoor environment of the zoo is often utilized by both zoo birds and wild bird populations from late spring through early fall and the zoo collections include exotic avian species bringing additional host factors into play. Cloacal swabs and blood samples were taken from birds in one avian zoo collection as they were moved to their outdoor pens in the spring. During late summer, environmental samples were collected on the grounds of five AZA institutions. Then in the fall, blood samples and cloacal swabs were collected from birds in the avian collections at two zoos as the birds were returned to their indoor winter quarters. No AIVs were recovered from the spring cloacal swabs, four type A influenza isolates were recovered from 129 environmental samples collected at two institutions, and results on fall cloacal swabs are pending. To date, no antibodies have been detected to the type specific antigen of AIVs. The 3.1% recovery rate from environmental samples indicates further surveillance efforts are warranted in following the dynamics of AIV introductions into zoo environments. By initiating AIV surveillance measures, these institutions have taken a proactive stance in protecting the health of their avian collections and possibly even visitors to their respective zoos.

Temporal and Spatial Comparisons of the Avian Influenza Virus NA-NA Combination Recovered from Ducks Sampled at Nearby Locations and in Different States over Two Years

Richard D. Slemons, Jacqueline M. Nolting, Vernon D. Stotts, H. Lloyd Alexander, Roy Kroll, Mark Shieldcastle and **Dennis A. Senne** Department of Veterinary Preventive Medicine, 1920 Coffey Road, Columbus, OH 43210

To date, low pathogenic type A influenza viruses circulating in wild birds have no documented impact on wild bird populations around the world. However, accumulating evidence implicates these viruses as being the ancestral origins of type A influenza viruses appearing, and causing disease, in domestic birds, lower mammals and humans. As a result, recommendations from numerous type A influenza virus symposia have identified a need to further define the natural history of type A influenza viruses in wild birds. Unfortunately, progress in this area has been modest due to limited funding and lack of systematic, coordinated efforts. After 35 years, it remains unclear how the antigenic and genetic diversity of these viruses is maintained in nature. In 2005-2006, two pairs of study sites were established. Each pair of study sites was located in a different state and the two sites in each pair were located approximately 18 miles from each other. Resident, wild mallards were the primary population at one set of sites and migratory, dabbling ducks were the primary populations at the other paired sites. Virus isolation attempts in embryonating-chicken eggs were conducted on either cloacal swabs or very fresh fecal samples and sequential sampling was conducted at each site over 2-3 months each year. The frequency of AIV recovery and distribution of NA-NA combinations varied markedly 1) over a few weeks at the same site, 2) during the same two month period at the paired sites, 3) during the same months in different states and 4) between years within a state. Merging surveillance and monitoring data by year and larger geographic regions provides critical, user friendly information on type A influenza viruses circulating in wild birds but, at the same time, data merging can result in the dynamic nature of type A influenza virus circulation in wild birds being overlooked. This dynamic must be taken into consideration when drawing conclusions from avian influenza virus surveillance efforts in wild birds.

Phenotypic variations in H1N1 low pathogenic avian influenza viruses over time and by location

Jacqueline M. Nolting, Vernon D. Stotts, H. Lloyd Alexander, Dennis A. Senne, and **Richard D. Slemons**,Department of Veterinary Preventive Medicine, the Ohio State University 1920 Coffey Road, Columbus, OH 43210

H1N1 type A influenza virus can be found in wild and domestic birds, swine and humans, which presents the possibility that individual genomic sequences from these viruses could move between the avian and mammalian hosts and this movement, if it occurs, could go undetected. Differences in replicative properties among waterfowl-origin H1N1 influenza viruses in embryonating chicken eggs might serve as an indicator for variations in the genomic constellations of the viruses and possibly identify isolates that are better suited to cross the species barrier from wildfowl to domestic poultry. It is hypothesized that growth rates of H1N1 water-fowl type A influenza viruses will vary in embryonating chicken egg culture. For initial screening, 5 first-egg passage H1N1 type A influenza virus stocks from our repository were selected for examination. They represented isolates from different years and geographic origins. A limiting dilution was performed on each of the isolates in ten-day-old embryonating chicken eggs and EID_{50} calculations were completed using the Reed and Muench method to determine the concentration of infectious virus in each of the respective first passage isolates. The EID_{50}/ml for the isolates ranged from $1x10^{3.67}$ EID_{50}/ml to $1x10^{7.22}$ EID_{50}/ml. There are several possible explanations for these different titers including, but not limited to, inhibitors and incomplete virus particles in original samples and first egg passage fluids, varying virus concentration in original samples and first egg passage fluids, varying virus concentration in original samples and different replicative properties among the viruses. Further investigation is underway to determine if there are real differences in the replicative properties of these isolates in embryonating chicken eggs.

Interspecies Transmission of H3N2 Influenza A viruses Between Swine and Turkeys

H.M. Yassine, C-W. Lee and **Y.M. Saif** ,Food Animal Health Research Program, Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691

Interspecies transmission of influenza A viruses is raising concerns for its potential threat to human and animal populations. Influenza A viruses belong to the family *Orthomyxoviradae* and are composed of segmented RNA genomes. They are usually host specific, depending mainly on the compatibility between the hemagglutinin (HA) protein and its corresponding receptor on the host cell. Pigs are considered as mixing vessels due to their susceptibility to infection by human and avian viruses, resulting in generation of new reassortant viruses.

Classical H1N1 lineage of influenza A viruses were the dominant cause of influenza in pigs for more than six decades in the United States (US). In 1998, H3N2 viruses emerged and rapidly spread through the swine population in the US. These viruses were double and triple reassortants, of which the latter became endemic in the swine population. Between 2003 and 2004, we isolated and characterized two H3N2 influenza A viruses from turkey breeder hens from Ohio and Illinois. The infected turkeys showed no clinical signs, but underwent complete cessation of egg production. Genetic analysis of these viruses revealed that they are triple reassortant viruses similar to those currently circulating in the swine population in the US. These findings prompted us to initiate a study on the interspecies transmission of these viruses between swine and turkeys.

Three viruses of turkey origin (TK/IL/04, TK/OH/04, TK/NC/03) and one virus of swine origin (SW/NC/03) vaccine strain) were tested for their transmissibility between swine and turkeys. All viruses were shown to replicate in both species (using real-time PCR on swab samples and HI-test on blood serum collected at day 15 post infection); however, only the Ohio strain was shown to transmit both ways between the two animal species that also showed seroconversion upon infection. The Illinois strain was shown previously to be more than 99% similar to the Ohio strain genetically and antigenically, however, it behaved differently in the transmission experiments. This indicates that minor changes in influenza viruses might totally change the behavior (replication, pathogenicity, etc.) of these viruses.

A Prime-boost approach to enhance DNA vaccination-mediated protection against infectious bursal disease

Ming K Hsieh, **Tsang Long Lin**, and **Ching Ching Wu**, Department of Comparative Pathobiology, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907

Multiple intramuscular injections with a large dose of DNA carrying the large segment gene of infectious bursal disease virus (IBDV) have been shown to provide effective protection of chickens against infectious bursal disease (IBD). The purpose of the present study was to determine if priming with DNA carrying the large segment gene of IBDV and boosting with killed IBD vaccine could adequately confer protection of chickens against IBD. One-day-old specific pathogen free (SPF) chickens were intramuscularly injected with DNA plasmid coding for the large segment gene of IBDV strain variant E (VE) (P/VP243/E) followed by intramuscular injection of killed IBD vaccine containing both standard and variant IBDV at 1 or 2 weeks of age. Chickens were orally challenged with IBDV strain VE or standard challenge strain (STC) at 3 weeks of age and observed for 10 days. Bursal lesion scores, bursa weight/ body weight (B/B ratios), protection efficacy, IBDV antigen in bursae, enzyme-linked immunosorbent assay (ELISA) titers to IBDV, and virus neutralization (VN) titers to IBDV were determined. Concanavalin (Con) A-induced spleen or blood lymphocyte proliferation responses were performed at 14, 21, 24 and 31 days after priming with P/VP243/E. Chickens primed with 50, 100, 200, or 400 μg of P/VP243/E at 1 day of age and boosted with 0.5 ml of killed IBD vaccine at 1 or 2 weeks of age had 80 to 100% protection against challenge by IBDV strain VE or 71 to 100% protection against challenge by IBDV strain STC. Chickens in the groups primed with P/VP243/E and boosted with killed vaccine had significantly higher ($P<0.05$) B/B ratios and significantly lower ($P<0.05$) bursal lesion scores than chickens in the challenge control (CC) groups and groups primed with saline or vector plasmid and boosted with killed IBD vaccine or only primed with P/VP243/E. No IBDV antigen was detected by immunofluorescent antibody assay (IFA) in bursae of chickens protected by priming with DNA vaccine and boosting with killed vaccine. Prior to challenge, chickens (21 days of age) in the groups rimed with P/VP243/E and boosted with killed IBD vaccine had significantly higher ($P<0.05$) ELISA and VN titers to IBDV and stimulation indices to ConA-induced spleen or blood lymphocyte proliferation than chickens in the CC groups or groups primed with vector plasmid or saline and boosted with killed IBD vaccine or only primed with P/VP243/E. In conclusion, SPF chickens at 1 day of age primed with IBDV large segment gene expressing DNA and boosted with killed IBD vaccine at either 1 or 2 weeks of age can be adequately protected against challenge by homologous variant or heterologous classical IBDV.

New Findings in Turkey Cellulitis

Dr. Daniel Karunakaran Agtech Products, 916 Wild Cherry Lane, Harrisonburg, VA 22801

Clostridial cellulitis has emerged recently as a significant concern for the turkey industry in the United States. Characterized by necrosis of the skin, subcutaneous tissue, and often the underlying musculature, the disease occurs suddenly and progresses rapidly with death occurring often before the first symptoms are observed.

Recent research findings with regard to the agents involved will be presented. Intervention strategy for prevention and control will be discussed.

Prevention of Turkey Cellulitis

Mahesh C. Kumar, B.V.Sc, M.S., PhD., A.C.P.V. Consultant, Poultry Health and Food Safety
St. Cloud, MN

Turkey cellulitis is a disease of turkeys characterized by swelling of tails, breasts and legs and it causes excess mortality in both toms and hens. There is a large amount of fluid under the skin. On necropsy, straw colored to blood-tinged fluid is observed. Birds start experiencing this disease around 14 to 15 weeks of age in toms and as young as 9 weeks of age in hens. Excess mortality runs at 1-2% per week in toms and 1/2% per week in hens until market. This excess mortality occurs despite being on treatment.

Etiology: The causative organism was isolated and identified by Carr (M.S. Thesis 1996) as *Clostridium perfringens* type A. It was also shown that this condition was reproduced by injecting Alpha Toxin under the skin. She also reproduced the cellulitis in turkeys by giving this organism by various routes.

Treatment: The birds respond to treatment with penicillin at 1,000,000 units per gallon of water. This is followed by chlortetracycline in the feed until market.

Control measures: The disease keeps on occurring in successive flocks despite various efforts made to control and prevent it from recurring. Control measures tried consist of complete clean out and disinfection of the buildings followed by bedding with fresh shavings. Various litter treatments have been tried without success. It sometimes reduces the severity of the subsequent outbreaks, but has not prevented the disease from recurring. Growth promotants were switched to prevent and control this disease with no success. However, one preventive measure was tried which consisted of cleaning out and disinfecting of buildings in between placement of flocks and providing feed treated with Termin-8® at 2lbs/ton of feed. The flocks given the treated feed did not break with cellulitis. Data will be presented at the meeting as to timing of using this feed treatment for complete prevention of this expensive disease.

Development of a Turkey Clostridium Cellulitis Inactivated Vaccine

Anil J. Thachil, **Binu T. Velayudhan**, **David A. Halvorson** and **Kakambi V. Nagaraja**
Department of Veterinary and Biomedical Sciences, University of Minnesota, 1971 Commonwealth Ave., St. Paul, MN 55108

Cellulitis has emerged as a major problem in the turkey industry over the last few years. It is one of the foremost causes of condemnation due to infectious disease contributing to severe economic loss. Cellulitis lesions in turkeys occur on the breast and ventral abdomen with apparent absence of any external wound. The infectious agents consistently isolated are *Clostridium perfringens* and *Clostridium septicum.* The objective of our study was to develop a formalin-inactivated vaccine against Clostridium cellulitis in turkeys. We selected isolates from cellulitis in field turkeys. The standardized formalin-inactivated vaccine preparations were evaluated experimentally for safety and efficacy in 6 and 8-week-old turkeys. The results of this experiment will be presented.

Effect of NuPro® and Growth Performance of Turkey Hens Grown to Market Age

R.M. Hulet, Penn State University, 222 Henning Building, University Park, PA 16802

Three studies were conducted to determine the effectiveness of NuPro® (NP) (Alltech) for improving the growth performance of turkey hens taken to either 84 or 95 days of age. NuPro® is a yeast extract derived from a select strain of yeast, grown under consistent and carefully controlled conditions. It provides an excellent source of protein, amino acids, nucleotides and important vitamins. Five hundred and twenty-eight female poults were randomized into 4 pens with 132 hens per pen for 1 week; At 1 week, they were spread into 12 pens (5.4 birds/m^2).

In study 1 and 2, hens were fed a 28% pre-starter (PS) diet for 4 wks, a 26% starter (S) for 2 wks, a 23% grower (G) for 4 wks, and an 18% finisher (F) until market age. There were 3 treatments fed in Study 1; NP at 4% in the PS followed by 2% (S), NP at 3% in both the PS and S diets, and a meat meal CN. In Study 2, the NP diet was fed at 4% PS and 2% S and compared to both a meat meal and a vegetable CN. For Study 3, hens fed NP at 4% for the first 11 days (28% CP) and then NP at 2% until 27 days of age (28% CO) was compared to Penicillin supplemented control diets from 1 to 27 days (28% CP). After 27 days, all hens were placed on the same diets for grower diets (28 and 24%) and a finisher diet (18%) CP). BW and feed consumption (FI) were measured at hatch, feed changes, and market age for all experiments.

Hens fed the NP diets had greater BW ($P < 0.05$) at 84 d when compared to the CN diet (Study 1) and at 95 d (Study 2, $P < 0.09$). Although the NP-fed hens had consistently less mortality, no significant difference in mortality or feed conversion was found in either study. The greatest effect appeared to be an increase in FI for those hens fed the NP treatments, resulting in increased growth. When compared to Penicillin feed hens in Study 3, NP supplemented hens were not significantly different in body weight, feed conversion or mortality at 84 days of age.

In summary, the addition of NP into the PS and S diets appeared to positively affect the growth of hen turkeys grown to 84 or 95 days of age when compared to commercial diets.

Comparison of Bio-Mos® and Flavomycin® as Growth Promoters

A.E. Sefton, Alltech, 20 Cutten Place, Guelph, ON Canada N1G-427

Antibiotic growth promoters (AGPs) have been a standard inclusion in meat bird rations since the 1940s. Recent events have seen a decrease in the number of antibiotics that can be used as growth promoters and it appears unlikely that new replacements will be forthcoming. One of the reasons for the move away from AGPs is that antibiotic resistant genes increase in prevalence during their use. These genes may be specific for the antibiotic in use or more general in nature. To maintain their effectiveness, it has been found best to rotate or shuttle AGPs. Bio-Mos® (Alltech Inc.), a source of yeast cell wall mannans, has been found to inhibit conjugation *in vitro*, decreasing the spread of antibiotic resistance transfer among *E. coli*, thus providing a strategy for the control or treatment of multi-drug resistance. Both broilers and turkeys fed Bio-Mos® have been shown to produce comparable body weight, FCR, and improved livability, when compared to birds fed rations containing AGPs. Specific data will be presented that shows this is the case for Bio-Mos® vs. Flavomycin®. Thus, Bio-Mos® is a useful substitute for Flavomycin® in rotational and shuttle growth promoter programs; it gives comparable growth promotion and decreases the spread of antibiotic resistant genes. The latter will increase the effectiveness of the AGP complement of the shuttle program.

Production losses associated with ionophore toxicosis in white leghorn pullets

Rob Porter, Wisconsin Veterinary Diagnostic Laboratory, Department of Pathobiological Sciences, University of Wisconsin School of Veterinary Medicine, Madison, WI **Dave Zoromski**, Wisconsin Veterinary Diagnostic Laboratory, Madison, WI , **John Brown**, Centurion Poultry, Inc., Lexington, GA

Coccidiosis was diagnosed in a flock of 4-week-old white leghorn pullets raised in three-tier, drop-through cages with nipple drinkers. At five weeks, shortly after delivery of feed containing an ionophore, many of the birds were depressed, laterally recumbent and unable to walk when manually positioned upright. Many of the affected pullets were thought to be dead, lying motionless in the cage and flattened laterally by the weight of other cagemates but, when examined, were actually alive. Pullets were recumbent but, when removed from cages and separated from other birds, were often alert and walking in several hours or less. At this time a preliminary diagnosis of ionophore toxicosis was made and 20 tons of replacement feed were delivered to the farm, but mortality continued for four days. Necropsy of affected birds revealed bruising on the ventral aspect of the wings and mild subcutaneous edema over the thigh and tibiotarsus. There was multifocal intramuscular hemorrhage of varying degrees in the caudal aspect of the thigh, digital flexor muscles of the tibiotarsus, and lumbosacral muscle (gluteal muscles). Histologic examination of affected muscles revealed attenuation, hyalinization and myocytolysis of muscle fibers with loss of cross-striations, varying infiltrates of mononuclear cells, mostly macrophages, and proliferation of satellite and fibroblast nuclei. A pre-mix error resulted in pullets consuming ionophore at five times the recommended concentration of 110 grams/ton. When replacement feed was delivered to the farm, the new feed was not mixed with the remaining 10,000 pounds of toxic feed, and pullets continued to consume toxic levels of ionophore for four days. Total mortality directly resulting from ionophore toxicosis was 3.9% and an additional 2.2% of the flock was culled over the next 13 weeks for lack of uniformity and poor growth. Persistent problems in the flock continued through lay and included cloacal prolapse comprising a significant portion of daily mortality. Additionally, egg size and case weights from this flock were below average, resulting in substantial loss in value. The persistent problems in the flock were attributed to long term effects of the toxicosis that had occurred months earlier.

The Minnesota Avian Pneumovirus Eradication Project

Dr. Dale Lauer, Poultry Program Director, Minnesota Board of Animal Health, 622 Business Hwy 71 HE P.O. Box 126, Willmar, MN 56201. **Drs. Will Hueston, Heather Case, Tim Goldsmith, Lee Nelson, Dave Halvorson, Sally Noll** and **Carol Trefry**, Center for Animal Health and Food, University of Minnesota, **Dr. Lindsey Garber, Bruce Wagner**, USDA APHIS VS CEAH, Minnesota Poultry Testing Laboratory, 622 Business Hwy 71 NE P.O. Box 126, Willmar, MN 56201

Introduction: Avian Pneumovirus (APV), an infectious respiratory disease of turkeys, has been a disease problem for the Minnesota turkey industry since the virus was identified in 1998. That year, a processing plant serological surveillance program was set up in Minnesota to identify positive APV turkey flocks using samples collected for Avian Influenza surveillance. The percentage of positive flocks/lots has ranged from 13% (March, 1999) to 66% (August 2003), but most months the percentage has been 40-60% positive.

Beginning on September 1, 2002, the Coordinated Area Control and Eradication Field Demonstration Project, known as the Morgan Area Project, was an attempt to use vaccination as an APV eradication tool in a geographic area. The project ran for one year and was a cooperative effort between the Minnesota Turkey Growers Association (MTGA), the University of Minnesota, the Board of Animal Health and the United States Department of Agriculture (USDA). The Avian Pneumovirus Eradication Project was designed to follow-up where the Morgan Area Project left off.

Avian Pneumovirus Eradication Project Objectives

- Can the modified-live vaccine be evaluated in controlled pen studies?
- Will turkey growers cooperate in a coordinated APV vaccination project?
- Can a coordinated vaccination program reduce APV clinical disease?
- Can risk factors for the initial introduction of APV be identified?
- Can a coordinated vaccination program reduce the economic impact of APV?
- Can a coordinated vaccination program serve as an APV eradication tool?

APV Eradication Project Components

Minnesota's Avian Pneumovirus Eradication Project was funded by the USDA (September 1, 2004-December 31, 2006) and directed by the Minnesota Board of Animal Health in cooperation with the University of Minnesota and the MTGA. The project consisted of three components: evaluating the APV vaccine in controlled pen studies, designing and implementing a field APV project and analyzing turkey health and production data to determine if a coordinated vaccination strategy is possible and successful.

1) **Vaccine Evaluation**

Results are reported at this conference in a separate presentation

2) Field Project Implementation

Within Minnesota, geographic regions or areas were identified (Morgan and Faribault areas) with similar turkey density, topographic features, and clinical disease caused by APV. Coordinated vaccination and comparison zones were identified in each area.. Both of these areas have experienced clinical APV—the Morgan area in 2000 and the Faribault area in 2003. A total of 400 flocks were identified as potential candidate flocks.

Coordinated Vaccination Field Project Analysis

The field project analysis evaluated the impact of coordinated vaccination strategies for the eradication of APV. Data was collected on the underlying biosecurity features of the participating premises as well as a series of production and processing measurements, including mortality (3-12 week), clinical respiratory disease, APV associated respiratory disease, growth rates, condemnation at processing, liveweight yield and economic data. The statistical analyses adjusted for differences associated with the gender of the flock (hens vs. toms), season of growout, age at processing, and plant where processed to control for potential confounding. Overall, data was collected from 380 flocks on 68 premises.

A) **Biosecurity Audits:** The participating premises face a number of potential threats from exposure to wild birds. Most of the participating premises are within three miles of a body of water. The presence of bodies of water on the premises was associated with significantly higher early mortality. Additionally, close proximity to other poultry farms was significantly associated with higher condemnation. Sharing equipment was a significant risk factor, negatively affecting a number of production measures with higher condemnation and lower liveweight yield for both toms and hens, and higher clinical respiratory disease in hens. Stockpiling manure was significantly associated with higher early mortality. One of the greatest risks for spreading disease tends to be contamination introduced by people and equipment. Opportunities to improve biosecurity include adding signs and actions restricting access, reducing visitors and increasing biosecurity practices required of visitors.

B) **Evaluation of coordinated vaccination zone effectiveness:** During the study period, tom flocks raised in the coordinated vaccination zone were significantly less likely to have high condemnations at processing and more likely to have high early mortality. Hen flocks raised in the coordinated vaccination zone had significantly higher condemnation, lower growth rate, lower liveweight yield, and higher earlier mortality. In general, vaccination did not improve production measures although the effectiveness of vaccination depended on the frequency, with two or three vaccinations achieving better performance than a single vaccination.

C) **Cautions for interpreting this field trial:** In an ideal situation, the coordinated vaccination strategy would be evaluated in a region of sustained APV field virus challenge. Producer reports and the serology surveillance demonstrated an apparent lack of APV field virus pressure during the year-long study. Evaluation of the effectiveness of coordinated vaccination was further complicated by the fact that a number of flocks in the comparison zone were also vaccinated, although not all followed the coordinated vaccination protocol. Finally, no hen flocks were produced in the Faribault coordinated vaccination zone, so the effectiveness of coordinated vaccination for hen flocks could only be evaluated for the Morgan area. All of these observations limited the full evaluation of the effectiveness of coordinated vaccination zone strategy in the face of a large scale APV outbreak.

Conclusions

- The USDA-funded coordinated vaccination field project was successfully designed and implemented. Independent growers and turkey integrators alike demonstrated a willingness to collaborate on a coordinated vaccination strategy in the designated geographic areas.
- Biosecurity audits provided participating growers valuable observations when comparing their turkey operations to others in the area
- While production statistics do not support sustained use of APV vaccine in the absence of field virus pressure, aggressive coordinated vaccination of all flocks in the geographic vicinity of a clinical APV outbreak appears to be a useful control and eradication tool.
- A successful APV eradication program is multi-faceted. An overall eradication strategy requires intervention measures that include, but are not limited to, breeder vaccination programs, biosecurity plans, and individual commercial premises eradication programs that may or may not include APV vaccination.

References

Lauer D.C., 2005 North Central Avian Disease Conference

Avian Pneumovirus Vaccine Evaluation Project

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Two experiments were conducted to evaluate the APV field vaccination program and other vaccination administration methods on post vaccination detection of vaccine virus, post vaccination transmission, post vaccination serology, post challenge virus shedding, and post challenge transmission, post challenge serology and post challenge clinical signs in a controlled setting. The vaccine used was Pneumomune® (Biomune). In each study there were two control groups (non-vaccinated and those vaccinated via eye-drop administration). The vaccine was also adminsitered by the following methods; low volume sprayer, high volume sprayer and spray cabinet. The vaccine was administered at 7 and 35 days of age for all groups with the exception of the spray cabinet where the vaccine was administered at 1 day of age followed by a second vaccination via sprayer at 35 days of age.

In each study, commercial hen turkey poults were obtained from a commercial hatchery from hatching eggs originating from APV naïve breeders and brooded in pens containing 400 poults each. Blood samples were taken at 2-wk intervals to market age. Swabs for PCR testing were taken periodically. Challenge studies were conducted three weeks after the first and second vaccinations of APV. A sample of poults were removed from the pens and moved to isolation facilities and challenged with a turbinate suspension prepared from 2-wk-old turkeys inoculated with a Minnesota isolate of aMPV (aMPV/Minnesota/Turkey/19/2003). In all challenge studies, unvaccinated controls developed clinical signs and eyedrop-vaccinated poults did not develop clinical signs to any significant degree. Based on clinical signs, only poults vaccinated via the spray cabinet showed protection against challenge equivalent to the eyedrop-vaccinated group when challenged at 4 weeks of age. However, all other vaccinated groups were protected regardless of vaccination method following the second vaccination at 5 weeks of age and challenged at 8 weeks of age. Titer increased in vaccinated (non-challenged) turkeys in both studies.

In summary, vaccine efficacy was dependent on method of administration. Protection after the first vaccination was obtained when administered via eyedrop and spray cabinet. Sprayer application required two vaccinations to provide protection. Titers in vaccinated turkeys that were not challenged increased with flock age suggesting cycling of the vaccine virus.

The attachment glycoprotein gene variation among avian metapneumovirus subtype C strains isolated from 1996 to 2003 in the United States.

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The objective of the present study was to examine the glycoprotein (G) gene of avian Metapneumovirus subtype C (aMPV-C) isolated from domestic turkeys from the United States for any variation in the size with respect to the year of isolation and level of passage in cell cultures. The virus was first detected in 1996 in the United States and continues to cause upper respiratory tract infection in turkeys. Twenty-one domestic turkey isolates of aMPV-C at different cell culture passages were examined by RT-PCR and gene sequencing. Results showed that there is extensive variation in the size of G gene with respect to the year of isolation and cell culture passage. The early isolates had a G gene size of 1978 nucleotides (nt) that coded for a predicted protein size of 585 aa and showed >97% nt similarity with that of aMPV-C isolated from Canada geese. The large G gene got truncated upon serial passage in cell cultures by a deletion of a 1015 nt long segment from the end of the G open reading frame. The recent isolates lacked the large G gene but instead had a small G gene of 782 nt in size, irrespective of the level of cell culture passages. Serial passages in cell culture, as well as natural passage in turkeys, led to truncation of the G gene. Some of the cell culture passages showed both large and small G gene products, which may indicate the existence of a mixed population of the virus. The present study warrants further investigation on the possibility of wild bird aMPV-C acting as a progenitor of turkey viruses, and also on the underlying mechanism of extensive variations in the size of G gene

Detection and quantitation of turkey coronavirus in turkey tissues by a specific real-time reverse transcription-polymerase chain reaction

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Outbreak of turkey coronavirus (TCoV) infection causes acute enteritis in turkey poults, leading to significant economic loss in the U.S. turkey industry. Rapid detection, differentiation, and quantitation of TCoV is critical to the diagnosis and control of the disease. A one-step real-time reverse transcription polymerase chain reaction (RT-PCR) assay for detection and quantitation of TCoV in turkey tissues was developed using a dual-labeled fluorescent probe. The fluorogenic probe labeled with a reporter dye (FAM, 6-carboxytetramethylrhodamin) and a quencher dye (AbsoluteQuencher™) was designed to bind to a 186 base-pair fragment flanked by the two PCR primers targeting 3' end spike gene of TCoV. The assay was performed on different avian viruses and bacteria to determine the specificity as well as the serial dilutions of TCoV for the sensitivity. Three animal trials were conducted to further validate the assay. Ten-day-old turkey poults were orally inoculated with 100 EID_{50} of TCoV Tissues (thymus, trachea, liver, kidney, spleen, duodenum, jejunum, ileum, cecum, colon, bursa, or cloaca), feces from cloacal swab, and/or feces from floor were collected at 12 hours, 1,2,3,5,7, and/or 14 days post inoculation (DPI). RNA was extracted from the samples and subjected to the real-time PCR assay, The designed primers and probe were specific for TCoV and other non-TCoV viruses and bacteria were not amplified by the real-time RT-PCR. The assay was also highly sensitive and could quantitate between 10^2 and10^{10} copies/µl of viral genome. TCoV was detected in the feces from cloacal swab or floor throughout 14 DPI; however, the viral RNA load varied among different turkey poults in different intervals from different trials. The highest amount of viral RNA, $2.8x10^{10}$ copies/µl , in the feces was the one from cloacal swab collected at 1 DPI. The average amount of TCoV RNA in cloacal fecal samples was 10-fold higher than that from floor. Quantitative level of TCoV RNA was obtained from the duodenum, jejunum, ileum, cecum, colon, cloaca, and bursa, but not thymus, trachea, liver, kidney, and spleen. The viral RNA in the intestine reached the highest level, $6x10^{15}$ copies/µl , in the jejunum at 5DPI. In addition, 84 intestine segments from one of the trials were assayed by the developed real-time RT-PCR and immunofluorescence antibody (IFA) assay. There were 6 segments negative for TCoV by both assays, 45 positive for TCoV by IFA, and 77 positive for TCoV by real-time RT-PCR. Taken together, the results indicated that the developed real-time RT-PCR assay is rapid, sensitive, and specific for detection and quantitation of TCoV in turkey tissues and will aid in monitoring the progression of disease outbreaks in turkey flocks.

Prevalence of Campylobacter and *Salmonella* in the Turkey Brooder House

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We have previously surveyed market weight turkeys for the effect of transport to and holding at the abattoir on the prevalence of *Campylobacter* and *Salmonella*. Whereas *Campylobacter* frequently colonizes the ceca of adult birds (~60-100%), the prevalence of *Salmonella* varies considerably from farm to farm. The purpose of this study was to evaluate the point of entry of these bacterial food-borne pathogens into the brooder house, the first stage of commercial turkey production. Two studies were conducted. In summer 2005, birds (100 per time point) were collected on-farm (0-,9– and 16-days of age), transported to the laboratory, euthanized, necropsied, and intestines cultured for *Campylobacter* and *Salmonella*. Whereas *Campylobacter* spp. were not detected in day of hatch poults, *Salmonella* was isolated from the ceca (3.9%) and yolk sac (0.1%). By day 9, whereas *Campylobacter* spp. were not detected, *Salmonella* was frequently isolated from the ceca (55%) and small intestine (45%). By day 16, the prevalence of *Salmonella* in the ceca (21%) and small intestine (5.1%) had declined. In contrast, *Campylobacter* spp. were infrequently isolated from either the ceca (2%) or small intestine (3%). In summer 2005, birds 5– to 33– days old were monitored on a second farm under the same ownership. This provided the opportunity to compare conventional culture with two commercially available real-time PCR detection formats. Whereas *Campylobacter* spp. were not detected in the ceca of 5-day old birds, it could be detected on days 20 (92%) and 33 (90%). *Salmonella* was isolated from nearly all of the 5-day old (98%) and 33-day old (96%) birds, at which time the turkeys were moved to the finisher house. At slaughter *Salmonella* (4.5%) and *Campylobacter* (92%) were isolated from the ceca of market weight (138-day old) birds originating from this flock. Overall, for *Salmonella*, conventional culture was more sensitive than either of the real-time platforms. For *Campylobacter*, the two real-time formats differed significantly in their sensitivity due to the frequency of error runs in one platform. We have previously observed high levels of ciprofloxacin resistance in *Campylobacter* in market weight birds. Antimicrobial profiles of the archived *Campylobacter* isolates indicated resistance to ciprofloxacin was already present in the young bird.

Efficacy Comparison of Three Commercial MG Vaccines

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This study was conducted at the University of Georgia to evaluate an inactivated *Mycoplasma gallisepticum* (MG) bacterin against two different types of MG vaccines, a modified-live vaccine and a recombinant pox-vectored MG vaccine.

Approximately 100 7-week-old SPF chickens were pre-screened to ensure they were free of mycoplasmas. At 10 weeks of age, 25 birds each were vaccinated with the three test vaccines and the remainder served as non-vaccinated controls. The chickens not given the pox-vectored vaccine also were vaccinated for fowl pox in case of shedding of the vector virus by the vector vaccine. Pox "takes" were confirmed a week later. Samples were collected 5 weeks following vaccination for serological evaluation using three tests. Serology of all chickens also was evaluated at the end of the trial, 10 days following challenge and 16 weeks following vaccination.

The timing of MG challenge was determined by egg production, which reached approximately 50% at 24 weeks of age. Aerosol challenge with the virulent R strain of MG followed at 25 weeks of age. Ten days following challenge, all the birds were necropsied and evaluated by gross examination of air sacs and ovaries, histopathology of tracheas and oviducts, and culture of air sacs and ovaries, as well as serology.

The trial was validated by the results observed in control chickens. It was found that the inactivated MG vaccine induced superior seroconversion, both at 5 weeks and 16 weeks following vaccination compared to the other test vaccines. The inactivated MG vaccine and the live MG vaccine both performed considerably better than the vectored product in providing protection against virulent MG challenge. This information is valuable in considering the use of a bacterin, a modified-live vaccine, a recombinant pox-vectored vaccine or a combination of these MG vaccines in a vaccination program.

Results:

At 5 weeks post-vaccination, the mean ELISA titers induced by the inactivated MG vaccine were significantly higher than the other two vaccines (table 1). Neither, the modified-live MG vaccine or the pox-vectored MG vaccine was significantly different from those of the non-vaccinates.

In both non-challenged and challenged chickens 16 weeks post-vaccination, the mean ELISA titers induced in chickens vaccinated with inactivated MG vaccines were significantly higher than those of the other vaccine groups.

Table 1. Summary Mean ELISA Serological Responses of Chickens 5 Weeks and 16 Weeks Post-Vaccination (PV) for MG.

Vaccine Group	Mean ELISA Serological Test Score (Mean sample to positive ratio)		
	5 weeks PV	16 Weeks PV No challenge	16 Weeks PV challenge
1 and 2 Controls	0.0^{a}	0.1^{a}	1.7^{b}
3 Inactivated MG vaccine	1.8^{b}	1.6^{b}	6.3^{d}
4 Pox-vectored MG vaccine	0.1^{a}	0.0^{a}	1.6^{b}
5 Modified-live MG vaccine	0.3^{a}	0.2^{a}	2.6^{c}

* Values within a column with a different superscript are significantly different ($\rho \leq 0.05$).

Both the inactivated MG vaccine and modified-live MG vaccine afforded significant protection against severity of air sac lesions, ovarian regression, and thickening of tracheal mucosa compared to non-vaccinated control chickens following virulent MG challenge. By comparison, the pox-vectored vaccine did not afford significant protection compared to non-vaccinate controls against air sac lesions, ovarian regression, or thickening of tracheal mucosa.

No vaccine in this trial afforded significant protection against re-isolation of the challenge organism from either air sac or oviduct. Vaccination for MG thus cannot be counted on to significantly prevent challenge colonization of those tissues. The challenge used in this trial was considered very severe based on the effects produced in controls, so the vaccines might be expected to perform better against a less severe challenge.

Table 2. Summary of necropsy findings 10 days following challenge with MG R strain, approximately 26 weeks of age: air sac lesion scores, incidence of ovarian regression (follicle atresia), tracheal mucosal measurements, and MG isolation

Challenge	Vaccine Group	Air sac Lesion Score[B]	Ovarian Regression	Tracheal mucosal thickness[C]	MG isolation	
					Air sacs	Oviduct
No	1 Not vaccinated	0/8[D] (0.0)[a]	0/8[a]	126.3 ± 37.9[a]	0/8[a]	0/8[a]
	3 Inactivated MG vaccine	0/5 (0.0)[a]	0/5[a]	113.7 ± 8.7[ab]	0/5[a]	0/5[a]
	4 Pox-vectored MG vaccine	0/5 (0.0)[a]	0/5[a]	125.7 ± 22.1[ab]	0/5[a]	0/5[a]
	5 Modified-live MG vaccine	0/4 (0.0)[a]	0/4[a]	130.2 ± 11.5[ab]	0/4[a]	0/4[a]
Yes	2 Not vaccinated	15/15 (3.6)[c]	13/15[c]	433.7 ± 85.0[d]	12/14[b]	15/15[b]
	3 Inactivated MG vaccine	20/20 (2.5)[b]	8/20[b]	294.4 ± 121.3[c]	20/20[b]	20/20[b]
	4 Pox-vectored MG vaccine	20/20 (3.5)[c]	19/20[c]	389.7 ± 165.9[cd]	20/20[b]	20/20[b]
	5 Modified-live MG vaccine	16/20 (2.1)[b]	8/20[b]	255.4 ± 172.8[bc]	17/19[b]	17/20[b]

Conclusions:

It is concluded from the results of this study that the inactivated MG vaccine and the modified-live MG vaccine both protect against a virulent MG challenge given near peak egg production. When considering the serological response, however, inactivated MG vaccine outperformed the modified-live MG vaccine, which may be an important factor under field conditions where birds are often kept for up to 2 years.

The recombinant pox-vectored MG vaccine failed to provide a serological response or protection against challenge that differed from that seen in non-vaccinates. While this vaccine resulted in milder fowl pox "takes" of variable size, it is worth noting that the poorer "take" quality of this vaccine has since been commonly reported in the field. However, discerning whether this may have played a role in its lack of efficacy was not within the scope of this study.

A three-year analysis of antibacterial susceptibility and resistance in chicken *Escherichia coli*, Salmonella spp., and *Pasteurella multocida* isolates

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Escherichia coli, Salmonella species, and *Pasteurella multocida* are the major bacterial pathogens in poultry flocks. Infections with bacteria are often required to be treated with antibiotics. However, susceptibility to the antimicrobial agents is not equal among all bacterial species as well as among different strains of the same bacterial species. Differences in susceptibility or resistance to different antibiotics has become a major factor in the successful treatment of bacterial diseases. Great concerns have been raised regarding emerging antimicrobial resistance among bacteria that may result in unpredictable antimicrobial susceptibility and untreatable bacterial infections. The primary objective of the present study was to determine the levels of antimicrobial susceptibility and resistance of *E. coli*, *Salmonella* species, and *P. multocida* isolated from chickens in a three-year period.. A total of 445 *E. coli* isolates, 387 *Salmonella* spp. isolates, and 80 *P. multocida* isolates from chickens isolated from 2001 to 2003 were obtained. Minimal inhibitory concentration (MIC) of 14 antimicrobial agents against each bacterial isolate was determined using a microbroth dilution assay according to the Clinical Laboratory Standards Institute (CLSI), formerly National Committee on Clinical Laboratory Standards (NCCLS) guidelines. Ninety-eight and twenty hundredths percent of *E. coli* isolates were resistant to tilmicosin, 79.33% to tetracycline, 51.46% to spectinomycin, 44.04% to gentamicin and 40% to ampicillin. All *Salmonella* spp. isolates were resistant to tilmicosin. Resistance to tetracycline was found in 72.61%, spectinomycin 68.48%, ampicillin 63.57%, gentamicin 63.31%, and ticarcillin 61.76% of *Salmonella* spp. isolates. The resistance rate of *P. multocida* isolates to all antimicrobials tested was less than 5% except 6.25% of resistance to tetracycline. There was decreased resistance to amoxicillin/clavulanic acid, ampicillin, ceftiofur, difloxacin, florfenicol, gentamicin, and spectinomycin, in E. coli isolates in a three-year period. Increased resistance to ampicillin, gentamicin, spectinomycin, ticarcillin, and trimethoprim/sulfadiazine was seen in Salmonella spp. Isolates in a three-year period. In summary, *E. coli* and *Salmonella* isolates were sensitive to ceftiofur and fluoroquinolones, while *P. multocida* isolates remained sensitive to all the antimicrobial agents tested in a three-year analysis.

Counteracting mycotoxin contamination:The effectiveness of *Saccharomyces cerevisiae* cell wall glucans for sequestering mycotoxins

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Introduction: Several natural strategies for controlling the disease processes associated with mycotoxins by sequestration of the mycotoxin within the gastrointestinal tract of an animal are being investigated. Some of these strategies target yeast cell wall as an efficient organic sequestering agent that decrease the toxicological properties of mycotoxins. It is important to understand the fundamental chemistry of the interaction to clarify the absorption process involved in toxin clearance.

Procedures: The chemical mechanisms involved in the sequestering activity of *Saccharomyces cerevisiae* cell wall components toward aflatoxin B1 (AFB1), deoxynivalenol (DON), zearalenone (ZEA), patulin (PAT) and ochratoxin A (OTA) were investigated *in vitro* mixing together increasing amounts of mycotoxins and a constant amount of sequestrants during 90 min at 37°C under orbital agitation. Quantification of the sequestered mycotoxins were achieved by HPLC analysis and evaluation of the interaction kinetic based on overall capacity and standardized affinity rate using Hill's model. The comparison of several sources of yeast cell wall differing in their relative glucan/mannan/chitin content was carried out.

The chemical interaction was approached using molecular mechanics investigation to evaluate the impact of the sequestrant 3D-structure. Together with complementary NMR, X-ray structural data analysis, molecular modeling assessed the overall stability of the modeled molecules in all their most stable possible conformations and allowed to evaluate the statistical probability of existence of each structure. Furthermore, the site-specific docking of a mycotoxin in the sequestrant was investigated so that translations, rotations, and up and down positioning were carefully explored.

Results: The evaluation of the affinity rate using Hill's equation and resultant biological parameters proved a strong efficacy of β-D-glucans, which are composed of linear chains of(1→3)-β-D-glucans branched with (1→6)-β-D-glucan side chains., for AFB1, ZEA, DON and PAT. Complementary work showed a significant efficacy for sequestering T-2 toxins, as well as endophytes associated toxins. Molecular mechanics investigations demonstrated the importance of single and/or triple helix organized structure of β-D-glucans. It was concluded that chemical interactions involved weak chemical linkages such as hydrogen and van der Waals bonds occurring between β-D-glucans and the hydroxyl and cyclic groups of mycotoxins. Several *in silico* models proposed a realistic view of mycotoxin molecules caged inside the helix-shaped (1→3)-β-D-glucans, which were firmly stabilized by (1→6)-β-D- glucan branched side chains due to high geometric similarities between mycotoxins and the active site on the β-D-glucan structure.

Conclusion: Basic science in biochemistry is required to complete and support in a meaningful way the advances in applied nutrition needed to extend our understanding of the beneficial role of *Saccharomyces cerevisiae* cell wall at limiting the toxicologic impact of mycotoxin. The plasticity of the structure of β-D-glucans exhibiting diverse stereochemistry was undoubtedly responsible for the affinity on a large range of mycotoxins. Affinity rates varied widely between toxins due to their structural and physico-chemical disparities. Nevertheless, we concluded that β-D-glucans may have strong affinities for mycotoxins exhibiting "aflatoxin-like" or "zearalenone-like" structures.

The Effect of Natustat® for Prevention of Histomoniasis in Turkeys

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Previous studies suggested that Natustat®, a natural product derived from yeast, had useful preventive activity against blackhead disease (histomoniasis) in turkeys kept on infected litter. The aim of this study was to further investigate the effect of Natustat® after experimental infection of turkey poults using a direct/indirect exposure model. Nitarsone was included as a positive control.

Natustat® was given in the feed at 250g, 500g, or 1000g/ton to 10-day-old turkeys in battery cages. Four days later three of eight birds in every cage were inoculated per cloaca with *Histomonas meleagridis* from cultures. The floor was covererd with heavy paper and the infection was allowed to spread to the other five birds. Mortality, weight gain, and lesion scores in ceca and liver were monitored.

Overall, Natustat® had little effect against the severe lesions caused by direct inoculations of poults. However, liver and cecal lesions of the indirectly exposed birds were reduced and weight gain was increased after treatment with the higher doses of Natustat® in comparison to the 250g/ton treatment. These results suggest that Natustat could be of value in reducing the spread of blackhead within a flock during an outbreak.

Poultry Vaccines: Labeling and Adverse Events

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Poultry vaccine labeling contains information important to the safe and effective use of the product. Information on the label includes:

- True Name—This indicates the disease(s) against which the vaccine is directed.
- Trade Name—The product may also have a name in addition to the True Name
- Name, address, and an Establishment Number—The Establishment Number is a unique identifying number for the company that made the product
- Distributor's Name—The labeling may also have information on the distributor of the vaccine (i.e., some companies only sell the product, not make it).
- Storage conditions.
- Usage instructions—Minimum age, route of vaccination, cautions, warnings, or other special restrictions are indicated
- Withholding statement—This is information on how long animals administered the product should be withheld from slaughter.
- Expiration date—Date the product should be used by for maximum effectiveness
- Antibiotics—While antibiotics are not used at therapeutic concentrations, they may be present in the final product

All labeling is reviewed by the Center for Veterinary Biologics (CVB) to ensure the claims are supported by data and are not false or misleading. Information on the veterinary biologics labeling requirements can be located on our website: http://www.aphis.usda.gov/vs/cvb/ under 9 CFR 112, Veterinary Services Memorandum 800.54 or 800.80

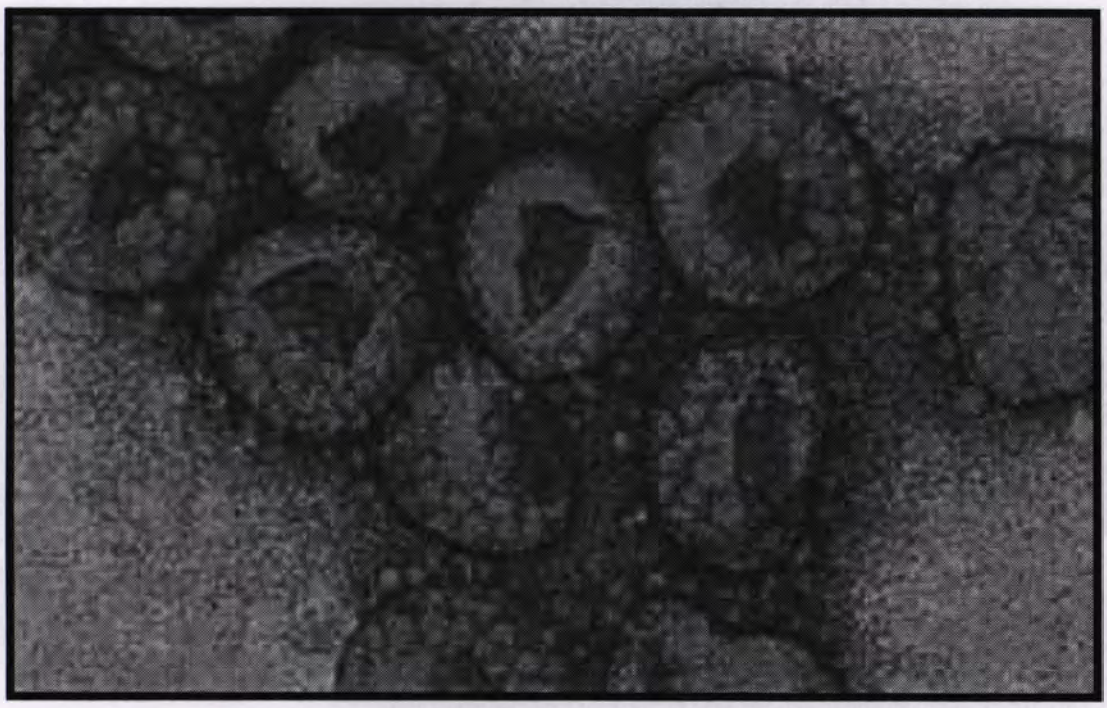
The CVB monitors adverse events following vaccination. An adverse event is defined as any undesirable occurrence after the use of a vaccine, including illness or reaction, whether or not the event was caused by the product. For products intended to diagnose disease, adverse events refer to anything that hinders discovery of the correct diagnosis.

On-line: Adverse Event Electronic Report Form
By fax or mail: Download the pdf form and FAX to area code (515) 232-7120 or by mail to CVB.
By telephone: Adverse events may also be reported by calling CVB at (800) 752-6255.

Things to be aware of when using vaccines:
- Manufacturers and Serial Numbers
- Approved routes and ages indicated on the label
- Warnings and cautions indicated on the label

Symposium

Lessons Learned from AI Preparation



Avian Influenza and Lessons Learned on AI Diagnosis

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Preparedness for outbreaks of avian influenza (AI) in poultry has never been more important than it is today. The rapid spread and reoccurring outbreaks of the highly pathogenic AI (HPAI) H5N1 virus in Asia, Europe, the Middle East and Africa emphasize the importance of having diagnostic assays available to ensure rapid detection should the virus be introduced into the United States. We know from past experiences with AI infections in poultry that the economic impact of outbreaks can be greatly reduced when infections are detected early and aggressive control measures are implemented. This is true for outbreaks caused by low pathogenicity AI (LPAI) viruses as well as for those caused by highly pathogenic AI (HPAI) viruses. However, the detection of LPAI can be especially difficult because clinical signs often cannot be used to identify infected flocks. Therefore, detection of AI requires good surveillance programs and diagnostic tests to confirm infections in poultry. This presentation will review recent advances in laboratory diagnosis of AI, identify the role of the National Animal Health laboratory Network (NAHLN) to conduct AI surveillance, and iterate the lessons learned from recent outbreaks of LPAI and HPAI in the United States.

NPIP Program, State Preparedness and Containment of Low Path AI

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High Path AI Responses and Update on Veterinary Stockpile

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AI Vaccine—A New Approach

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Live-virus vaccines have a distinct advantage over the conventional vaccine, which includes the administration by nasal spray. For humans, cold adapted live vaccines have been applied to millions of children with protective efficacy and without evidence of serious side effects. In the U.S. trivalent, cold-adapted, attenuated, live influenza vaccine (FluMist™) was approved for use on June 17, 2003. However, the use of live influenza vaccines in poultry has never been seriously considered for several reasons. First, there is the possibility of bird-to-bird or farm-to-farm transmission. This may establish AI virus as an endemic infection. Second, the use of live vaccine may cause vaccine-induced respiratory disease in commercial poultry populations. Third, and maybe most importantly, there is a potential for recombination with newly introduced AI virus strains to create a recombinant AI virus which is more pathogenic or has the capability of spreading to different hosts. However, with the advancement in biotechnology, it may be possible to consider new vaccine approaches using genetically engineered live attenuated virus. To be safer, it will be desirable to develop vaccine viruses that undergo only a few cycles of replication in chickens, but still induce a strong protective antibody response and stimulate a cell-mediated immune response without allowing the replication of a virus.

The NS1 protein of influenza virus functions as an interferon antagonist and is, thus, directly associated with the pathogenicity of the virus. From the TK/OR/71-del (H7N3) virus, we previously found that several variants with different sizes of the NS gene can be generated by serial passage of the virus in embryonating chicken eggs. We have further pursued the identification of different NS genes and have found 20 different NS genes that have unique deletions in different regions of the NS gene. Preliminary data shows that these naturally selected NS1 deletion variants will be useful in the development of live influenza vaccines both in their current state and with further modification of the NS1 protein. Furthermore, a deletion in the NS1 protein can also be useful as a negative marker for the DIVA (Differentiating Infected from Vaccinated Animals) approach.

In poultry, vaccine can be administered to eggs during later stages of embryonation, usually at 17-18 days of incubation. This *in ovo* vaccine delivery is easy and cost-effective and has replaced post-hatch injection of Marek's disease vaccine in most broiler chickens in the U.S. A potential use in the future would be for *in ovo* vaccination with attenuated or replication-defective live influenza virus that induces strong immune responses, but has no infectious virus present in the hatched chicks.

Lessons learned in the prevention and control of avian influenza: a scientific approach

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Preventing avian influenza (AI) exposure requires an understanding of the source of the virus, how the virus moves, knowledge of poultry population involved, cross over points and the exposure risk level. This knowledge allows the biosecurity tools of sanitation, isolation and traffic control to be directed appropriately. A logical source for this information is the scientific literature where it is possible to find knowledge gleaned by others while they were learning lessons from AI outbreaks.

Lesson 1. A labile virus. The AI virus (AIV) has an envelope that accounts for its susceptibility to detergents and disinfectants. Additionally it is susceptible to heat and drying; it is generally inactivated within one week at 21 C, but may survive for five weeks at 4 C (29,30). Moist feces collected from 1983 Pennsylvania H5N2 HPAI infected hens infected SPF chicks placed on the feces two days but not four days after collection (3). The Pennsylvania H7N2 LPAI virus from 1997 was inactivated in chickens manure in less than one week at 15-20 C (23).

Lesson 2. The natural reservoir. The known natural reservoir of AIV is wild waterfowl and shorebirds (orders anseriformes and charadriiformes) where the AI virus primarily causes an enteric infection and rarely a respiratory infection. A duck can shed virus for 30 days and can excrete 10^8 EID_{50}/ml of feces per day (34), contaminating surface water, sloughs, and shorelands (13). In its natural reservoir waterborne virus is transmitted most efficiently by the fecal oral route. Thus the water contamination, fecal oral transmission and movement of wild waterfowl and shore birds are the mechanisms that provide for AIV survival and dispersal in nature. The waterfowl-shorebird reservoir, the environment these birds occupy, and anything or anyone sharing that environment may be a source of AI virus for domestic poultry (29,30).

Lesson 3. The man made reservoir. The most important man-made reservoir of AI is the live poultry market system where the continual introduction of susceptible poultry can maintain the virus for years (27). Live poultry markets coupled with a village poultry production system with multi-species and non-confinement rearing are particularly likely to maintain AIV and recycle it back into wild birds. These aforementioned conditions in much of the world now contribute to the more complex epidemiology of highly pathogenic H5N1 and low pathogenicity H9N2 in much of the old world.

Lesson 3.1. Hong Kong authorities have demonstrated success in eliminating H5N1 in the live poultry market system by vaccination, by separating waterfowl from gallinaceous poultry, by enforcing monthly rest days in the markets and by active surveillance (11,21).

Lesson 4. Preventing accidental introduction. Apparently healthy wild or domestic waterfowl and poultry, particularly associated with live poultry markets or range production, may be infected with AIV. The virus is found in the droppings of infected waterfowl and each bird may excrete ten billion EID_{50} of virus per day (34). In gallinaceous poultry the virus is found

in both the respiratory secretions at up to 10^7 EID_{50} of virus per gram and in feces at up to 10^6 EID_{50} of virus per gram. An exception to this generalization was the observation of 10^9 EID_{50} of HPAI virus in the feces of laying hens experimentally infected with the 1983 H5N2 AI virus (3). To put this amount of virus in perspective, Tumpey et al (32) found that turkeys could be infected with less than 10^1 EID_{50} of H7N2 AI virus from the Virginia outbreak; so with the worst case scenario (10^9 EID_{50} of virus per gram of feces and 10^1 EID_{50} of virus equal to one turkey infectious dose 50%), one microgram of feces could contain 100 TID_{50} of AI virus. To prevent the introduction of AI any conceivable contact between the high risk contamination areas and commercial poultry must be avoided.

Lesson 4.1. Orders of birds other than anseriformes and charadriiformes may be infected but the virus is not maintained in them in nature; however, it is important to recognize that these birds may act as mechanical and biological vectors to move the virus between the waterfowl reservoir and domestic birds (29,30)

Lesson 4.2. Clothing or equipment used while hunting, trapping or fishing and travel directly from such environments can introduce AIV onto a poultry farm if precautions are not taken (14).

Lesson 4.3. Watering poultry from ponds can introduce AI virus to a farm (2,14).

Lesson 4.4. Clothing or equipment used while visiting live poultry markets or any other place where live poultry may be kept, sold, exhibited, or fought can introduce AI virus to a farm (14).

Lesson 4.5. Vehicles or crates or batteries that have been in contact with live poultry markets can introduce AIV onto a poultry farm (19,20).

Lesson 5. Preventing introductions associated with illegal behavior. Illegal behavior might introduce AI viruses onto a poultry farm. Farming is a business involving trust; routine deals involving thousands of dollars are sometimes done on a hand shake. The strong culture of trust in agribusiness is an obstacle to preventing potential illegal activity that could introduce disease agents such as influenza virus. Theft of birds from a commercial poultry farm for personal use or for sale at a live poultry market (Kradel, personal communication), smuggling birds or bird products, contact with fighting chickens, or intentional introduction of virus are all ways that people engaged in illegal activities could introduce avian influenza virus into a flock.

Lesson 5.1. At a bare minimum poultry farms need a security system that discourages visitors and includes locked doors (2,14).

Lesson 6. Preventing the spread. Once an AI outbreak has occurred attention must be shifted from the reservoirs of AI virus and the environments associated with them toward sources of virus within infected poultry populations.

Lesson 6.1. Because it is not possible to categorize with certainty that a given flock is not infected with AI, effort must be directed toward avoiding direct and indirect exposure to the documented sources of virus transmission: poultry manure, respiratory secretions, live poultry, dead poultry, re-used egg packing materials, and equipment or people contaminated with manure or respiratory secretions (4,5,7,14,17,25)).

Lesson 6.2. Other potential sources (flies, rodents, and people and equipment that have not contacted poultry or manure) are far less important and have not been documented as sources of transmission.

Lesson 6.3. In the U.S. and Canada pet birds and backyard poultry have not been associated with AI outbreaks in commercial poultry (Dennis Senne, personal communication, Victoria Bowes, personal communication) and the same conclusion was reached in the Netherlands following the HPAI outbreak in 2003 (9,18).

Lesson 7. The role of people in moving AI virus. In nature the avian influenza virus is moved from place to place by the movement of waterfowl and shorebirds. In domestic poultry the virus is moved from place to place by the activities of people. The existence of the virus in either the waterfowl or market reservoir is not the problem; rather it is movement of virus or infected birds into close proximity of susceptible birds that causes transmission of AI. Depending on the type of poultry, the type of poultry operation, and regional considerations (eg poultry density) for any influenza outbreak it is critical to quickly identify how the virus will be moved about by the activities of people. Recognizing the ways the virus is apt to be moved in a particular outbreak is the most important part of the control strategy.

Lesson 7.1. Eighty years ago it was recognized that contaminated poultry crates and rail cars spread fowl plague (HPAI) from New York to the Midwest (20).

Lesson 7.2. Twenty years ago in California it was documented that contaminated artificial insemination crews and equipment transmitted AI between five turkey breeder farms while leaving some 300 other poultry flocks in the same area untouched (24).

Lesson 7.3. In Minnesota live haul crews and equipment were implicated (2,14).

Lesson 7.4. In Virginia a common rendering plant and hired labor were involved (25)

Lesson 7.5. In Italy manure hauling contributed to spread (7)

Lesson 7.6. In California movement of spent hens and movement of egg handling material were identified (Cardona, personal communication).

Lesson 7.7. Feed trucks, eggs and day-old chicks have not been reported to be the source of spread.

Lesson 7.8. Airborne transmission has only been associated with human activity (trucking live infected birds, trucking carcasses, generation of dust and feathers during depopulation, trucking manure)

Lesson 8. Identify specific avian populations. Industry practices are specific for different types of poultry. Some types of poultry are moved from one place to another, have access to the outdoors, have contact with live poultry markets, or have contact with wild birds. Recognition of these practices has an impact on control measures that must be initiated (29,30). The susceptibility of different poultry groups to AI outbreaks differs greatly from one group to another and depends to a great extent on exposure to natural or man-made sources of AIV, on the virus itself, on movement of birds, their products and their manure, and on the extent of human activity during the production period.

Lesson 8.1. For example, during the 2002 Virginia LPAI outbreak it was observed that turkeys in the growing facilities (after being moved) were far more frequently infected than those in brooder facilities (having never been moved)(25).

Lesson 8.2. Totally confined populations. The commercial poultry industries reduce their risk of AI introduction by avoiding exposure to the wild bird or live poultry market reservoirs; if AI is introduced their risk is related to the high population density and intra-establishment movement

Lesson 8.3. Non-confined populations. In general owners of non-confinement birds reduce their risk of AI loss by their small size and low population density; their risk of exposure is great and is related to direct or indirect contact with the one of the reservoirs of AI and inter-establishment movement.

Lesson 8.4. The term, backyard birds, has no specific meaning in determining AI risk because they may consist of a single age and species kept in confinement with no contact with live poultry markets, or they may consist of multi-age, multi-species reared outdoors and with live poultry market contact. Thus, it is clear that backyard flocks have to be characterized in order to make meaningful decisions about their potential contribution to AI spread and control. Backyard flocks in the U.S. probably have little resemblance to backyard flocks in East Asia or Africa.

Lesson 8.5. Upland game and waterfowl that are raised for release; organic, range, and pastured poultry; backyard, exhibition, or zoo birds and game fowl (fighting chickens) may have exposure to wild birds. In addition live birds may be transferred from one location to another (birds raised for release, exhibition birds, zoo birds and fighting cocks), although there may be little or no movement of backyard birds. Other poultry may be grown for the live poultry marketing system where the exposure comes from the transfer of contaminated materials or rarely live birds from market to farm, where susceptible poultry then multiply the infection and become a source for additional contamination of the market.

Lesson 9. Identify cross over between populations. The new World Animal Health Association (OIE) chapter on avian influenza promotes the concept of compartmentalization for maintaining poultry trade in the event of a notifiable avian influenza outbreak. Although poultry establishments make great effort to distance themselves from others producing the same type or different types of poultry, sometimes there are opportunities for exposure due to cross over. Cross over occurs where production related activities of one establishment utilize the same resources as other establishments. For examples, a rendering truck may visit broiler, layer and turkey farms, contract labor (crews) may provide services to many different companies, individuals may work on more than one crew, pullet moving dollies may be used on multiple farms, egg moving materials often go to different farms, and feed trucks may provide feed to multiple establishments. Eliminating these cross over points demonstrates compartmentalization and is critical for any establishment that is interested in exporting poultry meat or eggs.

Lesson 10. Identify specific exposure risk level. In the past determining the risk of disease exposure has been a subjective exercise for poultry professionals. Recently an exposure risk index (ERI) has been proposed as a way to make this determination more objective (16). An ERI ≥ 7 is correlated with intolerable or high risk according to a group of poultry veterinarians, while an ERI of ≤ 4 was considered moderate or low risk. A high ERI also is correlated with documented sources of AI transmission risk (eg manure or sick or dead birds) and a low ERI is correlated with low transmission risk (eg pet birds or washed eggs).

Lesson 10.1. The ERI can help evaluate mitigation strategies. For example, the ERI indicates that pullet moving equipment contaminated with one Kg of manure with 10^6 AI viruses per gram still has an unacceptable ERI even after cleaning and disinfecting remove 99.99% (4 logs) of the contamination. This indicates that the cleaning and disinfecting procedure requires modification (16).

Lesson 10.2. Once exposure risk is estimated, investments in fixed and variable costs of biosecurity can be evaluated in light of possible economic impact of avian influenza outbreaks. In the past the biosecurity costs were weighed against risk of disease effects on performance: mortality, body weight, feed efficiency, egg production, etc. As the poultry industries have evolved from meat and egg production-oriented companies to global consumer-oriented companies, today an avian influenza outbreak has the potential to have far greater impact than just on poultry production. Food safety may be called into question, exports may be at risk, public health may be questioned, and brand name survival may be threatened. This increased impact means that increased spending for biosecurity is justified in the global economy (16).

Lesson 11. Control measures that have worked. Avian influenza control programs that have incorporated education, preventing introduction, surveillance & reporting and response have been successful in preventing and controlling AI (14,17,29,30).

Lesson 11.1. Early detection and reporting were found to be key to control in British Columbia, Netherlands, and Minnesota (4,5,10,14).

Lesson 11.2. Industry infrastructure was key to control in British Columbia (4,5).

Lesson 11.3. Containment was dependent on lockdown response in British Columbia and Minnesota (4,5,14) and reducing number of susceptible flocks in Netherlands (28).

Lesson 11.4. Abandoning range production resulted in a sharp decline in AI in turkeys (28,29).

Lesson 11.5. In Utah, Connecticut, Italy and Hong Kong blanket vaccination coupled with biosecurity aided in control (1,6,8,11,15).

Lesson 11.6. In British Columbia it was learned not to move carcasses outside or off farm before time-temperature treatment (4,5)

Lesson 12. Control measures that have caused problems. Some measures have been identified that have caused problems by contributing to virus spread.

Lesson 12.1. A delay in detection results in delayed response and a larger outbreak (4,5,10).

Lesson 12.2. Grinding and trucking carcasses off the farm spread the virus in BC (26).

Lesson 12.3. Trucking actively infected birds spread the virus in Minnesota (2).

Lesson 12.4. Some control activities such as increased sampling spread the virus (19).

Lesson 12.5. Killing pet birds, backyard birds and healthy poultry reduced support for eradication and caused an emotional toll in Netherlands, BC & Italy (4,5,7,12,18,22)

Lesson 12.6. Moving manure spread the virus in Italy (7).

Lesson 12.7. Depopulation efforts (generation of dust and feathers, failure to completely control depopulation crews) led to increasing spread in Pennsylvania in 83 and 97 (17,19).

Lesson 12.8. Destruction costs approximately 10 to 100 times more than controlled marketing.

Lesson 12.9. Vaccination alone has not been a satisfactory control strategy in Mexico (33).

Conclusion

On a global scale AI control is very complex; on a local level it is likely to be simple, but rarely easy. In either case, scientific studies, in the laboratory and in the field, have greatly added to our knowledge of avian influenza prevention and control. Drawing from this body of knowledge to understand the virus, the reservoirs, the movement, the characteristics of specific populations, the cross over between populations and the exposure risk level, it is possible to construct and justify scientifically sound prevention and control programs.

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The Use of Vaccines and Biologics in Controlling AI and the Concept of Compartmentalization and Regionalization

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USDA, APHIS, VS, Mission

The USDA, APHIS, VS, protects and improves the health, quality and marketability of our nation's animals and animal products, and veterinary biologics by preventing, controlling, and/ or eliminating animal disease, and monitoring and promoting animal health and productivity, and licensing veterinary biological products intended for use in the treatment or diagnosis of diseases in animals.

Background

Eradication and biosecurity are the first line of defense against all AI viruses. Preventing the introduction of AI by eliminating all contact between commercial poultry and wild birds, swine farms, and live bird markets is a common and successful practice. However, occasionally, AI is introduced into the commercial poultry population.

Under conditions of high poultry density or multiple poultry establishments in one area, eradication and biosecurity alone are not likely to be successful control strategies (5). A successful strategy requires reducing the susceptibility and density of the poultry population. The components of a control strategy can vary, but generally include five categories: (i) biosecurity (including quarantine); (ii) diagnostic and surveillance; (iii) elimination of infected poultry; (iv) decreasing host susceptibility to the pathogen (for example, through vaccination); and (v) education of personnel in the animal production chain and allied industries to better understand how diseases are transmitted so personnel with responsibility to prevent transmission or spread can be incorporated into action plans.(3)

For many years, APHIS policy regarding the control of AI and the related production and distribution of AI vaccines remained unchanged. Then, in 1995, restriction on the production and use of H7 subtype vaccines was added to the restriction that existed since 1983 on the use of H5 subtype vaccines. At the same time, APHIS concluded that the restrictions imposed on AI vaccine production and use during the 1983 high pathogenicity avian influenza (HPAI) eradication campaign should be modified to allow H5 and H7 vaccines to be used as a tool for combating any potential outbreak of HPAI

As we approach the eradication of specific animal diseases in the United States, it will be necessary to refine regulations, policies, and procedures pertaining to veterinary biological product availability for these diseases.

Current APHIS policy

This presentation will review the current USDA, APHIS policy on the use of vaccines and biologics in controlling AI and the concept of Compartmentalization and Regionalization or Zoning. The current APHIS policy, as described in VS Memorandum No. 565.12 allows "H5 and H7 vaccines to be used as a tool for combating any potential outbreak of HPAI in the United States" (6). AI vaccines may be prepared from any serotype, including H5 and H7, and may be recommended for use in chickens or turkeys subject to the requirements and restrictions specified in VS Memorandum No. 800.85 (6). VS Memorandum No. 800.85 allows H5 and H7 vaccines to only be used under the supervision or control of USDA, APHIS, VS, as part of an official USDA animal disease control program. The USDA, APHIS, VS Center for Veterinary Biologics (CVB) implements the provisions of the Virus-Serum-Toxin Act to ensure that veterinary biologics available for the diagnosis, prevention and treatment of animal disease are pure, safe, potent, and effective.

APHIS supports the general concept of vaccination as a tool in the eradication of notifiable AI. However, vaccination should be available as part of a science-based influenza control strategy that includes: (i) enhanced biosecurity; (ii) controlled vaccination for flocks deemed to be at risk; (iii) suitable monitoring of all flocks at risk and of all vaccinated flocks; and ((iv) a repopulation plan (2,3,4). The management of AI must continue to be based on sound scientific principles. However, innovative strategies will be required to eliminate these persistent and adaptive viruses.

The Concept of Compartmentalization and Regionalization or Zoning

The following definitions have been adopted from the new Office International des Epizooties (OIE) Terrestrial Code for Avian Influenza

Compartment means one or more establishments (premises in which animals are kept) under a common biosecurity management system containing an animal subpopulation with a distinct health status with respect to a specific disease or specific diseases for which required surveillance, control and biosecurity measures have been applied for the porpose of internation trade (1).

Region/Zone means a clearly defined part of a country containing an animal subpopulation with a distinct health status with respect to a specific disease for which required surveillance, control and biosecurity measures have been applied for the purpose of international trade (1).

The procedures used to establish and maintain the distinct health status of a compartment or zone should be appropriate to the particular circumstances, and will depend on (1):

- The epidemiology of the disease (including methods of disease spread and species affected);
- Environmental factors (including the presence of natural barriers);
- Appropriate and applicable biosecurity measures (including movement controls, use of natural and artificial boundaries, commercial management and husbandry practices); and
- Disease surveillance.

Both Compartmentalization and Zoning are tools that may be applied to facilitate trade. Fundamental to their application is the free exchange of information necessary to provide confidence to the importing country that the risk of disease introduction from trade is minimized. Therefore, the procedures for establishing trade based upon the compartmentalization concept should be similar to those practiced for regionalization or zoning. However, the ultimate trade decisions are determined by the importing country's analysis and their acceptable level of risk.

Risk analysis, research and development are urgently needed to obtain a better understanding of risk factors in order to implement more effective control measures. A need exists for significant sustained financial investment in national and regional infrastructures to ensure that most countries and states work in a coordinated and harmonized manner to implement these concepts and required surveillance and control measures. It is in the interest of USDA, APHIS, VS and states to make these investments to protect animal health.

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Handling Emerging Disease Issues When You Are In Charge

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When a disease issue affects your poultry farm operation how prepared are you to respond? This session overviews the steps needed to take action should a disease issue affect your poultry operation.

Having a networking plan in place beyond internal employee communication is critical in addressing emerging disease issues. Crisis management coaching is necessary because we are often unprepared for the harsh reality of dealing with disease issues which become the focus of media and public scrutiny. Such public scrutiny can be surprising and overwhelming for employees and managers. Therefore, developing effective responses to the media and general public are crucial for poultry operations. Actions will be outlined to prepare for external public communication and to develop an internal communications plan. This session serves to establish the foundation for effective management of disease issues and assists in responses to the media and the general public's concerns.

AI Preparedness—the Texas Experience

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Avian Influenza is an ever present challenge and it thrives in the clash between new and old poultry husbandry/marketing practices. In various states of the country, including Texas, Live Bird Markets (LBMs) have become potential or known reservoirs of Avian Influenza (AI). The multi-age, multi-source and multi-species nature of LBMs provides a suitable environment for the emergence of poultry-adapted AI viruses. This poultry production, distribution and marketing system represents a risk in addition to the well known risk from wild birds. Commercial poultry are immunologically naïve to AI so they are always at risk. Biosecurity is the key to separate commercial poultry from high risk bird populations. If a breach in biosecurity occurs then disease control requires detection, containment and prevention. In 1995, in response to the presence of highly pathogenic AI in Mexico, the Texas Poultry Federation (TPF) developed an active AI surveillance program and an industry-based indemnity program to encourage eradication of the disease. In addition, poultry industry, regulatory and laboratory personnel cooperated in the development of an AI response plan. Just last year, Texas AI surveillance generated approximately 99,000 AI serology tests in the Gonzales laboratory alone. Since the establishment of the Texas AI response plan, we have had several opportunities to put theory into practice. On the bright side, preparedness, cooperation and the combined experience of laboratory, regulatory and industry personnel yielded positive results. On the other hand, AI has a heavy toll on all involved. No person, business, agency, city, state or country is ever completely ready to deal with the consequences of an AI outbreak. Preparedness and experience are key elements to the effectiveness of the response. Active surveillance is the key to early detection and a quick resolution. Our experiences from 2004 will be used to illustrate AI preparedness and response.

Actions and Lessons Learned in Preparing the Broiler and Broiler Breeder Industry for AI

Susan Williams, DVM, PhD, Poultry Diagnostic and and Research Center, Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA 30602

Preparing for an Avian Influenza outbreak has been a long process with numerous plans undergoing revisions as new regulations are enacted. State regulations also influence plan development and whether or not the state has had previous experience with AI or even Exotic Newcastle. Covered in this presentation are 3 states in the southeastern United States with large broiler and broiler breeder industries and what has occurred in the development of their plans to manage High Path AI and Low Path AI outbreaks.

South Carolina has about equal industries of broilers, layers, and commercial turkeys as the major poultry producers in the state. There are smaller operations with quail and game birds, along with backyard producers. To develop a plan, a small core of representatives first met and developed an outline which was then debated and revised. After getting a consensus from representatives from the table egg industry, broiler industry and turkey industry along with NPIP and State Veterinarian's office, they presented a draft to the entire industry to discuss and revise. Representatives from all companies were able to review and raise concerns, questions, etc., about the documents and come to consensus about what to do if AI were to occur. For the broiler industry, this means composting in the house for 2 weeks, then testing for virus presence. If negative, they will continue composting out of the house and clean and disinfect the house. New farms are now required to get pre-approval for an on-farm burial site for mass euthanasia from the environmental protection department. Older farms are now going through the process so that if or when the need for mass euthanasia occurs, they will not have to wait on the environmental protection agency to approve their site. Their reasoning for on-site burial is that the landfills in South Carolina are privately owned and they can turn away the poultry industry if they desire for whatever reason. There is one rendering plant that will take poultry in the state and it operates 5-6 days a week already so it would not be feasible to bring more poultry for rendering in the event of an outbreak. There has been a small foam euthanasia demonstration with chickens on the floor in a pen to see how this would work. Results were good and this practice for broilers and broiler breeders on the floor would most likely be employed. South Carolina has applied for a grant that requests a foam machine for the industry to use and is waiting for funding results. The biggest lesson learned for South Carolina was to include everyone in the process so that no particular sect of the poultry industry felt ignored or left out.

Alabama has predominantly broiler and broiler breeder industry with several large layer complexes and a large quail production industry. They also have a game bird industry that imports birds from all over the United States and abroad. The experience of having a pullorum outbreak in the 1990s and having to euthanize large numbers of birds and dispose of them has prepared them in setting up a plan for the state. The Alabama Avian Health Advisory Board has representatives from industry, the State Veterinarian's office and the Commissioner of Agriculture office and has developed a plan that takes into account what happened during the pullorum outbreak. A member of the State Veterinarian's office participated in the California END outbreak surveillance and learned how important communication is for compliance. They have in the past used CO_2 for euthanasia and plan to use it again. However, some companies

have bought their own foam machine for mass euthanasia and the State Veterinarian's office encourages them to do so. Each company is encouraged to have its own AI plan ready that will help their workers know what to do in the event of an outbreak. Disposal will vary depending on location in the state. The Alabama environmental protection agency has agreed that mass burial is to be instituted where possible. Burial on site is preferred but, in some areas, there is not enough topsoil to cover the birds properly with 2 feet of dirt. In these instances, composting or incineration or landfill disposal are the preferred methods. They are also investigating a mobile tissue digester that could do smaller flocks up to 1500 lbs a day. They have a digester at the State Laboratory in Auburn that handles more poundage. Vaccination will depend on what USDA allows. Since there is a primary breeder company in Alabama, compartmentalization is being looked into so that they can continue to export chicks, eggs, etc., to US and world customers. They also have addressed what to do if companies have complexes in other states that may be infected and how the company can still operate without endangering the Alabama industry. Again, communication within the industry and state officials will be key.

Georgia has a large broiler and broiler breeder industry in the northern half of the state along with pedigree farms. There are layer operations also in the northern half and one company in southern Georgia. There is a quail operation in the northern half. There is a large game bird industry throughout the state. The southern half is mainly broiler companies. The Georgia Poultry Laboratory Network has 9 labs for industry use along with Athens Diagnostic Laboratory (USDA regional testing lab for foreign animal diseases with BSL 3 labs) and Poultry and Research Center at UGA. To prepare for Avian Influenza, an industry wide survey occurred last May-Dec where a representative from GPL and APHIS met with all companies in the state. They made a presentation to the companies about the proposed low path AI plan and determined if the companies had any plans for their company. Afterwards, they presented the revised low path plan to the industry and voted on the items that everyone did not agree on. The high path plan will be voted upon in March this year. For mass euthanasia, the State Veterinarian's office has a foam machine that has been used on houses with birds that were going to be euthanized to make sure everything was working properly and personnel know what to do. Another machine is going to be bought that will be available for companies to use without going through the State Veterinarian's office. For disposal, composting and incineration are most likely going to be employed in a high path outbreak. Burial is available, but many growers may not want to bury HPAI birds on their farms due to the stigma attached. Georgia has a mobile incineration unit that is smokeless and odorless, is quite large, and holds large amounts of birds. Ashes can then be buried in landfills or trenches. Import and export issues will be handled by the State Veterinarian's office in cases of low path AI.

Overall, communication with the companies and getting everyone to agree to the plans has been the most important lesson learned for the meat bird industry.

The North Central Experience

Mick Fulton, Diagnostic Center for Population and Animal Health, Michigan State University, 4125 Beaumont Street, Lansing, MI 48910

Pennsylvania Experience: Live Bird Market and Back Yard Birds

Eric Gingerich, DVM, University of Pennsylvania School of Veterinary Medicine, New Bolton Center Laboratory of Avian Medicine and Pathology, Kennett Square PA 19348

The production and selling of poultry for the live bird market (LBM) system in new York and New Jersey is a big business in Pennsylvania. A majority of the 25 to 30 million birds sold are raised in PA. Most of this production is raised under contract by distributors who either have a marketing contract for selling a grower's birds or the distributor owns that birds that the grower raises for a fee. The distributor sets up the placement and marketing schedule and arranges for transportation to market. The grower is responsible for care and housing of the birds. Labor to gather the birds for transport is either arranged by the grower or by the distributor depending on the situation.

As many of the LBM markets have been positive for low pathogenic avian influenza (LPAI) virus in the past, the key to maintaining negative production flocks in PA is to be sure the flocks going to market are free of LPAI and that any equipment used coming back to PA is sanitized prior to reuse. The spread of LPAI among flocks or between wild birds and LBM supply flocks has not been the source of LPAI for LBMs in PA LBM supply flocks.

AI testing prior to market is required for both states, NY and NJ. A PA certified poultry "Test Prior to Market or "Monitored Flock Program". The "Test Prior to Market" program requires 30 samples of sera for AI agar gel immunodiffusion (AGID) testing (30 cloacal swabs for virus isolation if waterfowl) within 10 days of marketing. The "Monitored Flock Program" requires 30 sera samples (30 cloacal swabs for waterfowl flocks) every month for any flocks on the farm over 4 weeks of age.

All coops and trucks used to transport LBM poultry are required to use a USDA approved coop and truck sanitation system to clean and disinfect (C&D) the coops, crates, and trucks between uses. Receipts are kept as proof that the C&D was done. USDA personnel perform monthly audits of each distributor to maintain a check on the production and marketing system. During these monthly visits, flock testing records, truck and coop C&D records, and other practices used by the distributor are monitored. Swab testing of equipment used for transport is also collected for AI virus isolation.

The PA AI response plans treats the LBM producers the same as commercial poultry producers as many of the LBM producers are similar in size to commercial units; most broiler supply flocks are 15-20,000 in size. Waterfowl flocks tend to be smaller in the range of 1000 birds. Spent egg layers tend to be brown egg commercial flocks. Geographic Information system (GIS) and contact information is kept in a computerized database for both commercial and LBM supply flocks by the PennAg Industries Poultry Council at the University of Pennsylvania New Bolton Center Laboratory of Avian Medicine and Pathology in Kennett Square PA. Much of this information is updated from information gathered from sample submissions for flock testing.

Should an LPAI outbreak occur, the commercial and LBM supply flocks will be identified in the 3 and 10 km diameter zones surrounding the index case, a map produced with the location of each flock identified by number on the map, and a list of the companies, distributors, or independent growers to contact who own the flocks in these zones is produced. The map along

with the contacts list will be sent to the Pennsylvania Department of Agriculture (PDA) so they may initiate necessary actions in order to control the outbreak.

PDA may establish quarantine zones based on established roadways, geographical, or political boundaries. The contact information will be used to shut down any transport of poultry or eggs into or out of the zone and to establish a plan for testing of dead birds from each flock as part of the surveillance plan. As most of the contacts in the database are contractors of the flocks and not the growers themselves, these contractors then are responsible for calling the grower to inform them of the actions to be taken; limit movement of people on and off the farm and how and when to collect dead birds for surveillance. The contractors are also the most knowledgeable for controlling the movement of feed, eggs, flock supervisors, etc.

Surveillance of dead birds from all flocks for the presence of LPAI within the 3 km diameter zone (the protection zone) is planned within 48 hours and surveillance of all flocks within the 10 km zone (the surveillance zone) is to be accomplished within 72 hours. In order to do this, the growers will need to place 15 dead or freshly killed birds from each flock at the entry to the farm where one of a number of teams will be dispatched to sample these birds for AI testing (oropharyngeal swabs from gallinaceous birds for the RRT-PCR or cloacal swabs from waterfowl for virus isolation). Coordination of these efforts will be through PDA and the contractors.

Once a flock within the protection or surveillance zones has tested negative, movement of birds or eggs can commence by permit issued by PDA under the conditions that any obvious clinical signs of LPAI be reported immediately. Continued dead bird surveillance of flocks in the protection and surveillance zones will be performed on a weekly basis.

A most important aspect of surveillance is to determine possible links to the outbreak, both prior to or after the outbreak, as possible sources of LPAI or recipients of virus (dangerous contacts). PDA personnel will need to determine these dangerous contacts from two weeks prior to the break onward through interviews and viewing of records with the index case flock personnel. These dangerous contacts are prioritized in order of risk of spread. The top priority would be persons who actually entered the flocks and visited other flocks (flock supervisors, caretakers, repairmen, catch crews, vaccinators, etc). Second in priority would be egg pickup trucks and drivers. The third in priority would be deliveries of various sorts where activity is close to the buildings, i.e. feed trucks and drivers, gas delivery, etc. Appropriate samples will be taken from these flocks to determine if these flocks served as a source of the LPAI infection or spread from the index case occurred to that flock.

In all of the above actions, LBM supply flocks would be included in the plan. As concerns backyard flocks, an effort has not been made to my knowledge by PDA or any other agency to identify or collect information on backyard flocks. Backyard flock surveillance, if an outbreak occurs at this time, would need to be done in the zones as the occasion arises.

In summary, the prior collection of up-to-date contact information in regard to all flocks, commercial and LBM supply, is essential for a quick response to an LPAI outbreak. Efforts to gather information about the location and contact information for backyard flocks is needed to improve the control of an outbreak.

Business Continuity in the Face of an AI Outbreak

Will Hueston, Center for Animal Health and Food Safety, University of Minnesota, 1354 Eckles Avenue, St. Paul, MN 55108

Appendices

North Central Avian Disease Conference By-Laws

Revised September 2002

I. Membership

The North Central Avian Disease Conference shall include the geographic area composed of the following states: Illinois, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota and Wisconsin. Other states or provinces contiguous to the area may affiliate upon request. Any individual interested in poultry disease control and eradication, research, or diagnosis, and residing in the above named geographic region shall be considered eligible for membership. Membership will be maintained by attendance at the annual meeting or by request of the member. Voting privileges will be extended to those members who have attended at least two annual meetings in the past five years.

II. Guests

Host institutions shall have the privilege of inviting individuals from their organization to attend sessions when the meeting is held on their campus or facility.

III. Conference Officials and Committees

The executive committee shall be the governing body of this conference. The committee shall be composed of the chairperson, the secretary-treasurer, the chairperson-elect, two members-at-large, **the chair of the fund raising committee, the chair of the publications committee** and the past chairperson as an ex-officio member.

A. Terms of Office

The chairperson, chairperson-elect and two members-at-large shall be on a rotational term basis. The term of office for the chairperson and chairperson-elect shall be a period of two years. A new member of the executive committee shall enter as a member-at-large. New terms of office shall take place at the annual business meeting; wherein, the past chairperson will step down and the chairperson-elect will become the new chairperson. The member-at-large who is at the end of his/her second year (of a 2-year-term) will then become the new chairperson-elect and a new member-at-large will be elected annually. The new member-at-large shall be nominated annually to be the executive committee and approved by a majority vote of the membership at the annual business meeting. **Members-at-large are usually future meeting hosts.** The secretary-treasurer shall be nominated by the executive committee and approved by a majority vote of the membership at the annual business meeting.

B. Duties

1. Executive Committee

It shall be the duty of the executive committee to select the location of the annual meeting. This shall be for the meeting four years in advance when possible. The executive committee will select the location of the meeting and present it to the membership for approval by a majority vote at the annual business meeting. The executive committee will function as the program committee in preparing the annual meeting. The executive committee shall initiate and activate such functions as may be considered necessary during the interim between annual meetings. The executive committee shall meet at the North Central Avian Disease Conference prior to the annual business meeting.

2. Chairperson

The chairperson shall preside over the annual business meeting and perform the duties of such office. The chairperson will be responsible for arranging the time and place of the meetings and shall approve the program and arrange for publication of the proceedings. The chairperson may assign duties or appoint such committees as deemed necessary to conduct the affairs of this conference.

3. Secretary-Treasurer

The secretary-treasurer shall maintain the records and files of the North Central Avian Disease Conference. The secretary-treasurer shall maintain a list of the names and addresses of members in attendance. He shall keep minutes of the meetings and perform the usual duties of a secretary-treasurer and such other duties as assigned by the chairperson and/or the executive committee.

4. Standing Committees

Awards Committee: This committee will be composed of 3 members appointed by the executive committee. Membership will be for 3 years with a new member appointed annually. The longest serving member will serve as chair. The committee will select the recipients of the Service Award, the Ben Pomeroy Award, and any other awards approved by the membership.

Fund Raising Committee: The executive committee will appoint members of the committee annually. The committee will solicit funds to support the annual meeting, publications, awards, and other activities designated by the membership.

Publications Committee: The individual in charge of the publication of the proceeding of the annual meeting will serve as chair of this committee. One or two other members will be appointed by the executive committee, one of which will be in charge of the conference website.

Minutes

57th North Central Avian Disease Conference
March 20, 2006

Program Chairman Mick Fulton called the meeting to order at 2:12 pm. 2005 minutes were amended and approved. The low attendance concern was addressed by various members. Possible solutions discussed were: logistics of NCADC during the MPF, closer ties to the NEADC, advertising complete program w/symposium further in advance, more "take home" information, timing (WPDC), industry concern over total number of meetings attended, symposium questionnaire for membership.

2007 symposium committee is to consist of Drs. Wu, Fulton and Saif

2008 program chair is Dr. Richard Slemons
2009 program chair is Dr. Mahesh Kumar
2010 program chair is Dr. Dan Shaw

Need was expressed for NCADC to urge AAAP to take a major role in Avian Influenza Control education due to others' experiences and perceived urgency. Interested members drafted proposal to immediately establish a committee to recommend the best method of appropriate response, control and prevention of a High Path AI outbreak in commercial and noncommercial poultry, and exotic, captive and wild birds. This information should be disseminated to the membership and public in a timely manner no later than the fall of 2006. It should be done in conjunction with appropriate governmental agencies. 2007 Program Chairperson Ching Ching Wu sent attached letter to AAAO President Robert Owen.

Chairman Fulton thanked the membership, executive committee (Wu, Halvorson, Slemons, Kumar and Bryan), the fundraising committee (Lippert, Bryan and Saif),and the awards committee for their input for the 2006 program. Secretary/Treasurer position will remain the same until the 2007 meeting.

After 2006 NCADC expenses, there remained approximately $10,000 in the checking account.

Dr. Fulton introduced 2007 Program Chairperson Ching Ching Wu. Dr. Wu expressed thanks for Dr. Fulton's leadership and presented a plaque of recognition. 2007 symposium topic was briefly discussed followed by adjournment.

At the Awards dinner, Dr. Fulton presented the Ben S. Pomeroy Award to Dr. Hadi Yassine.

Dr. Mahesh Kumar was presented the 2006 Meritorious Service Award *in absentia*.

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ANIMAL DISEASE DIAGNOSTIC LABORATORY

March 31, 2006

Dr. Robert Owen
President
AAAP
953 College Station Road,
Athens, GA 30602-4875

Dear President Owen:

The membership of the 57th North Central Avian Disease Conference urgently and unanimously recommends that the American Association of Avian Pathologists (AAAP) Board of Directors proceed immediately to establish a committee to recommend methods of appropriate response, control and prevention of an high pathogenic Avian Influenza (high path AI) outbreak in commercial, non-commercial poultry and exotic, captive and wild birds. This information should be disseminated to the AAAP membership, appropriate governmental agencies and the public in a timely manner, no later than the fall of 2006.

We appreciate your timely attention on this matter. We believe this is an urgent matter and requires immediate plan and actions from AAAP.

Sincerely,

Ching Ching Wu, DVM, PhD,
Chair,
North Central Avian Disease Conference

406 S. University Street ※ West Lafayette, IN 47907-2065 ※ (765) 494-7440 ※ Fax: (765) 494-9181

Minutes

56th NCADC Business Meeting
March 15, 2005

The 56th NCADC business meeting was called to order by program cochair, Dr. David Halvorson at 3:15pm with 29 members present. Dr. Halvorson called for review of the 2004 minutes and approval. Minutes were approved. Thanks were expressed to program committee, fundraising committee (Lippert, Mills, Bryan) and awards committee (Fulton, Pedersen, Wu), Jan Swanson and Sarah Sommerbelle (University of Minnesota Continuing Education). Executive committee proposal was read as follows: "NCADC extends appreciation to Midwest Poultry Federation for sharing meeting rooms with NCADC at no cost to NCADC. It is proposed that NCADC hold its meetings in coordination with MPF and that NCADC will be willing to share its speakers or members with MPF in educational workshops. NCADC recognizes that the timing of the meeting may present weather unknowns, necessitating air travel for some graduate students presenting papers; therefore, additional revenues will be sought as well as reducing graduate student registration costs 50% without presentation and to no registration cost with presentation." Proposal was seconded by Dr. Kumar. Discussion followed. Proposal passed. Dr. YM Saif agreed to assist fundraising committee on behalf of graduate student travel support.

2007 program chair is Dr. Ching Ching Wu
2008 program chair is Dr. Richard Slemons
2009 program chair is Dr. Mahesh Kumar

2006 Sec/Treasurer election went to Dr. Tom Bryan. Dr. Halvorson introduced 2006 program chair, Dr. Mick Fulton. Dr. Fulton thanked Drs. Halvorson and Njenga (in absentia) for increasing the NCADC attendance with fine symposium and program. Dr. Fulton reminded attendees of the 2006 symposium topic of vaccines and vaccine delivery systems to be held in coordination with the MPF, March 20-21, 2006. Announcement was made of the Poultry Health School at Michigan State University May 9-11, 2005. 2006 Executive Committee consists of Drs. Fulton, Wu, Halvorson, Bryan, two at-large members (Dr. Slemons and Dr. Kumar), fundraising chair, and publications chair. Fundraising committee consists of Drs. Lippert, Mills, Saif and Bryan. Awards committee will be appointed. Dr. Fulton adjourned the meeting at 4pm.

**At the Awards Luncheon, Dr. Nagaraja presented the Ben S Pomeroy Awards to Dr. Binu Velayudham, UMN, "Studies on a recent isolate of avian metapneumovirus type C in Minnesota", and Ms. Pam Olah, UMN, "Analysis of Salmonella sp and Campylobacter sp isolated from freshly processed turkeys". Dr. Ron Lippert was presented the Meritorious Service Award.*

**Amendments to minutes*

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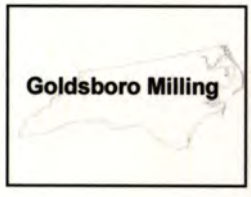

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Proceedings

58th North Central Avian Disease Conference and Symposium on Lessons Learned from AI Preparation

58th North Central Avian Disease Conference
and
Symposium on
"Lessons Learned from AI Preparation"

March 11-13, 2007

Sunday, March 11 **St. Paul River Centre, Room 4**

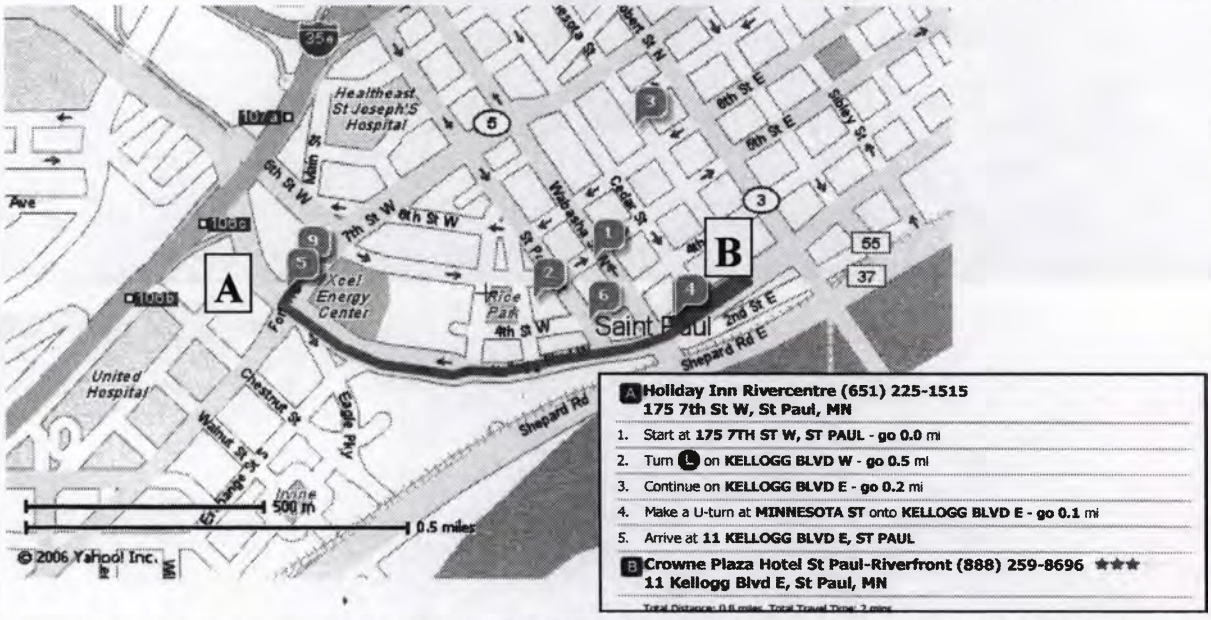
1-2 pm	On-site Registration
2-5:40 pm	Scientific presentations
6-8 pm	**Welcome Dinner at St. Paul River Centre, Room 5**
	— Sponsored by Maple Leaf Farms

Monday, March 12 **St. Paul River Centre, Rooms 4 and 5**

7-8 am	Continental Breakfast	
8-11:40 am	Scientific Presentations	
1-4 pm	Symposium	
4:25 pm	Y. M. Saif	NCADC Disease Round Table
5:00 pm	Ching Ching Wu	NCADC Business Meeting
6-9 pm	**Award Dinner at Crown Plaza Hotel, Kellogg Room**	
	— Sponsored by Alltech	

Tuesday, March 13 **St. Paul River Centre, Rooms 4 and 5**

7-7:30 am	Continental breakfast
7:30-11 am	Symposium, continued



Scientific Presentations

Sunday, March 11, 2007 ***St. Paul River Centre, Room 4***

Moderators: Mick Fulton, Tom Bryan

2:00	L. Long	Avian Influenza Virus Surveillance in Ohio Zoological Institutions
2:20	R. Slemons	Temporal and Spatial Comparisons of the AI Virus Combinations Recovered from Ducks Sampled at nearby Locations in different states over 2 years
2:40	J. Nolting	Phenotypic variations in H1N1 low pathogenic avian influenza viruses over time and by location
3:00	H. Yassine	Interspecies Transmission of H3N2 Influenza A viruses between Swine and Turkeys
3:20	M.K. Hsieh	A prime-boost approach to enhance DNA vaccination-mediated protection against infectious bursal disease
3:40-4:00	**BREAK**	
4:00	D. Karunakaran	New findings in turkey cellulitis
4:20	M. C. Kumar	Prevention of turkey cellulitis
4:40	A. Thachil	Development of a turkey Clostridium cellulitis inactivated vaccine
5:00	R.M. Hulet	Effect of NuPro® and Growth Performance of Turkey Hens Grown to Market Age
5:20	A.E. Sefton	Comparison of Bio-Mos® and Flavomycin® as Growth Promoters
5:40	R. Porter	Production losses associated with ionophore toxicosis in white leghorn pullets

Scientific Presentations, continued

Monday, March 12, 2007 ***St. Paul River Centre, Rooms 4 and 5***

Moderators: Rob Porter, Tsang Long Lin

8:00a	D. Lauer	The Minnesota Avian Pneumovirus Eradication Project
8:20	S. Noll	Avian Pneumovirus Vaccine Evaluation Project
8:40	B. Velayudhan	The attachment glycoprotein gene variation among avian metapneumovirus subtype C strains isolated from 1996-2003 in the United States
9:00	Y.N. Chen	Detection and quantitation of turkey coronavirus in turkey tissues by a specific real-time reverse transcription-polymerase chain reaction
9:20	Irene Lesley	Prevalence of *Campylobacter* and *Salmonella* in the Turkey Brooder House
9:40-10:00	**BREAK**	
10:00	C. Gustafson	Efficacy Comparison of Three Commercial MG Vaccines
10:20	T.M. Huang	A Three-Year analysis of antibacterial susceptibility and resistance in chicken *Escherichia coli*, *Salmonella* spp. and *Pasteurella multocida* isolates
10:40	A. Yiannikouris	Counteracting mycotoxin contamination: the effectiveness of *Saccharomyces cerevisiae* cell wall glucans for sequestering mycotoxins
11:00	R. Hauck	The Effect of Natustat® for Prevention of Histomoniasis in Turkeys
11:20	S. Taylor/C. Schmelik	Poultry Vaccines Labeling and Adverse Events

Symposium

Monday, March 12 ***St. Paul River Centre, Rooms 4 and 5***

Moderators: K.V. Nagavaja, Susan Williams

1:00 pm	Welcome	Y. M. Saif
1:05 pm	Dennis Senne	Avian Influenza and Lessons Learned on AI Diagnosis
1:35 pm	Andy Rhorer	NPIP Program, State Preparedness and Containment of Low Path AI
2:05 pm	Randall Levings	High Path AI Responses and Update of Veterinary Stockpile
2:35 pm	BREAK	
2:45 pm	Chang-Won Lee	AI Vaccine—A New Approach
3:15 pm	Dave Halvorson	Lessons Learned on AI Prevention and Control
3:45 pm	T.J. Meyers	The Use of Vaccines and Biologics in Controlling AI and the Concept of Compartmentalization and Regionalization
4:15 pm	BREAK	(Symposium will continue Tuesday morning)

4:25pm	Y. M. Saif	NCADC Disease Roundtable
5:00pm	Ching Ching Wu	NCADC Business meeting

6:00pm Award Dinner at Crown Plaza Hotel *Sponsored by Alltech*

Symposium, *continued*

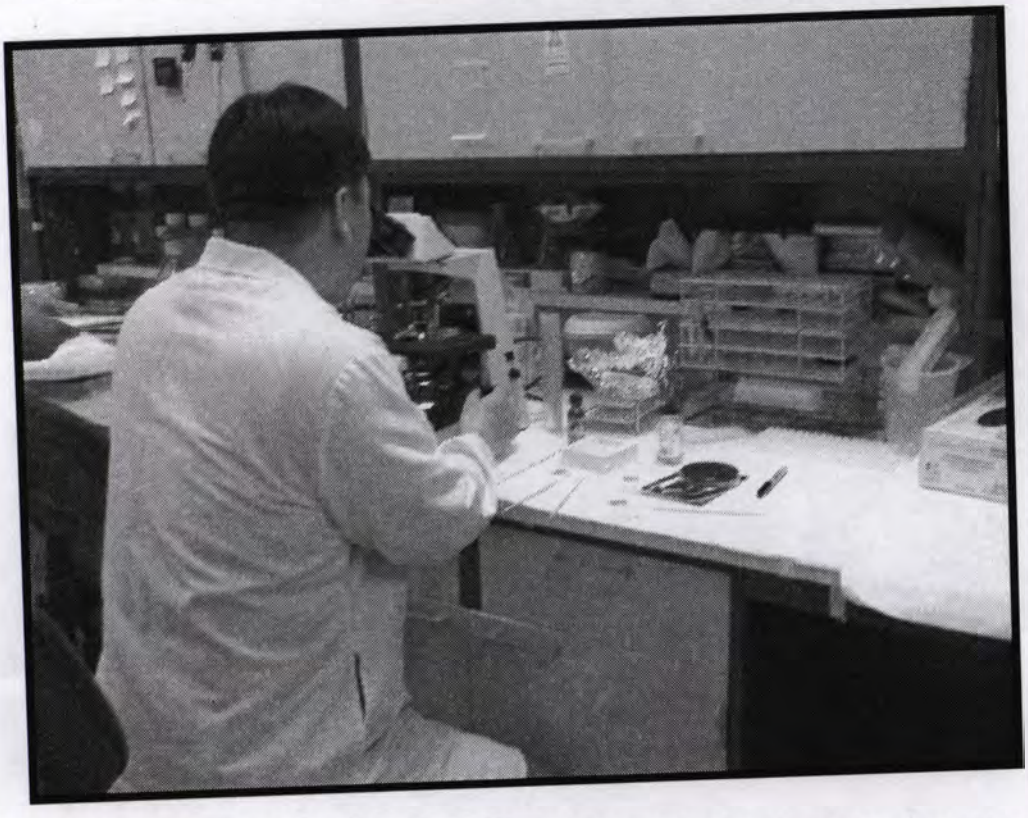
Tuesday, March 13

Moderators: Dave Halvorson, Jose Linares

7:30 am	Alice Black	Dealing with Media When You Are In Charge of Disease Issues
8:00 am	Jose Linares	AI Preparedness—the Texas Experience
8:30 am	Susan Williams	Actions and Lessons Learned in Preparing the Broiler and Broiler Breeder Industry for AI
9:00 am	BREAK	
9:15 am	Mick Fulton	The North Central Experience
9:45 am	Eric Gingerich	Pennsylvania Experience: LBM and Backyard Birds
10:15 am	Will Hueston	Business Continuity in the face of an AI Outbreak
10:45 am	Richard Slemons	Conclusion
11:00 am		**NCADC Conference ends and Midwest Poultry Consortium Research Summit begins**

Scientific

Presentations



Avian Influenza Virus Surveillance in Ohio Zoological Institutions

Lindsey J. Long, Patricia H. Dennis, Jacqueline M. Nolting, Dennis A. Senne, William Saville and Richard D. Slemons, Department of Veterinary Preventive Medicine, the Ohio State University 1920 Coffey Road, Columbus, OH 43210

Avian collections in zoological institutions accredited by the Association of Zoos and Aquariums (AZA) serve as unique sentinels for emerging infectious diseases in North America. Found in urban and rural settings across the United States, AZA institutions quarantine all animals entering their collections, house individually identified animals which receive regular health examinations, and veterinarians perform complete necropsies on collection animals that die. During 2005, AZA avian collections in Ohio were incorporated into our ongoing avian influenza virus (AIV) surveillance efforts. The rationale was that the outdoor environment of the zoo is often utilized by both zoo birds and wild bird populations from late spring through early fall and the zoo collections include exotic avian species bringing additional host factors into play. Cloacal swabs and blood samples were taken from birds in one avian zoo collection as they were moved to their outdoor pens in the spring. During late summer, environmental samples were collected on the grounds of five AZA institutions. Then in the fall, blood samples and cloacal swabs were collected from birds in the avian collections at two zoos as the birds were returned to their indoor winter quarters. No AIVs were recovered from the spring cloacal swabs, four type A influenza isolates were recovered from 129 environmental samples collected at two institutions, and results on fall cloacal swabs are pending. To date, no antibodies have been detected to the type specific antigen of AIVs. The 3.1% recovery rate from environmental samples indicates further surveillance efforts are warranted in following the dynamics of AIV introductions into zoo environments. By initiating AIV surveillance measures, these institutions have taken a proactive stance in protecting the health of their avian collections and possibly even visitors to their respective zoos.

Temporal and Spatial Comparisons of the Avian Influenza Virus NA-NA Combination Recovered from Ducks Sampled at Nearby Locations and in Different States over Two Years

Richard D. Slemons, Jacqueline M. Nolting, Vernon D. Stotts, H. Lloyd Alexander, Roy Kroll, Mark Shieldcastle and **Dennis A. Senne** Department of Veterinary Preventive Medicine, 1920 Coffey Road, Columbus, OH 43210

To date, low pathogenic type A influenza viruses circulating in wild birds have no documented impact on wild bird populations around the world. However, accumulating evidence implicates these viruses as being the ancestral origins of type A influenza viruses appearing, and causing disease, in domestic birds, lower mammals and humans. As a result, recommendations from numerous type A influenza virus symposia have identified a need to further define the natural history of type A influenza viruses in wild birds. Unfortunately, progress in this area has been modest due to limited funding and lack of systematic, coordinated efforts. After 35 years, it remains unclear how the antigenic and genetic diversity of these viruses is maintained in nature. In 2005-2006, two pairs of study sites were established. Each pair of study sites was located in a different state and the two sites in each pair were located approximately 18 miles from each other. Resident, wild mallards were the primary population at one set of sites and migratory, dabbling ducks were the primary populations at the other paired sites. Virus isolation attempts in embryonating-chicken eggs were conducted on either cloacal swabs or very fresh fecal samples and sequential sampling was conducted at each site over 2-3 months each year. The frequency of AIV recovery and distribution of NA-NA combinations varied markedly 1) over a few weeks at the same site, 2) during the same two month period at the paired sites, 3) during the same months in different states and 4) between years within a state. Merging surveillance and monitoring data by year and larger geographic regions provides critical, user friendly information on type A influenza viruses circulating in wild birds but, at the same time, data merging can result in the dynamic nature of type A influenza virus circulation in wild birds being overlooked. This dynamic must be taken into consideration when drawing conclusions from avian influenza virus surveillance efforts in wild birds.

Phenotypic variations in H1N1 low pathogenic avian influenza viruses over time and by location

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H1N1 type A influenza virus can be found in wild and domestic birds, swine and humans, which presents the possibility that individual genomic sequences from these viruses could move between the avian and mammalian hosts and this movement, if it occurs, could go undetected. Differences in replicative properties among waterfowl-origin H1N1 influenza viruses in embryonating chicken eggs might serve as an indicator for variations in the genomic constellations of the viruses and possibly identify isolates that are better suited to cross the species barrier from wildfowl to domestic poultry. It is hypothesized that growth rates of H1N1 water-fowl type A influenza viruses will vary in embryonating chicken egg culture. For initial screening, 5 first-egg passage H1N1 type A influenza virus stocks from our repository were selected for examination. They represented isolates from different years and geographic origins. A limiting dilution was performed on each of the isolates in ten-day-old embryonating chicken eggs and EID_{50} calculations were completed using the Reed and Muench method to determine the concentration of infectious virus in each of the respective first passage isolates. The EID_{50}/ml for the isolates ranged from $1x10^{3.67}$ EID_{50}/ml to $1x10^{7.22}$ EID_{50}/ml. There are several possible explanations for these different titers including, but not limited to, inhibitors and incomplete virus particles in original samples and first egg passage fluids, varying virus concentration in original samples and first egg passage fluids, varying virus concentration in original samples and different replicative properties among the viruses. Further investigation is underway to determine if there are real differences in the replicative properties of these isolates in embryonating chicken eggs.

Interspecies Transmission of H3N2 Influenza A viruses Between Swine and Turkeys

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Interspecies transmission of influenza A viruses is raising concerns for its potential threat to human and animal populations. Influenza A viruses belong to the family *Orthomyxoviradae* and are composed of segmented RNA genomes. They are usually host specific, depending mainly on the compatibility between the hemagglutinin (HA) protein and its corresponding receptor on the host cell. Pigs are considered as mixing vessels due to their susceptibility to infection by human and avian viruses, resulting in generation of new reassortant viruses.

Classical H1N1 lineage of influenza A viruses were the dominant cause of influenza in pigs for more than six decades in the United States (US). In 1998, H3N2 viruses emerged and rapidly spread through the swine population in the US. These viruses were double and triple reassortants, of which the latter became endemic in the swine population. Between 2003 and 2004, we isolated and characterized two H3N2 influenza A viruses from turkey breeder hens from Ohio and Illinois. The infected turkeys showed no clinical signs, but underwent complete cessation of egg production. Genetic analysis of these viruses revealed that they are triple reassortant viruses similar to those currently circulating in the swine population in the US. These findings prompted us to initiate a study on the interspecies transmission of these viruses between swine and turkeys.

Three viruses of turkey origin (TK/IL/04, TK/OH/04, TK/NC/03) and one virus of swine origin (SW/NC/03) vaccine strain) were tested for their transmissibility between swine and turkeys. All viruses were shown to replicate in both species (using real-time PCR on swab samples and HI-test on blood serum collected at day 15 post infection); however, only the Ohio strain was shown to transmit both ways between the two animal species that also showed seroconversion upon infection. The Illinois strain was shown previously to be more than 99% similar to the Ohio strain genetically and antigenically, however, it behaved differently in the transmission experiments. This indicates that minor changes in influenza viruses might totally change the behavior (replication, pathogenicity, etc.) of these viruses.

A Prime-boost approach to enhance DNA vaccination-mediated protection against infectious bursal disease

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Multiple intramuscular injections with a large dose of DNA carrying the large segment gene of infectious bursal disease virus (IBDV) have been shown to provide effective protection of chickens against infectious bursal disease (IBD). The purpose of the present study was to determine if priming with DNA carrying the large segment gene of IBDV and boosting with killed IBD vaccine could adequately confer protection of chickens against IBD. One-day-old specific pathogen free (SPF) chickens were intramuscularly injected with DNA plasmid coding for the large segment gene of IBDV strain variant E (VE) (P/VP243/E) followed by intramuscular injection of killed IBD vaccine containing both standard and variant IBDV at 1 or 2 weeks of age. Chickens were orally challenged with IBDV strain VE or standard challenge strain (STC) at 3 weeks of age and observed for 10 days. Bursal lesion scores, bursa weight/ body weight (B/B ratios), protection efficacy, IBDV antigen in bursae, enzyme-linked immunosorbent assay (ELISA) titers to IBDV, and virus neutralization (VN) titers to IBDV were determined. Concanavalin (Con) A-induced spleen or blood lymphocyte proliferation responses were performed at 14, 21, 24 and 31 days after priming with P/VP243/E. Chickens primed with 50, 100, 200, or 400 μg of P/VP243/E at 1 day of age and boosted with 0.5 ml of killed IBD vaccine at 1 or 2 weeks of age had 80 to 100% protection against challenge by IBDV strain VE or 71 to 100% protection against challenge by IBDV strain STC. Chickens in the groups primed with P/VP243/E and boosted with killed vaccine had significantly higher ($P<0.05$) B/B ratios and significantly lower ($P<0.05$) bursal lesion scores than chickens in the challenge control (CC) groups and groups primed with saline or vector plasmid and boosted with killed IBD vaccine or only primed with P/VP243/E. No IBDV antigen was detected by immunofluorescent antibody assay (IFA) in bursae of chickens protected by priming with DNA vaccine and boosting with killed vaccine. Prior to challenge, chickens (21 days of age) in the groups rimed with P/VP243/E and boosted with killed IBD vaccine had significantly higher ($P<0.05$) ELISA and VN titers to IBDV and stimulation indices to ConA-induced spleen or blood lymphocyte proliferation than chickens in the CC groups or groups primed with vector plasmid or saline and boosted with killed IBD vaccine or only primed with P/VP243/E. In conclusion, SPF chickens at 1 day of age primed with IBDV large segment gene expressing DNA and boosted with killed IBD vaccine at either 1 or 2 weeks of age can be adequately protected against challenge by homologous variant or heterologous classical IBDV.

New Findings in Turkey Cellulitis

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Clostridial cellulitis has emerged recently as a significant concern for the turkey industry in the United States. Characterized by necrosis of the skin, subcutaneous tissue, and often the underlying musculature, the disease occurs suddenly and progresses rapidly with death occurring often before the first symptoms are observed.

Recent research findings with regard to the agents involved will be presented. Intervention strategy for prevention and control will be discussed.

Prevention of Turkey Cellulitis

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Turkey cellulitis is a disease of turkeys characterized by swelling of tails, breasts and legs and it causes excess mortality in both toms and hens. There is a large amount of fluid under the skin. On necropsy, straw colored to blood-tinged fluid is observed. Birds start experiencing this disease around 14 to 15 weeks of age in toms and as young as 9 weeks of age in hens. Excess mortality runs at 1-2% per week in toms and 1/2% per week in hens until market. This excess mortality occurs despite being on treatment.

Etiology: The causative organism was isolated and identified by Carr (M.S. Thesis 1996) as *Clostridium perfringens* type A. It was also shown that this condition was reproduced by injecting Alpha Toxin under the skin. She also reproduced the cellulitis in turkeys by giving this organism by various routes.

Treatment: The birds respond to treatment with penicillin at 1,000,000 units per gallon of water. This is followed by chlortetracycline in the feed until market.

Control measures: The disease keeps on occurring in successive flocks despite various efforts made to control and prevent it from recurring. Control measures tried consist of complete clean out and disinfection of the buildings followed by bedding with fresh shavings. Various litter treatments have been tried without success. It sometimes reduces the severity of the subsequent outbreaks, but has not prevented the disease from recurring. Growth promotants were switched to prevent and control this disease with no success. However, one preventive measure was tried which consisted of cleaning out and disinfecting of buildings in between placement of flocks and providing feed treated with Termin-8® at 2lbs/ton of feed. The flocks given the treated feed did not break with cellulitis. Data will be presented at the meeting as to timing of using this feed treatment for complete prevention of this expensive disease.

Development of a Turkey Clostridium Cellulitis Inactivated Vaccine

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Cellulitis has emerged as a major problem in the turkey industry over the last few years. It is one of the foremost causes of condemnation due to infectious disease contributing to severe economic loss. Cellulitis lesions in turkeys occur on the breast and ventral abdomen with apparent absence of any external wound. The infectious agents consistently isolated are *Clostridium perfringens* and *Clostridium septicum.* The objective of our study was to develop a formalin-inactivated vaccine against Clostridium cellulitis in turkeys. We selected isolates from cellulitis in field turkeys. The standardized formalin-inactivated vaccine preparations were evaluated experimentally for safety and efficacy in 6 and 8-week-old turkeys. The results of this experiment will be presented.

Effect of NuPro® and Growth Performance of Turkey Hens Grown to Market Age

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Three studies were conducted to determine the effectiveness of NuPro® (NP) (Alltech) for improving the growth performance of turkey hens taken to either 84 or 95 days of age. NuPro® is a yeast extract derived from a select strain of yeast, grown under consistent and carefully controlled conditions. It provides an excellent source of protein, amino acids, nucleotides and important vitamins. Five hundred and twenty-eight female poults were randomized into 4 pens with 132 hens per pen for 1 week; At 1 week, they were spread into 12 pens (5.4 birds/m^2).

In study 1 and 2, hens were fed a 28% pre-starter (PS) diet for 4 wks, a 26% starter (S) for 2 wks, a 23% grower (G) for 4 wks, and an 18% finisher (F) until market age. There were 3 treatments fed in Study 1; NP at 4% in the PS followed by 2% (S), NP at 3% in both the PS and S diets, and a meat meal CN. In Study 2, the NP diet was fed at 4% PS and 2% S and compared to both a meat meal and a vegetable CN. For Study 3, hens fed NP at 4% for the first 11 days (28% CP) and then NP at 2% until 27 days of age (28% CO) was compared to Penicillin supplemented control diets from 1 to 27 days (28% CP). After 27 days, all hens were placed on the same diets for grower diets (28 and 24%) and a finisher diet (18%) CP). BW and feed consumption (FI) were measured at hatch, feed changes, and market age for all experiments.

Hens fed the NP diets had greater BW ($P < 0.05$) at 84 d when compared to the CN diet (Study 1) and at 95 d (Study 2, $P < 0.09$). Although the NP-fed hens had consistently less mortality, no significant difference in mortality or feed conversion was found in either study. The greatest effect appeared to be an increase in FI for those hens fed the NP treatments, resulting in increased growth. When compared to Penicillin feed hens in Study 3, NP supplemented hens were not significantly different in body weight, feed conversion or mortality at 84 days of age.

In summary, the addition of NP into the PS and S diets appeared to positively affect the growth of hen turkeys grown to 84 or 95 days of age when compared to commercial diets.

Comparison of Bio-Mos® and Flavomycin® as Growth Promoters

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Antibiotic growth promoters (AGPs) have been a standard inclusion in meat bird rations since the 1940s. Recent events have seen a decrease in the number of antibiotics that can be used as growth promoters and it appears unlikely that new replacements will be forthcoming. One of the reasons for the move away from AGPs is that antibiotic resistant genes increase in prevalence during their use. These genes may be specific for the antibiotic in use or more general in nature. To maintain their effectiveness, it has been found best to rotate or shuttle AGPs. Bio-Mos® (Alltech Inc.), a source of yeast cell wall mannans, has been found to inhibit conjugation *in vitro*, decreasing the spread of antibiotic resistance transfer among *E. coli*, thus providing a strategy for the control or treatment of multi-drug resistance. Both broilers and turkeys fed Bio-Mos® have been shown to produce comparable body weight, FCR, and improved livability, when compared to birds fed rations containing AGPs. Specific data will be presented that shows this is the case for Bio-Mos® vs. Flavomycin®. Thus, Bio-Mos® is a useful substitute for Flavomycin® in rotational and shuttle growth promoter programs; it gives comparable growth promotion and decreases the spread of antibiotic resistant genes. The latter will increase the effectiveness of the AGP complement of the shuttle program.

Production losses associated with ionophore toxicosis in white leghorn pullets

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Coccidiosis was diagnosed in a flock of 4-week-old white leghorn pullets raised in three-tier, drop-through cages with nipple drinkers. At five weeks, shortly after delivery of feed containing an ionophore, many of the birds were depressed, laterally recumbent and unable to walk when manually positioned upright. Many of the affected pullets were thought to be dead, lying motionless in the cage and flattened laterally by the weight of other cagemates but, when examined, were actually alive. Pullets were recumbent but, when removed from cages and separated from other birds, were often alert and walking in several hours or less. At this time a preliminary diagnosis of ionophore toxicosis was made and 20 tons of replacement feed were delivered to the farm, but mortality continued for four days. Necropsy of affected birds revealed bruising on the ventral aspect of the wings and mild subcutaneous edema over the thigh and tibiotarsus. There was multifocal intramuscular hemorrhage of varying degrees in the caudal aspect of the thigh, digital flexor muscles of the tibiotarsus, and lumbosacral muscle (gluteal muscles). Histologic examination of affected muscles revealed attenuation, hyalinization and myocytolysis of muscle fibers with loss of cross-striations, varying infiltrates of mononuclear cells, mostly macrophages, and proliferation of satellite and fibroblast nuclei. A pre-mix error resulted in pullets consuming ionophore at five times the recommended concentration of 110 grams/ton. When replacement feed was delivered to the farm, the new feed was not mixed with the remaining 10,000 pounds of toxic feed, and pullets continued to consume toxic levels of ionophore for four days. Total mortality directly resulting from ionophore toxicosis was 3.9% and an additional 2.2% of the flock was culled over the next 13 weeks for lack of uniformity and poor growth. Persistent problems in the flock continued through lay and included cloacal prolapse comprising a significant portion of daily mortality. Additionally, egg size and case weights from this flock were below average, resulting in substantial loss in value. The persistent problems in the flock were attributed to long term effects of the toxicosis that had occurred months earlier.

The Minnesota Avian Pneumovirus Eradication Project

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Introduction: Avian Pneumovirus (APV), an infectious respiratory disease of turkeys, has been a disease problem for the Minnesota turkey industry since the virus was identified in 1998. That year, a processing plant serological surveillance program was set up in Minnesota to identify positive APV turkey flocks using samples collected for Avian Influenza surveillance. The percentage of positive flocks/lots has ranged from 13% (March, 1999) to 66% (August 2003), but most months the percentage has been 40-60% positive.

Beginning on September 1, 2002, the Coordinated Area Control and Eradication Field Demonstration Project, known as the Morgan Area Project, was an attempt to use vaccination as an APV eradication tool in a geographic area. The project ran for one year and was a cooperative effort between the Minnesota Turkey Growers Association (MTGA), the University of Minnesota, the Board of Animal Health and the United States Department of Agriculture (USDA). The Avian Pneumovirus Eradication Project was designed to follow-up where the Morgan Area Project left off.

Avian Pneumovirus Eradication Project Objectives

- Can the modified-live vaccine be evaluated in controlled pen studies?
- Will turkey growers cooperate in a coordinated APV vaccination project?
- Can a coordinated vaccination program reduce APV clinical disease?
- Can risk factors for the initial introduction of APV be identified?
- Can a coordinated vaccination program reduce the economic impact of APV?
- Can a coordinated vaccination program serve as an APV eradication tool?

APV Eradication Project Components

Minnesota's Avian Pneumovirus Eradication Project was funded by the USDA (September 1, 2004-December 31, 2006) and directed by the Minnesota Board of Animal Health in cooperation with the University of Minnesota and the MTGA. The project consisted of three components: evaluating the APV vaccine in controlled pen studies, designing and implementing a field APV project and analyzing turkey health and production data to determine if a coordinated vaccination strategy is possible and successful.

1) **Vaccine Evaluation**

Results are reported at this conference in a separate presentation

2) Field Project Implementation

Within Minnesota, geographic regions or areas were identified (Morgan and Faribault areas) with similar turkey density, topographic features, and clinical disease caused by APV. Coordinated vaccination and comparison zones were identified in each area.. Both of these areas have experienced clinical APV—the Morgan area in 2000 and the Faribault area in 2003. A total of 400 flocks were identified as potential candidate flocks.

Coordinated Vaccination Field Project Analysis

The field project analysis evaluated the impact of coordinated vaccination strategies for the eradication of APV. Data was collected on the underlying biosecurity features of the participating premises as well as a series of production and processing measurements, including mortality (3-12 week), clinical respiratory disease, APV associated respiratory disease, growth rates, condemnation at processing, liveweight yield and economic data. The statistical analyses adjusted for differences associated with the gender of the flock (hens vs. toms), season of growout, age at processing, and plant where processed to control for potential confounding. Overall, data was collected from 380 flocks on 68 premises.

A) **Biosecurity Audits:** The participating premises face a number of potential threats from exposure to wild birds. Most of the participating premises are within three miles of a body of water. The presence of bodies of water on the premises was associated with significantly higher early mortality. Additionally, close proximity to other poultry farms was significantly associated with higher condemnation. Sharing equipment was a significant risk factor, negatively affecting a number of production measures with higher condemnation and lower liveweight yield for both toms and hens, and higher clinical respiratory disease in hens. Stockpiling manure was significantly associated with higher early mortality. One of the greatest risks for spreading disease tends to be contamination introduced by people and equipment. Opportunities to improve biosecurity include adding signs and actions restricting access, reducing visitors and increasing biosecurity practices required of visitors.

B) **Evaluation of coordinated vaccination zone effectiveness:** During the study period, tom flocks raised in the coordinated vaccination zone were significantly less likely to have high condemnations at processing and more likely to have high early mortality. Hen flocks raised in the coordinated vaccination zone had significantly higher condemnation, lower growth rate, lower liveweight yield, and higher earlier mortality. In general, vaccination did not improve production measures although the effectiveness of vaccination depended on the frequency, with two or three vaccinations achieving better performance than a single vaccination.

C) **Cautions for interpreting this field trial:** In an ideal situation, the coordinated vaccination strategy would be evaluated in a region of sustained APV field virus challenge. Producer reports and the serology surveillance demonstrated an apparent lack of APV field virus pressure during the year-long study. Evaluation of the effectiveness of coordinated vaccination was further complicated by the fact that a number of flocks in the comparison zone were also vaccinated, although not all followed the coordinated vaccination protocol. Finally, no hen flocks were produced in the Faribault coordinated vaccination zone, so the effectiveness of coordinated vaccination for hen flocks could only be evaluated for the Morgan area. All of these observations limited the full evaluation of the effectiveness of coordinated vaccination zone strategy in the face of a large scale APV outbreak.

Conclusions

- The USDA-funded coordinated vaccination field project was successfully designed and implemented. Independent growers and turkey integrators alike demonstrated a willingness to collaborate on a coordinated vaccination strategy in the designated geographic areas.
- Biosecurity audits provided participating growers valuable observations when comparing their turkey operations to others in the area
- While production statistics do not support sustained use of APV vaccine in the absence of field virus pressure, aggressive coordinated vaccination of all flocks in the geographic vicinity of a clinical APV outbreak appears to be a useful control and eradication tool.
- A successful APV eradication program is multi-faceted. An overall eradication strategy requires intervention measures that include, but are not limited to, breeder vaccination programs, biosecurity plans, and individual commercial premises eradication programs that may or may not include APV vaccination.

References

Lauer D.C., 2005 North Central Avian Disease Conference

Avian Pneumovirus Vaccine Evaluation Project

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Two experiments were conducted to evaluate the APV field vaccination program and other vaccination administration methods on post vaccination detection of vaccine virus, post vaccination transmission, post vaccination serology, post challenge virus shedding, and post challenge transmission, post challenge serology and post challenge clinical signs in a controlled setting. The vaccine used was Pneumomune® (Biomune). In each study there were two control groups (non-vaccinated and those vaccinated via eye-drop administration). The vaccine was also adminsitered by the following methods; low volume sprayer, high volume sprayer and spray cabinet. The vaccine was administered at 7 and 35 days of age for all groups with the exception of the spray cabinet where the vaccine was administered at 1 day of age followed by a second vaccination via sprayer at 35 days of age.

In each study, commercial hen turkey poults were obtained from a commercial hatchery from hatching eggs originating from APV naïve breeders and brooded in pens containing 400 poults each. Blood samples were taken at 2-wk intervals to market age. Swabs for PCR testing were taken periodically. Challenge studies were conducted three weeks after the first and second vaccinations of APV. A sample of poults were removed from the pens and moved to isolation facilities and challenged with a turbinate suspension prepared from 2-wk-old turkeys inoculated with a Minnesota isolate of aMPV (aMPV/Minnesota/Turkey/19/2003). In all challenge studies, unvaccinated controls developed clinical signs and eyedrop-vaccinated poults did not develop clinical signs to any significant degree. Based on clinical signs, only poults vaccinated via the spray cabinet showed protection against challenge equivalent to the eyedrop-vaccinated group when challenged at 4 weeks of age. However, all other vaccinated groups were protected regardless of vaccination method following the second vaccination at 5 weeks of age and challenged at 8 weeks of age. Titer increased in vaccinated (non-challenged) turkeys in both studies.

In summary, vaccine efficacy was dependent on method of administration. Protection after the first vaccination was obtained when administered via eyedrop and spray cabinet. Sprayer application required two vaccinations to provide protection. Titers in vaccinated turkeys that were not challenged increased with flock age suggesting cycling of the vaccine virus.

The attachment glycoprotein gene variation among avian metapneumovirus subtype C strains isolated from 1996 to 2003 in the United States.

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The objective of the present study was to examine the glycoprotein (G) gene of avian Metapneumovirus subtype C (aMPV-C) isolated from domestic turkeys from the United States for any variation in the size with respect to the year of isolation and level of passage in cell cultures. The virus was first detected in 1996 in the United States and continues to cause upper respiratory tract infection in turkeys. Twenty-one domestic turkey isolates of aMPV-C at different cell culture passages were examined by RT-PCR and gene sequencing. Results showed that there is extensive variation in the size of G gene with respect to the year of isolation and cell culture passage. The early isolates had a G gene size of 1978 nucleotides (nt) that coded for a predicted protein size of 585 aa and showed >97% nt similarity with that of aMPV-C isolated from Canada geese. The large G gene got truncated upon serial passage in cell cultures by a deletion of a 1015 nt long segment from the end of the G open reading frame. The recent isolates lacked the large G gene but instead had a small G gene of 782 nt in size, irrespective of the level of cell culture passages. Serial passages in cell culture, as well as natural passage in turkeys, led to truncation of the G gene. Some of the cell culture passages showed both large and small G gene products, which may indicate the existence of a mixed population of the virus. The present study warrants further investigation on the possibility of wild bird aMPV-C acting as a progenitor of turkey viruses, and also on the underlying mechanism of extensive variations in the size of G gene

Detection and quantitation of turkey coronavirus in turkey tissues by a specific real-time reverse transcription-polymerase chain reaction

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Outbreak of turkey coronavirus (TCoV) infection causes acute enteritis in turkey poults, leading to significant economic loss in the U.S. turkey industry. Rapid detection, differentiation, and quantitation of TCoV is critical to the diagnosis and control of the disease. A one-step real-time reverse transcription polymerase chain reaction (RT-PCR) assay for detection and quantitation of TCoV in turkey tissues was developed using a dual-labeled fluorescent probe. The fluorogenic probe labeled with a reporter dye (FAM, 6-carboxytetramethylrhodamin) and a quencher dye (AbsoluteQuencher™) was designed to bind to a 186 base-pair fragment flanked by the two PCR primers targeting 3' end spike gene of TCoV. The assay was performed on different avian viruses and bacteria to determine the specificity as well as the serial dilutions of TCoV for the sensitivity. Three animal trials were conducted to further validate the assay. Ten-day-old turkey poults were orally inoculated with 100 EID_{50} of TCoV Tissues (thymus, trachea, liver, kidney, spleen, duodenum, jejunum, ileum, cecum, colon, bursa, or cloaca), feces from cloacal swab, and/or feces from floor were collected at 12 hours, 1,2,3,5,7, and/or 14 days post inoculation (DPI). RNA was extracted from the samples and subjected to the real-time PCR assay, The designed primers and probe were specific for TCoV and other non-TCoV viruses and bacteria were not amplified by the real-time RT-PCR. The assay was also highly sensitive and could quantitate between 10^2 and10^{10} copies/µl of viral genome. TCoV was detected in the feces from cloacal swab or floor throughout 14 DPI; however, the viral RNA load varied among different turkey poults in different intervals from different trials. The highest amount of viral RNA, $2.8x10^{10}$ copies/µl , in the feces was the one from cloacal swab collected at 1 DPI. The average amount of TCoV RNA in cloacal fecal samples was 10-fold higher than that from floor. Quantitative level of TCoV RNA was obtained from the duodenum, jejunum, ileum, cecum, colon, cloaca, and bursa, but not thymus, trachea, liver, kidney, and spleen. The viral RNA in the intestine reached the highest level, $6x10^{15}$ copies/µl , in the jejunum at 5DPI. In addition, 84 intestine segments from one of the trials were assayed by the developed real-time RT-PCR and immunofluorescence antibody (IFA) assay. There were 6 segments negative for TCoV by both assays, 45 positive for TCoV by IFA, and 77 positive for TCoV by real-time RT-PCR. Taken together, the results indicated that the developed real-time RT-PCR assay is rapid, sensitive, and specific for detection and quantitation of TCoV in turkey tissues and will aid in monitoring the progression of disease outbreaks in turkey flocks.

Prevalence of Campylobacter and *Salmonella* in the Turkey Brooder House

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We have previously surveyed market weight turkeys for the effect of transport to and holding at the abattoir on the prevalence of *Campylobacter* and *Salmonella*. Whereas *Campylobacter* frequently colonizes the ceca of adult birds (~60-100%), the prevalence of *Salmonella* varies considerably from farm to farm. The purpose of this study was to evaluate the point of entry of these bacterial food-borne pathogens into the brooder house, the first stage of commercial turkey production. Two studies were conducted. In summer 2005, birds (100 per time point) were collected on-farm (0-,9– and 16-days of age), transported to the laboratory, euthanized, necropsied, and intestines cultured for *Campylobacter* and *Salmonella*. Whereas *Campylobacter* spp. were not detected in day of hatch poults, *Salmonella* was isolated from the ceca (3.9%) and yolk sac (0.1%). By day 9, whereas *Campylobacter* spp. were not detected, *Salmonella* was frequently isolated from the ceca (55%) and small intestine (45%). By day 16, the prevalence of *Salmonella* in the ceca (21%) and small intestine (5.1%) had declined. In contrast, *Campylobacter* spp. were infrequently isolated from either the ceca (2%) or small intestine (3%). In summer 2005, birds 5– to 33– days old were monitored on a second farm under the same ownership. This provided the opportunity to compare conventional culture with two commercially available real-time PCR detection formats. Whereas *Campylobacter* spp. were not detected in the ceca of 5-day old birds, it could be detected on days 20 (92%) and 33 (90%). *Salmonella* was isolated from nearly all of the 5-day old (98%) and 33-day old (96%) birds, at which time the turkeys were moved to the finisher house. At slaughter *Salmonella* (4.5%) and *Campylobacter* (92%) were isolated from the ceca of market weight (138-day old) birds originating from this flock. Overall, for *Salmonella*, conventional culture was more sensitive than either of the real-time platforms. For *Campylobacter*, the two real-time formats differed significantly in their sensitivity due to the frequency of error runs in one platform. We have previously observed high levels of ciprofloxacin resistance in *Campylobacter* in market weight birds. Antimicrobial profiles of the archived *Campylobacter* isolates indicated resistance to ciprofloxacin was already present in the young bird.

Efficacy Comparison of Three Commercial MG Vaccines

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This study was conducted at the University of Georgia to evaluate an inactivated *Mycoplasma gallisepticum* (MG) bacterin against two different types of MG vaccines, a modified-live vaccine and a recombinant pox-vectored MG vaccine.

Approximately 100 7-week-old SPF chickens were pre-screened to ensure they were free of mycoplasmas. At 10 weeks of age, 25 birds each were vaccinated with the three test vaccines and the remainder served as non-vaccinated controls. The chickens not given the pox-vectored vaccine also were vaccinated for fowl pox in case of shedding of the vector virus by the vector vaccine. Pox "takes" were confirmed a week later. Samples were collected 5 weeks following vaccination for serological evaluation using three tests. Serology of all chickens also was evaluated at the end of the trial, 10 days following challenge and 16 weeks following vaccination.

The timing of MG challenge was determined by egg production, which reached approximately 50% at 24 weeks of age. Aerosol challenge with the virulent R strain of MG followed at 25 weeks of age. Ten days following challenge, all the birds were necropsied and evaluated by gross examination of air sacs and ovaries, histopathology of tracheas and oviducts, and culture of air sacs and ovaries, as well as serology.

The trial was validated by the results observed in control chickens. It was found that the inactivated MG vaccine induced superior seroconversion, both at 5 weeks and 16 weeks following vaccination compared to the other test vaccines. The inactivated MG vaccine and the live MG vaccine both performed considerably better than the vectored product in providing protection against virulent MG challenge. This information is valuable in considering the use of a bacterin, a modified-live vaccine, a recombinant pox-vectored vaccine or a combination of these MG vaccines in a vaccination program.

Results:

At 5 weeks post-vaccination, the mean ELISA titers induced by the inactivated MG vaccine were significantly higher than the other two vaccines (table 1). Neither, the modified-live MG vaccine or the pox-vectored MG vaccine was significantly different from those of the non-vaccinates.

In both non-challenged and challenged chickens 16 weeks post-vaccination, the mean ELISA titers induced in chickens vaccinated with inactivated MG vaccines were significantly higher than those of the other vaccine groups.

Table 1. Summary Mean ELISA Serological Responses of Chickens 5 Weeks and 16 Weeks Post-Vaccination (PV) for MG.

Vaccine Group	Mean ELISA Serological Test Score (Mean sample to positive ratio)		
	5 weeks PV	16 Weeks PV No challenge	16 Weeks PV challenge
1 and 2 Controls	0.0^{a}	0.1^{a}	1.7^{b}
3 Inactivated MG vaccine	1.8^{b}	1.6^{b}	6.3^{d}
4 Pox-vectored MG vaccine	0.1^{a}	0.0^{a}	1.6^{b}
5 Modified-live MG vaccine	0.3^{a}	0.2^{a}	2.6^{c}

* Values within a column with a different superscript are significantly different ($\rho \leq 0.05$).

Both the inactivated MG vaccine and modified-live MG vaccine afforded significant protection against severity of air sac lesions, ovarian regression, and thickening of tracheal mucosa compared to non-vaccinated control chickens following virulent MG challenge. By comparison, the pox-vectored vaccine did not afford significant protection compared to non-vaccinate controls against air sac lesions, ovarian regression, or thickening of tracheal mucosa.

No vaccine in this trial afforded significant protection against re-isolation of the challenge organism from either air sac or oviduct. Vaccination for MG thus cannot be counted on to significantly prevent challenge colonization of those tissues. The challenge used in this trial was considered very severe based on the effects produced in controls, so the vaccines might be expected to perform better against a less severe challenge.

Table 2. Summary of necropsy findings 10 days following challenge with MG R strain, approximately 26 weeks of age: air sac lesion scores, incidence of ovarian regression (follicle atresia), tracheal mucosal measurements, and MG isolation

Challenge	Vaccine Group	Air sac Lesion Score[B]	Ovarian Regression	Tracheal mucosal thickness[C]	MG isolation	
					Air sacs	Oviduct
No	1 Not vaccinated	0/8[D] (0.0)[a]	0/8[a]	126.3 ± 37.9[a]	0/8[a]	0/8[a]
	3 Inactivated MG vaccine	0/5 (0.0)[a]	0/5[a]	113.7 ± 8.7[ab]	0/5[a]	0/5[a]
	4 Pox-vectored MG vaccine	0/5 (0.0)[a]	0/5[a]	125.7 ± 22.1[ab]	0/5[a]	0/5[a]
	5 Modified-live MG vaccine	0/4 (0.0)[a]	0/4[a]	130.2 ± 11.5[ab]	0/4[a]	0/4[a]
Yes	2 Not vaccinated	15/15 (3.6)[c]	13/15[c]	433.7 ± 85.0[d]	12/14[b]	15/15[b]
	3 Inactivated MG vaccine	20/20 (2.5)[b]	8/20[b]	294.4 ± 121.3[c]	20/20[b]	20/20[b]
	4 Pox-vectored MG vaccine	20/20 (3.5)[c]	19/20[c]	389.7 ± 165.9[cd]	20/20[b]	20/20[b]
	5 Modified-live MG vaccine	16/20 (2.1)[b]	8/20[b]	255.4 ± 172.8[bc]	17/19[b]	17/20[b]

Conclusions:

It is concluded from the results of this study that the inactivated MG vaccine and the modified-live MG vaccine both protect against a virulent MG challenge given near peak egg production. When considering the serological response, however, inactivated MG vaccine outperformed the modified-live MG vaccine, which may be an important factor under field conditions where birds are often kept for up to 2 years.

The recombinant pox-vectored MG vaccine failed to provide a serological response or protection against challenge that differed from that seen in non-vaccinates. While this vaccine resulted in milder fowl pox "takes" of variable size, it is worth noting that the poorer "take" quality of this vaccine has since been commonly reported in the field. However, discerning whether this may have played a role in its lack of efficacy was not within the scope of this study.

A three-year analysis of antibacterial susceptibility and resistance in chicken *Escherichia coli*, Salmonella spp., and *Pasteurella multocida* isolates

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Escherichia coli, Salmonella species, and *Pasteurella multocida* are the major bacterial pathogens in poultry flocks. Infections with bacteria are often required to be treated with antibiotics. However, susceptibility to the antimicrobial agents is not equal among all bacterial species as well as among different strains of the same bacterial species. Differences in susceptibility or resistance to different antibiotics has become a major factor in the successful treatment of bacterial diseases. Great concerns have been raised regarding emerging antimicrobial resistance among bacteria that may result in unpredictable antimicrobial susceptibility and untreatable bacterial infections. The primary objective of the present study was to determine the levels of antimicrobial susceptibility and resistance of *E. coli*, *Salmonella* species, and *P. multocida* isolated from chickens in a three-year period.. A total of 445 *E. coli* isolates, 387 *Salmonella* spp. isolates, and 80 *P. multocida* isolates from chickens isolated from 2001 to 2003 were obtained. Minimal inhibitory concentration (MIC) of 14 antimicrobial agents against each bacterial isolate was determined using a microbroth dilution assay according to the Clinical Laboratory Standards Institute (CLSI), formerly National Committee on Clinical Laboratory Standards (NCCLS) guidelines. Ninety-eight and twenty hundredths percent of *E. coli* isolates were resistant to tilmicosin, 79.33% to tetracycline, 51.46% to spectinomycin, 44.04% to gentamicin and 40% to ampicillin. All *Salmonella* spp. isolates were resistant to tilmicosin. Resistance to tetracycline was found in 72.61%, spectinomycin 68.48%, ampicillin 63.57%, gentamicin 63.31%, and ticarcillin 61.76% of *Salmonella* spp. isolates. The resistance rate of *P. multocida* isolates to all antimicrobials tested was less than 5% except 6.25% of resistance to tetracycline. There was decreased resistance to amoxicillin/clavulanic acid, ampicillin, ceftiofur, difloxacin, florfenicol, gentamicin, and spectinomycin, in E. coli isolates in a three-year period. Increased resistance to ampicillin, gentamicin, spectinomycin, ticarcillin, and trimethoprim/sulfadiazine was seen in Salmonella spp. Isolates in a three-year period. In summary, *E. coli* and *Salmonella* isolates were sensitive to ceftiofur and fluoroquinolones, while *P. multocida* isolates remained sensitive to all the antimicrobial agents tested in a three-year analysis.

Counteracting mycotoxin contamination:The effectiveness of *Saccharomyces cerevisiae* cell wall glucans for sequestering mycotoxins

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Introduction: Several natural strategies for controlling the disease processes associated with mycotoxins by sequestration of the mycotoxin within the gastrointestinal tract of an animal are being investigated. Some of these strategies target yeast cell wall as an efficient organic sequestering agent that decrease the toxicological properties of mycotoxins. It is important to understand the fundamental chemistry of the interaction to clarify the absorption process involved in toxin clearance.

Procedures: The chemical mechanisms involved in the sequestering activity of *Saccharomyces cerevisiae* cell wall components toward aflatoxin B1 (AFB1), deoxynivalenol (DON), zearalenone (ZEA), patulin (PAT) and ochratoxin A (OTA) were investigated *in vitro* mixing together increasing amounts of mycotoxins and a constant amount of sequestrants during 90 min at 37°C under orbital agitation. Quantification of the sequestered mycotoxins were achieved by HPLC analysis and evaluation of the interaction kinetic based on overall capacity and standardized affinity rate using Hill's model. The comparison of several sources of yeast cell wall differing in their relative glucan/mannan/chitin content was carried out.

The chemical interaction was approached using molecular mechanics investigation to evaluate the impact of the sequestrant 3D-structure. Together with complementary NMR, X-ray structural data analysis, molecular modeling assessed the overall stability of the modeled molecules in all their most stable possible conformations and allowed to evaluate the statistical probability of existence of each structure. Furthermore, the site-specific docking of a mycotoxin in the sequestrant was investigated so that translations, rotations, and up and down positioning were carefully explored.

Results: The evaluation of the affinity rate using Hill's equation and resultant biological parameters proved a strong efficacy of β-D-glucans, which are composed of linear chains of(1→3)-β-D-glucans branched with (1→6)-β-D-glucan side chains., for AFB1, ZEA, DON and PAT. Complementary work showed a significant efficacy for sequestering T-2 toxins, as well as endophytes associated toxins. Molecular mechanics investigations demonstrated the importance of single and/or triple helix organized structure of β-D-glucans. It was concluded that chemical interactions involved weak chemical linkages such as hydrogen and van der Waals bonds occurring between β-D-glucans and the hydroxyl and cyclic groups of mycotoxins. Several *in silico* models proposed a realistic view of mycotoxin molecules caged inside the helix-shaped (1→3)-β-D-glucans, which were firmly stabilized by (1→6)-β-D- glucan branched side chains due to high geometric similarities between mycotoxins and the active site on the β-D-glucan structure.

Conclusion: Basic science in biochemistry is required to complete and support in a meaningful way the advances in applied nutrition needed to extend our understanding of the beneficial role of *Saccharomyces cerevisiae* cell wall at limiting the toxicologic impact of mycotoxin. The plasticity of the structure of β-D-glucans exhibiting diverse stereochemistry was undoubtedly responsible for the affinity on a large range of mycotoxins. Affinity rates varied widely between toxins due to their structural and physico-chemical disparities. Nevertheless, we concluded that β-D-glucans may have strong affinities for mycotoxins exhibiting "aflatoxin-like" or "zearalenone-like" structures.

The Effect of Natustat® for Prevention of Histomoniasis in Turkeys

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Previous studies suggested that Natustat®, a natural product derived from yeast, had useful preventive activity against blackhead disease (histomoniasis) in turkeys kept on infected litter. The aim of this study was to further investigate the effect of Natustat® after experimental infection of turkey poults using a direct/indirect exposure model. Nitarsone was included as a positive control.

Natustat® was given in the feed at 250g, 500g, or 1000g/ton to 10-day-old turkeys in battery cages. Four days later three of eight birds in every cage were inoculated per cloaca with *Histomonas meleagridis* from cultures. The floor was covererd with heavy paper and the infection was allowed to spread to the other five birds. Mortality, weight gain, and lesion scores in ceca and liver were monitored.

Overall, Natustat® had little effect against the severe lesions caused by direct inoculations of poults. However, liver and cecal lesions of the indirectly exposed birds were reduced and weight gain was increased after treatment with the higher doses of Natustat® in comparison to the 250g/ton treatment. These results suggest that Natustat could be of value in reducing the spread of blackhead within a flock during an outbreak.

Poultry Vaccines: Labeling and Adverse Events

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Poultry vaccine labeling contains information important to the safe and effective use of the product. Information on the label includes:

- True Name—This indicates the disease(s) against which the vaccine is directed.
- Trade Name—The product may also have a name in addition to the True Name
- Name, address, and an Establishment Number—The Establishment Number is a unique identifying number for the company that made the product
- Distributor's Name—The labeling may also have information on the distributor of the vaccine (i.e., some companies only sell the product, not make it).
- Storage conditions.
- Usage instructions—Minimum age, route of vaccination, cautions, warnings, or other special restrictions are indicated
- Withholding statement—This is information on how long animals administered the product should be withheld from slaughter.
- Expiration date—Date the product should be used by for maximum effectiveness
- Antibiotics—While antibiotics are not used at therapeutic concentrations, they may be present in the final product

All labeling is reviewed by the Center for Veterinary Biologics (CVB) to ensure the claims are supported by data and are not false or misleading. Information on the veterinary biologics labeling requirements can be located on our website: http://www.aphis.usda.gov/vs/cvb/ under 9 CFR 112, Veterinary Services Memorandum 800.54 or 800.80

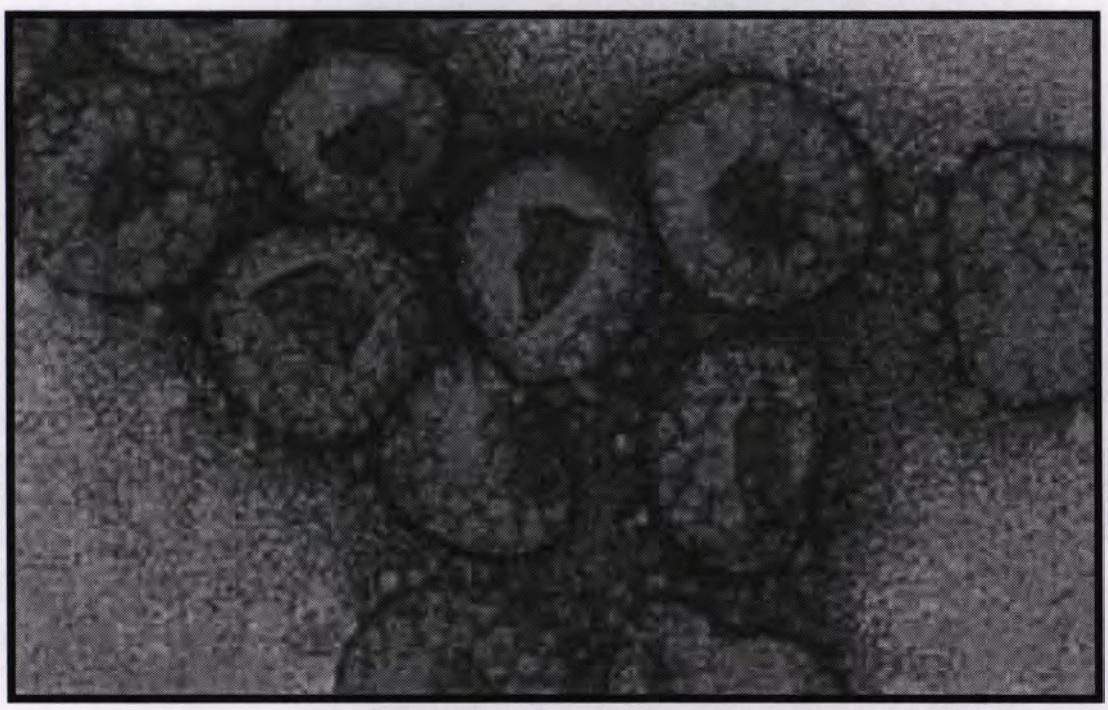
The CVB monitors adverse events following vaccination. An adverse event is defined as any undesirable occurrence after the use of a vaccine, including illness or reaction, whether or not the event was caused by the product. For products intended to diagnose disease, adverse events refer to anything that hinders discovery of the correct diagnosis.

On-line: Adverse Event Electronic Report Form
By fax or mail: Download the pdf form and FAX to area code (515) 232-7120 or by mail to CVB.
By telephone: Adverse events may also be reported by calling CVB at (800) 752-6255.

Things to be aware of when using vaccines:
- Manufacturers and Serial Numbers
- Approved routes and ages indicated on the label
- Warnings and cautions indicated on the label

Symposium

Lessons Learned from AI Preparation



Avian Influenza and Lessons Learned on AI Diagnosis

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Preparedness for outbreaks of avian influenza (AI) in poultry has never been more important than it is today. The rapid spread and reoccurring outbreaks of the highly pathogenic AI (HPAI) H5N1 virus in Asia, Europe, the Middle East and Africa emphasize the importance of having diagnostic assays available to ensure rapid detection should the virus be introduced into the United States. We know from past experiences with AI infections in poultry that the economic impact of outbreaks can be greatly reduced when infections are detected early and aggressive control measures are implemented. This is true for outbreaks caused by low pathogenicity AI (LPAI) viruses as well as for those caused by highly pathogenic AI (HPAI) viruses. However, the detection of LPAI can be especially difficult because clinical signs often cannot be used to identify infected flocks. Therefore, detection of AI requires good surveillance programs and diagnostic tests to confirm infections in poultry. This presentation will review recent advances in laboratory diagnosis of AI, identify the role of the National Animal Health laboratory Network (NAHLN) to conduct AI surveillance, and iterate the lessons learned from recent outbreaks of LPAI and HPAI in the United States.

NPIP Program, State Preparedness and Containment of Low Path AI

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High Path AI Responses and Update on Veterinary Stockpile

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AI Vaccine—A New Approach

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Live-virus vaccines have a distinct advantage over the conventional vaccine, which includes the administration by nasal spray. For humans, cold adapted live vaccines have been applied to millions of children with protective efficacy and without evidence of serious side effects. In the U.S. trivalent, cold-adapted, attenuated, live influenza vaccine (FluMist™) was approved for use on June 17, 2003. However, the use of live influenza vaccines in poultry has never been seriously considered for several reasons. First, there is the possibility of bird-to-bird or farm-to-farm transmission. This may establish AI virus as an endemic infection. Second, the use of live vaccine may cause vaccine-induced respiratory disease in commercial poultry populations. Third, and maybe most importantly, there is a potential for recombination with newly introduced AI virus strains to create a recombinant AI virus which is more pathogenic or has the capability of spreading to different hosts. However, with the advancement in biotechnology, it may be possible to consider new vaccine approaches using genetically engineered live attenuated virus. To be safer, it will be desirable to develop vaccine viruses that undergo only a few cycles of replication in chickens, but still induce a strong protective antibody response and stimulate a cell-mediated immune response without allowing the replication of a virus.

The NS1 protein of influenza virus functions as an interferon antagonist and is, thus, directly associated with the pathogenicity of the virus. From the TK/OR/71-del (H7N3) virus, we previously found that several variants with different sizes of the NS gene can be generated by serial passage of the virus in embryonating chicken eggs. We have further pursued the identification of different NS genes and have found 20 different NS genes that have unique deletions in different regions of the NS gene. Preliminary data shows that these naturally selected NS1 deletion variants will be useful in the development of live influenza vaccines both in their current state and with further modification of the NS1 protein. Furthermore, a deletion in the NS1 protein can also be useful as a negative marker for the DIVA (Differentiating Infected from Vaccinated Animals) approach.

In poultry, vaccine can be administered to eggs during later stages of embryonation, usually at 17-18 days of incubation. This *in ovo* vaccine delivery is easy and cost-effective and has replaced post-hatch injection of Marek's disease vaccine in most broiler chickens in the U.S. A potential use in the future would be for *in ovo* vaccination with attenuated or replication-defective live influenza virus that induces strong immune responses, but has no infectious virus present in the hatched chicks.

Lessons learned in the prevention and control of avian influenza: a scientific approach

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Preventing avian influenza (AI) exposure requires an understanding of the source of the virus, how the virus moves, knowledge of poultry population involved, cross over points and the exposure risk level. This knowledge allows the biosecurity tools of sanitation, isolation and traffic control to be directed appropriately. A logical source for this information is the scientific literature where it is possible to find knowledge gleaned by others while they were learning lessons from AI outbreaks.

Lesson 1. A labile virus. The AI virus (AIV) has an envelope that accounts for its susceptibility to detergents and disinfectants. Additionally it is susceptible to heat and drying; it is generally inactivated within one week at 21 C, but may survive for five weeks at 4 C (29,30). Moist feces collected from 1983 Pennsylvania H5N2 HPAI infected hens infected SPF chicks placed on the feces two days but not four days after collection (3). The Pennsylvania H7N2 LPAI virus from 1997 was inactivated in chickens manure in less than one week at 15-20 C (23).

Lesson 2. The natural reservoir. The known natural reservoir of AIV is wild waterfowl and shorebirds (orders anseriformes and charadriiformes) where the AI virus primarily causes an enteric infection and rarely a respiratory infection. A duck can shed virus for 30 days and can excrete 10^8 EID_{50}/ml of feces per day (34), contaminating surface water, sloughs, and shorelands (13). In its natural reservoir waterborne virus is transmitted most efficiently by the fecal oral route. Thus the water contamination, fecal oral transmission and movement of wild waterfowl and shore birds are the mechanisms that provide for AIV survival and dispersal in nature. The waterfowl-shorebird reservoir, the environment these birds occupy, and anything or anyone sharing that environment may be a source of AI virus for domestic poultry (29,30).

Lesson 3. The man made reservoir. The most important man-made reservoir of AI is the live poultry market system where the continual introduction of susceptible poultry can maintain the virus for years (27). Live poultry markets coupled with a village poultry production system with multi-species and non-confinement rearing are particularly likely to maintain AIV and recycle it back into wild birds. These aforementioned conditions in much of the world now contribute to the more complex epidemiology of highly pathogenic H5N1 and low pathogenicity H9N2 in much of the old world.

Lesson 3.1. Hong Kong authorities have demonstrated success in eliminating H5N1 in the live poultry market system by vaccination, by separating waterfowl from gallinaceous poultry, by enforcing monthly rest days in the markets and by active surveillance (11,21).

Lesson 4. Preventing accidental introduction. Apparently healthy wild or domestic waterfowl and poultry, particularly associated with live poultry markets or range production, may be infected with AIV. The virus is found in the droppings of infected waterfowl and each bird may excrete ten billion EID_{50} of virus per day (34). In gallinaceous poultry the virus is found

in both the respiratory secretions at up to 10^7 EID_{50} of virus per gram and in feces at up to 10^6 EID_{50} of virus per gram. An exception to this generalization was the observation of 10^9 EID_{50} of HPAI virus in the feces of laying hens experimentally infected with the 1983 H5N2 AI virus (3). To put this amount of virus in perspective, Tumpey et al (32) found that turkeys could be infected with less than 10^1 EID_{50} of H7N2 AI virus from the Virginia outbreak; so with the worst case scenario (10^9 EID_{50} of virus per gram of feces and 10^1 EID_{50} of virus equal to one turkey infectious dose 50%), one microgram of feces could contain 100 TID_{50} of AI virus. To prevent the introduction of AI any conceivable contact between the high risk contamination areas and commercial poultry must be avoided.

Lesson 4.1. Orders of birds other than anseriformes and charadriiformes may be infected but the virus is not maintained in them in nature; however, it is important to recognize that these birds may act as mechanical and biological vectors to move the virus between the waterfowl reservoir and domestic birds (29,30)

Lesson 4.2. Clothing or equipment used while hunting, trapping or fishing and travel directly from such environments can introduce AIV onto a poultry farm if precautions are not taken (14).

Lesson 4.3. Watering poultry from ponds can introduce AI virus to a farm (2,14).

Lesson 4.4. Clothing or equipment used while visiting live poultry markets or any other place where live poultry may be kept, sold, exhibited, or fought can introduce AI virus to a farm (14).

Lesson 4.5. Vehicles or crates or batteries that have been in contact with live poultry markets can introduce AIV onto a poultry farm (19,20).

Lesson 5. Preventing introductions associated with illegal behavior. Illegal behavior might introduce AI viruses onto a poultry farm. Farming is a business involving trust; routine deals involving thousands of dollars are sometimes done on a hand shake. The strong culture of trust in agribusiness is an obstacle to preventing potential illegal activity that could introduce disease agents such as influenza virus. Theft of birds from a commercial poultry farm for personal use or for sale at a live poultry market (Kradel, personal communication), smuggling birds or bird products, contact with fighting chickens, or intentional introduction of virus are all ways that people engaged in illegal activities could introduce avian influenza virus into a flock.

Lesson 5.1. At a bare minimum poultry farms need a security system that discourages visitors and includes locked doors (2,14).

Lesson 6. Preventing the spread. Once an AI outbreak has occurred attention must be shifted from the reservoirs of AI virus and the environments associated with them toward sources of virus within infected poultry populations.

Lesson 6.1. Because it is not possible to categorize with certainty that a given flock is not infected with AI, effort must be directed toward avoiding direct and indirect exposure to the documented sources of virus transmission: poultry manure, respiratory secretions, live poultry, dead poultry, re-used egg packing materials, and equipment or people contaminated with manure or respiratory secretions (4,5,7,14,17,25)).

Lesson 6.2. Other potential sources (flies, rodents, and people and equipment that have not contacted poultry or manure) are far less important and have not been documented as sources of transmission.

Lesson 6.3. In the U.S. and Canada pet birds and backyard poultry have not been associated with AI outbreaks in commercial poultry (Dennis Senne, personal communication, Victoria Bowes, personal communication) and the same conclusion was reached in the Netherlands following the HPAI outbreak in 2003 (9,18).

Lesson 7. The role of people in moving AI virus. In nature the avian influenza virus is moved from place to place by the movement of waterfowl and shorebirds. In domestic poultry the virus is moved from place to place by the activities of people. The existence of the virus in either the waterfowl or market reservoir is not the problem; rather it is movement of virus or infected birds into close proximity of susceptible birds that causes transmission of AI. Depending on the type of poultry, the type of poultry operation, and regional considerations (eg poultry density) for any influenza outbreak it is critical to quickly identify how the virus will be moved about by the activities of people. Recognizing the ways the virus is apt to be moved in a particular outbreak is the most important part of the control strategy.

Lesson 7.1. Eighty years ago it was recognized that contaminated poultry crates and rail cars spread fowl plague (HPAI) from New York to the Midwest (20).

Lesson 7.2. Twenty years ago in California it was documented that contaminated artificial insemination crews and equipment transmitted AI between five turkey breeder farms while leaving some 300 other poultry flocks in the same area untouched (24).

Lesson 7.3. In Minnesota live haul crews and equipment were implicated (2,14).

Lesson 7.4. In Virginia a common rendering plant and hired labor were involved (25)

Lesson 7.5. In Italy manure hauling contributed to spread (7)

Lesson 7.6. In California movement of spent hens and movement of egg handling material were identified (Cardona, personal communication).

Lesson 7.7. Feed trucks, eggs and day-old chicks have not been reported to be the source of spread.

Lesson 7.8. Airborne transmission has only been associated with human activity (trucking live infected birds, trucking carcasses, generation of dust and feathers during depopulation, trucking manure)

Lesson 8. Identify specific avian populations. Industry practices are specific for different types of poultry. Some types of poultry are moved from one place to another, have access to the outdoors, have contact with live poultry markets, or have contact with wild birds. Recognition of these practices has an impact on control measures that must be initiated (29,30). The susceptibility of different poultry groups to AI outbreaks differs greatly from one group to another and depends to a great extent on exposure to natural or man-made sources of AIV, on the virus itself, on movement of birds, their products and their manure, and on the extent of human activity during the production period.

Lesson 8.1. For example, during the 2002 Virginia LPAI outbreak it was observed that turkeys in the growing facilities (after being moved) were far more frequently infected than those in brooder facilities (having never been moved)(25).

Lesson 8.2. Totally confined populations. The commercial poultry industries reduce their risk of AI introduction by avoiding exposure to the wild bird or live poultry market reservoirs; if AI is introduced their risk is related to the high population density and intra-establishment movement

Lesson 8.3. Non-confined populations. In general owners of non-confinement birds reduce their risk of AI loss by their small size and low population density; their risk of exposure is great and is related to direct or indirect contact with the one of the reservoirs of AI and inter-establishment movement.

Lesson 8.4. The term, backyard birds, has no specific meaning in determining AI risk because they may consist of a single age and species kept in confinement with no contact with live poultry markets, or they may consist of multi-age, multi-species reared outdoors and with live poultry market contact. Thus, it is clear that backyard flocks have to be characterized in order to make meaningful decisions about their potential contribution to AI spread and control. Backyard flocks in the U.S. probably have little resemblance to backyard flocks in East Asia or Africa.

Lesson 8.5. Upland game and waterfowl that are raised for release; organic, range, and pastured poultry; backyard, exhibition, or zoo birds and game fowl (fighting chickens) may have exposure to wild birds. In addition live birds may be transferred from one location to another (birds raised for release, exhibition birds, zoo birds and fighting cocks), although there may be little or no movement of backyard birds. Other poultry may be grown for the live poultry marketing system where the exposure comes from the transfer of contaminated materials or rarely live birds from market to farm, where susceptible poultry then multiply the infection and become a source for additional contamination of the market.

Lesson 9. Identify cross over between populations. The new World Animal Health Association (OIE) chapter on avian influenza promotes the concept of compartmentalization for maintaining poultry trade in the event of a notifiable avian influenza outbreak. Although poultry establishments make great effort to distance themselves from others producing the same type or different types of poultry, sometimes there are opportunities for exposure due to cross over. Cross over occurs where production related activities of one establishment utilize the same resources as other establishments. For examples, a rendering truck may visit broiler, layer and turkey farms, contract labor (crews) may provide services to many different companies, individuals may work on more than one crew, pullet moving dollies may be used on multiple farms, egg moving materials often go to different farms, and feed trucks may provide feed to multiple establishments. Eliminating these cross over points demonstrates compartmentalization and is critical for any establishment that is interested in exporting poultry meat or eggs.

Lesson 10. Identify specific exposure risk level. In the past determining the risk of disease exposure has been a subjective exercise for poultry professionals. Recently an exposure risk index (ERI) has been proposed as a way to make this determination more objective (16). An ERI ≥ 7 is correlated with intolerable or high risk according to a group of poultry veterinarians, while an ERI of ≤ 4 was considered moderate or low risk. A high ERI also is correlated with documented sources of AI transmission risk (eg manure or sick or dead birds) and a low ERI is correlated with low transmission risk (eg pet birds or washed eggs).

Lesson 10.1. The ERI can help evaluate mitigation strategies. For example, the ERI indicates that pullet moving equipment contaminated with one Kg of manure with 10^6 AI viruses per gram still has an unacceptable ERI even after cleaning and disinfecting remove 99.99% (4 logs) of the contamination. This indicates that the cleaning and disinfecting procedure requires modification (16).

Lesson 10.2. Once exposure risk is estimated, investments in fixed and variable costs of biosecurity can be evaluated in light of possible economic impact of avian influenza outbreaks. In the past the biosecurity costs were weighed against risk of disease effects on performance: mortality, body weight, feed efficiency, egg production, etc. As the poultry industries have evolved from meat and egg production-oriented companies to global consumer-oriented companies, today an avian influenza outbreak has the potential to have far greater impact than just on poultry production. Food safety may be called into question, exports may be at risk, public health may be questioned, and brand name survival may be threatened. This increased impact means that increased spending for biosecurity is justified in the global economy (16).

Lesson 11. Control measures that have worked. Avian influenza control programs that have incorporated education, preventing introduction, surveillance & reporting and response have been successful in preventing and controlling AI (14,17,29,30).

Lesson 11.1. Early detection and reporting were found to be key to control in British Columbia, Netherlands, and Minnesota (4,5,10,14).

Lesson 11.2. Industry infrastructure was key to control in British Columbia (4,5).

Lesson 11.3. Containment was dependent on lockdown response in British Columbia and Minnesota (4,5,14) and reducing number of susceptible flocks in Netherlands (28).

Lesson 11.4. Abandoning range production resulted in a sharp decline in AI in turkeys (28,29).

Lesson 11.5. In Utah, Connecticut, Italy and Hong Kong blanket vaccination coupled with biosecurity aided in control (1,6,8,11,15).

Lesson 11.6. In British Columbia it was learned not to move carcasses outside or off farm before time-temperature treatment (4,5)

Lesson 12. Control measures that have caused problems. Some measures have been identified that have caused problems by contributing to virus spread.

Lesson 12.1. A delay in detection results in delayed response and a larger outbreak (4,5,10).

Lesson 12.2. Grinding and trucking carcasses off the farm spread the virus in BC (26).

Lesson 12.3. Trucking actively infected birds spread the virus in Minnesota (2).

Lesson 12.4. Some control activities such as increased sampling spread the virus (19).

Lesson 12.5. Killing pet birds, backyard birds and healthy poultry reduced support for eradication and caused an emotional toll in Netherlands, BC & Italy (4,5,7,12,18,22)

Lesson 12.6. Moving manure spread the virus in Italy (7).

Lesson 12.7. Depopulation efforts (generation of dust and feathers, failure to completely control depopulation crews) led to increasing spread in Pennsylvania in 83 and 97 (17,19).

Lesson 12.8. Destruction costs approximately 10 to 100 times more than controlled marketing.

Lesson 12.9. Vaccination alone has not been a satisfactory control strategy in Mexico (33).

Conclusion

On a global scale AI control is very complex; on a local level it is likely to be simple, but rarely easy. In either case, scientific studies, in the laboratory and in the field, have greatly added to our knowledge of avian influenza prevention and control. Drawing from this body of knowledge to understand the virus, the reservoirs, the movement, the characteristics of specific populations, the cross over between populations and the exposure risk level, it is possible to construct and justify scientifically sound prevention and control programs.

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The Use of Vaccines and Biologics in Controlling AI and the Concept of Compartmentalization and Regionalization

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USDA, APHIS, VS, Mission

The USDA, APHIS, VS, protects and improves the health, quality and marketability of our nation's animals and animal products, and veterinary biologics by preventing, controlling, and/ or eliminating animal disease, and monitoring and promoting animal health and productivity, and licensing veterinary biological products intended for use in the treatment or diagnosis of diseases in animals.

Background

Eradication and biosecurity are the first line of defense against all AI viruses. Preventing the introduction of AI by eliminating all contact between commercial poultry and wild birds, swine farms, and live bird markets is a common and successful practice. However, occasionally, AI is introduced into the commercial poultry population.

Under conditions of high poultry density or multiple poultry establishments in one area, eradication and biosecurity alone are not likely to be successful control strategies (5). A successful strategy requires reducing the susceptibility and density of the poultry population. The components of a control strategy can vary, but generally include five categories: (i) biosecurity (including quarantine); (ii) diagnostic and surveillance; (iii) elimination of infected poultry; (iv) decreasing host susceptibility to the pathogen (for example, through vaccination); and (v) education of personnel in the animal production chain and allied industries to better understand how diseases are transmitted so personnel with responsibility to prevent transmission or spread can be incorporated into action plans.(3)

For many years, APHIS policy regarding the control of AI and the related production and distribution of AI vaccines remained unchanged. Then, in 1995, restriction on the production and use of H7 subtype vaccines was added to the restriction that existed since 1983 on the use of H5 subtype vaccines. At the same time, APHIS concluded that the restrictions imposed on AI vaccine production and use during the 1983 high pathogenicity avian influenza (HPAI) eradication campaign should be modified to allow H5 and H7 vaccines to be used as a tool for combating any potential outbreak of HPAI

As we approach the eradication of specific animal diseases in the United States, it will be necessary to refine regulations, policies, and procedures pertaining to veterinary biological product availability for these diseases.

Current APHIS policy

This presentation will review the current USDA, APHIS policy on the use of vaccines and biologics in controlling AI and the concept of Compartmentalization and Regionalization or Zoning. The current APHIS policy, as described in VS Memorandum No. 565.12 allows "H5 and H7 vaccines to be used as a tool for combating any potential outbreak of HPAI in the United States" (6). AI vaccines may be prepared from any serotype, including H5 and H7, and may be recommended for use in chickens or turkeys subject to the requirements and restrictions specified in VS Memorandum No. 800.85 (6). VS Memorandum No. 800.85 allows H5 and H7 vaccines to only be used under the supervision or control of USDA, APHIS, VS, as part of an official USDA animal disease control program. The USDA, APHIS, VS Center for Veterinary Biologics (CVB) implements the provisions of the Virus-Serum-Toxin Act to ensure that veterinary biologics available for the diagnosis, prevention and treatment of animal disease are pure, safe, potent, and effective.

APHIS supports the general concept of vaccination as a tool in the eradication of notifiable AI. However, vaccination should be available as part of a science-based influenza control strategy that includes: (i) enhanced biosecurity; (ii) controlled vaccination for flocks deemed to be at risk; (iii) suitable monitoring of all flocks at risk and of all vaccinated flocks; and ((iv) a repopulation plan (2,3,4). The management of AI must continue to be based on sound scientific principles. However, innovative strategies will be required to eliminate these persistent and adaptive viruses.

The Concept of Compartmentalization and Regionalization or Zoning

The following definitions have been adopted from the new Office International des Epizooties (OIE) Terrestrial Code for Avian Influenza

Compartment means one or more establishments (premises in which animals are kept) under a common biosecurity management system containing an animal subpopulation with a distinct health status with respect to a specific disease or specific diseases for which required surveillance, control and biosecurity measures have been applied for the porpose of internation trade (1).

Region/Zone means a clearly defined part of a country containing an animal subpopulation with a distinct health status with respect to a specific disease for which required surveillance, control and biosecurity measures have been applied for the purpose of international trade (1).

The procedures used to establish and maintain the distinct health status of a compartment or zone should be appropriate to the particular circumstances, and will depend on (1):

- The epidemiology of the disease (including methods of disease spread and species affected);
- Environmental factors (including the presence of natural barriers);
- Appropriate and applicable biosecurity measures (including movement controls, use of natural and artificial boundaries, commercial management and husbandry practices); and
- Disease surveillance.

Both Compartmentalization and Zoning are tools that may be applied to facilitate trade. Fundamental to their application is the free exchange of information necessary to provide confidence to the importing country that the risk of disease introduction from trade is minimized. Therefore, the procedures for establishing trade based upon the compartmentalization concept should be similar to those practiced for regionalization or zoning. However, the ultimate trade decisions are determined by the importing country's analysis and their acceptable level of risk.

Risk analysis, research and development are urgently needed to obtain a better understanding of risk factors in order to implement more effective control measures. A need exists for significant sustained financial investment in national and regional infrastructures to ensure that most countries and states work in a coordinated and harmonized manner to implement these concepts and required surveillance and control measures. It is in the interest of USDA, APHIS, VS and states to make these investments to protect animal health.

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Handling Emerging Disease Issues When You Are In Charge

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When a disease issue affects your poultry farm operation how prepared are you to respond? This session overviews the steps needed to take action should a disease issue affect your poultry operation.

Having a networking plan in place beyond internal employee communication is critical in addressing emerging disease issues. Crisis management coaching is necessary because we are often unprepared for the harsh reality of dealing with disease issues which become the focus of media and public scrutiny. Such public scrutiny can be surprising and overwhelming for employees and managers. Therefore, developing effective responses to the media and general public are crucial for poultry operations. Actions will be outlined to prepare for external public communication and to develop an internal communications plan. This session serves to establish the foundation for effective management of disease issues and assists in responses to the media and the general public's concerns.

AI Preparedness—the Texas Experience

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Avian Influenza is an ever present challenge and it thrives in the clash between new and old poultry husbandry/marketing practices. In various states of the country, including Texas, Live Bird Markets (LBMs) have become potential or known reservoirs of Avian Influenza (AI). The multi-age, multi-source and multi-species nature of LBMs provides a suitable environment for the emergence of poultry-adapted AI viruses. This poultry production, distribution and marketing system represents a risk in addition to the well known risk from wild birds. Commercial poultry are immunologically naïve to AI so they are always at risk. Biosecurity is the key to separate commercial poultry from high risk bird populations. If a breach in biosecurity occurs then disease control requires detection, containment and prevention. In 1995, in response to the presence of highly pathogenic AI in Mexico, the Texas Poultry Federation (TPF) developed an active AI surveillance program and an industry-based indemnity program to encourage eradication of the disease. In addition, poultry industry, regulatory and laboratory personnel cooperated in the development of an AI response plan. Just last year, Texas AI surveillance generated approximately 99,000 AI serology tests in the Gonzales laboratory alone. Since the establishment of the Texas AI response plan, we have had several opportunities to put theory into practice. On the bright side, preparedness, cooperation and the combined experience of laboratory, regulatory and industry personnel yielded positive results. On the other hand, AI has a heavy toll on all involved. No person, business, agency, city, state or country is ever completely ready to deal with the consequences of an AI outbreak. Preparedness and experience are key elements to the effectiveness of the response. Active surveillance is the key to early detection and a quick resolution. Our experiences from 2004 will be used to illustrate AI preparedness and response.

Actions and Lessons Learned in Preparing the Broiler and Broiler Breeder Industry for AI

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Preparing for an Avian Influenza outbreak has been a long process with numerous plans undergoing revisions as new regulations are enacted. State regulations also influence plan development and whether or not the state has had previous experience with AI or even Exotic Newcastle. Covered in this presentation are 3 states in the southeastern United States with large broiler and broiler breeder industries and what has occurred in the development of their plans to manage High Path AI and Low Path AI outbreaks.

South Carolina has about equal industries of broilers, layers, and commercial turkeys as the major poultry producers in the state. There are smaller operations with quail and game birds, along with backyard producers. To develop a plan, a small core of representatives first met and developed an outline which was then debated and revised. After getting a consensus from representatives from the table egg industry, broiler industry and turkey industry along with NPIP and State Veterinarian's office, they presented a draft to the entire industry to discuss and revise. Representatives from all companies were able to review and raise concerns, questions, etc., about the documents and come to consensus about what to do if AI were to occur. For the broiler industry, this means composting in the house for 2 weeks, then testing for virus presence. If negative, they will continue composting out of the house and clean and disinfect the house. New farms are now required to get pre-approval for an on-farm burial site for mass euthanasia from the environmental protection department. Older farms are now going through the process so that if or when the need for mass euthanasia occurs, they will not have to wait on the environmental protection agency to approve their site. Their reasoning for on-site burial is that the landfills in South Carolina are privately owned and they can turn away the poultry industry if they desire for whatever reason. There is one rendering plant that will take poultry in the state and it operates 5-6 days a week already so it would not be feasible to bring more poultry for rendering in the event of an outbreak. There has been a small foam euthanasia demonstration with chickens on the floor in a pen to see how this would work. Results were good and this practice for broilers and broiler breeders on the floor would most likely be employed. South Carolina has applied for a grant that requests a foam machine for the industry to use and is waiting for funding results. The biggest lesson learned for South Carolina was to include everyone in the process so that no particular sect of the poultry industry felt ignored or left out.

Alabama has predominantly broiler and broiler breeder industry with several large layer complexes and a large quail production industry. They also have a game bird industry that imports birds from all over the United States and abroad. The experience of having a pullorum outbreak in the 1990s and having to euthanize large numbers of birds and dispose of them has prepared them in setting up a plan for the state. The Alabama Avian Health Advisory Board has representatives from industry, the State Veterinarian's office and the Commissioner of Agriculture office and has developed a plan that takes into account what happened during the pullorum outbreak. A member of the State Veterinarian's office participated in the California END outbreak surveillance and learned how important communication is for compliance. They have in the past used CO_2 for euthanasia and plan to use it again. However, some companies

have bought their own foam machine for mass euthanasia and the State Veterinarian's office encourages them to do so. Each company is encouraged to have its own AI plan ready that will help their workers know what to do in the event of an outbreak. Disposal will vary depending on location in the state. The Alabama environmental protection agency has agreed that mass burial is to be instituted where possible. Burial on site is preferred but, in some areas, there is not enough topsoil to cover the birds properly with 2 feet of dirt. In these instances, composting or incineration or landfill disposal are the preferred methods. They are also investigating a mobile tissue digester that could do smaller flocks up to 1500 lbs a day. They have a digester at the State Laboratory in Auburn that handles more poundage. Vaccination will depend on what USDA allows. Since there is a primary breeder company in Alabama, compartmentalization is being looked into so that they can continue to export chicks, eggs, etc., to US and world customers. They also have addressed what to do if companies have complexes in other states that may be infected and how the company can still operate without endangering the Alabama industry. Again, communication within the industry and state officials will be key.

Georgia has a large broiler and broiler breeder industry in the northern half of the state along with pedigree farms. There are layer operations also in the northern half and one company in southern Georgia. There is a quail operation in the northern half. There is a large game bird industry throughout the state. The southern half is mainly broiler companies. The Georgia Poultry Laboratory Network has 9 labs for industry use along with Athens Diagnostic Laboratory (USDA regional testing lab for foreign animal diseases with BSL 3 labs) and Poultry and Research Center at UGA. To prepare for Avian Influenza, an industry wide survey occurred last May-Dec where a representative from GPL and APHIS met with all companies in the state. They made a presentation to the companies about the proposed low path AI plan and determined if the companies had any plans for their company. Afterwards, they presented the revised low path plan to the industry and voted on the items that everyone did not agree on. The high path plan will be voted upon in March this year. For mass euthanasia, the State Veterinarian's office has a foam machine that has been used on houses with birds that were going to be euthanized to make sure everything was working properly and personnel know what to do. Another machine is going to be bought that will be available for companies to use without going through the State Veterinarian's office. For disposal, composting and incineration are most likely going to be employed in a high path outbreak. Burial is available, but many growers may not want to bury HPAI birds on their farms due to the stigma attached. Georgia has a mobile incineration unit that is smokeless and odorless, is quite large, and holds large amounts of birds. Ashes can then be buried in landfills or trenches. Import and export issues will be handled by the State Veterinarian's office in cases of low path AI.

Overall, communication with the companies and getting everyone to agree to the plans has been the most important lesson learned for the meat bird industry.

The North Central Experience

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Pennsylvania Experience: Live Bird Market and Back Yard Birds

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The production and selling of poultry for the live bird market (LBM) system in new York and New Jersey is a big business in Pennsylvania. A majority of the 25 to 30 million birds sold are raised in PA. Most of this production is raised under contract by distributors who either have a marketing contract for selling a grower's birds or the distributor owns that birds that the grower raises for a fee. The distributor sets up the placement and marketing schedule and arranges for transportation to market. The grower is responsible for care and housing of the birds. Labor to gather the birds for transport is either arranged by the grower or by the distributor depending on the situation.

As many of the LBM markets have been positive for low pathogenic avian influenza (LPAI) virus in the past, the key to maintaining negative production flocks in PA is to be sure the flocks going to market are free of LPAI and that any equipment used coming back to PA is sanitized prior to reuse. The spread of LPAI among flocks or between wild birds and LBM supply flocks has not been the source of LPAI for LBMs in PA LBM supply flocks.

AI testing prior to market is required for both states, NY and NJ. A PA certified poultry "Test Prior to Market or "Monitored Flock Program". The "Test Prior to Market" program requires 30 samples of sera for AI agar gel immunodiffusion (AGID) testing (30 cloacal swabs for virus isolation if waterfowl) within 10 days of marketing. The "Monitored Flock Program" requires 30 sera samples (30 cloacal swabs for waterfowl flocks) every month for any flocks on the farm over 4 weeks of age.

All coops and trucks used to transport LBM poultry are required to use a USDA approved coop and truck sanitation system to clean and disinfect (C&D) the coops, crates, and trucks between uses. Receipts are kept as proof that the C&D was done. USDA personnel perform monthly audits of each distributor to maintain a check on the production and marketing system. During these monthly visits, flock testing records, truck and coop C&D records, and other practices used by the distributor are monitored. Swab testing of equipment used for transport is also collected for AI virus isolation.

The PA AI response plans treats the LBM producers the same as commercial poultry producers as many of the LBM producers are similar in size to commercial units; most broiler supply flocks are 15-20,000 in size. Waterfowl flocks tend to be smaller in the range of 1000 birds. Spent egg layers tend to be brown egg commercial flocks. Geographic Information system (GIS) and contact information is kept in a computerized database for both commercial and LBM supply flocks by the PennAg Industries Poultry Council at the University of Pennsylvania New Bolton Center Laboratory of Avian Medicine and Pathology in Kennett Square PA. Much of this information is updated from information gathered from sample submissions for flock testing.

Should an LPAI outbreak occur, the commercial and LBM supply flocks will be identified in the 3 and 10 km diameter zones surrounding the index case, a map produced with the location of each flock identified by number on the map, and a list of the companies, distributors, or independent growers to contact who own the flocks in these zones is produced. The map along

with the contacts list will be sent to the Pennsylvania Department of Agriculture (PDA) so they may initiate necessary actions in order to control the outbreak.

PDA may establish quarantine zones based on established roadways, geographical, or political boundaries. The contact information will be used to shut down any transport of poultry or eggs into or out of the zone and to establish a plan for testing of dead birds from each flock as part of the surveillance plan. As most of the contacts in the database are contractors of the flocks and not the growers themselves, these contractors then are responsible for calling the grower to inform them of the actions to be taken; limit movement of people on and off the farm and how and when to collect dead birds for surveillance. The contractors are also the most knowledgeable for controlling the movement of feed, eggs, flock supervisors, etc.

Surveillance of dead birds from all flocks for the presence of LPAI within the 3 km diameter zone (the protection zone) is planned within 48 hours and surveillance of all flocks within the 10 km zone (the surveillance zone) is to be accomplished within 72 hours. In order to do this, the growers will need to place 15 dead or freshly killed birds from each flock at the entry to the farm where one of a number of teams will be dispatched to sample these birds for AI testing (oropharyngeal swabs from gallinaceous birds for the RRT-PCR or cloacal swabs from waterfowl for virus isolation). Coordination of these efforts will be through PDA and the contractors.

Once a flock within the protection or surveillance zones has tested negative, movement of birds or eggs can commence by permit issued by PDA under the conditions that any obvious clinical signs of LPAI be reported immediately. Continued dead bird surveillance of flocks in the protection and surveillance zones will be performed on a weekly basis.

A most important aspect of surveillance is to determine possible links to the outbreak, both prior to or after the outbreak, as possible sources of LPAI or recipients of virus (dangerous contacts). PDA personnel will need to determine these dangerous contacts from two weeks prior to the break onward through interviews and viewing of records with the index case flock personnel. These dangerous contacts are prioritized in order of risk of spread. The top priority would be persons who actually entered the flocks and visited other flocks (flock supervisors, caretakers, repairmen, catch crews, vaccinators, etc). Second in priority would be egg pickup trucks and drivers. The third in priority would be deliveries of various sorts where activity is close to the buildings, i.e. feed trucks and drivers, gas delivery, etc. Appropriate samples will be taken from these flocks to determine if these flocks served as a source of the LPAI infection or spread from the index case occurred to that flock.

In all of the above actions, LBM supply flocks would be included in the plan. As concerns backyard flocks, an effort has not been made to my knowledge by PDA or any other agency to identify or collect information on backyard flocks. Backyard flock surveillance, if an outbreak occurs at this time, would need to be done in the zones as the occasion arises.

In summary, the prior collection of up-to-date contact information in regard to all flocks, commercial and LBM supply, is essential for a quick response to an LPAI outbreak. Efforts to gather information about the location and contact information for backyard flocks is needed to improve the control of an outbreak.

Business Continuity in the Face of an AI Outbreak

Will Hueston, Center for Animal Health and Food Safety, University of Minnesota, 1354 Eckles Avenue, St. Paul, MN 55108

Appendices

North Central Avian Disease Conference By-Laws

Revised September 2002

I. Membership

The North Central Avian Disease Conference shall include the geographic area composed of the following states: Illinois, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota and Wisconsin. Other states or provinces contiguous to the area may affiliate upon request. Any individual interested in poultry disease control and eradication, research, or diagnosis, and residing in the above named geographic region shall be considered eligible for membership. Membership will be maintained by attendance at the annual meeting or by request of the member. Voting privileges will be extended to those members who have attended at least two annual meetings in the past five years.

II. Guests

Host institutions shall have the privilege of inviting individuals from their organization to attend sessions when the meeting is held on their campus or facility.

III. Conference Officials and Committees

The executive committee shall be the governing body of this conference. The committee shall be composed of the chairperson, the secretary-treasurer, the chairperson-elect, two members-at-large, **the chair of the fund raising committee, the chair of the publications committee** and the past chairperson as an ex-officio member.

A. Terms of Office

The chairperson, chairperson-elect and two members-at-large shall be on a rotational term basis. The term of office for the chairperson and chairperson-elect shall be a period of two years. A new member of the executive committee shall enter as a member-at-large. New terms of office shall take place at the annual business meeting; wherein, the past chairperson will step down and the chairperson-elect will become the new chairperson. The member-at-large who is at the end of his/her second year (of a 2-year-term) will then become the new chairperson-elect and a new member-at-large will be elected annually. The new member-at-large shall be nominated annually to be the executive committee and approved by a majority vote of the membership at the annual business meeting. **Members-at-large are usually future meeting hosts.** The secretary-treasurer shall be nominated by the executive committee and approved by a majority vote of the membership at the annual business meeting.

B. Duties

1. Executive Committee

It shall be the duty of the executive committee to select the location of the annual meeting. This shall be for the meeting four years in advance when possible. The executive committee will select the location of the meeting and present it to the membership for approval by a majority vote at the annual business meeting. The executive committee will function as the program committee in preparing the annual meeting. The executive committee shall initiate and activate such functions as may be considered necessary during the interim between annual meetings. The executive committee shall meet at the North Central Avian Disease Conference prior to the annual business meeting.

2. Chairperson

The chairperson shall preside over the annual business meeting and perform the duties of such office. The chairperson will be responsible for arranging the time and place of the meetings and shall approve the program and arrange for publication of the proceedings. The chairperson may assign duties or appoint such committees as deemed necessary to conduct the affairs of this conference.

3. Secretary-Treasurer

The secretary-treasurer shall maintain the records and files of the North Central Avian Disease Conference. The secretary-treasurer shall maintain a list of the names and addresses of members in attendance. He shall keep minutes of the meetings and perform the usual duties of a secretary-treasurer and such other duties as assigned by the chairperson and/or the executive committee.

4. Standing Committees

Awards Committee: This committee will be composed of 3 members appointed by the executive committee. Membership will be for 3 years with a new member appointed annually. The longest serving member will serve as chair. The committee will select the recipients of the Service Award, the Ben Pomeroy Award, and any other awards approved by the membership.

Fund Raising Committee: The executive committee will appoint members of the committee annually. The committee will solicit funds to support the annual meeting, publications, awards, and other activities designated by the membership.

Publications Committee: The individual in charge of the publication of the proceeding of the annual meeting will serve as chair of this committee. One or two other members will be appointed by the executive committee, one of which will be in charge of the conference website.

Minutes

57th North Central Avian Disease Conference
March 20, 2006

Program Chairman Mick Fulton called the meeting to order at 2:12 pm. 2005 minutes were amended and approved. The low attendance concern was addressed by various members. Possible solutions discussed were: logistics of NCADC during the MPF, closer ties to the NEADC, advertising complete program w/symposium further in advance, more "take home" information, timing (WPDC), industry concern over total number of meetings attended, symposium questionnaire for membership.

2007 symposium committee is to consist of Drs. Wu, Fulton and Saif

2008 program chair is Dr. Richard Slemons
2009 program chair is Dr. Mahesh Kumar
2010 program chair is Dr. Dan Shaw

Need was expressed for NCADC to urge AAAP to take a major role in Avian Influenza Control education due to others' experiences and perceived urgency. Interested members drafted proposal to immediately establish a committee to recommend the best method of appropriate response, control and prevention of a High Path AI outbreak in commercial and noncommercial poultry, and exotic, captive and wild birds. This information should be disseminated to the membership and public in a timely manner no later than the fall of 2006. It should be done in conjunction with appropriate governmental agencies. 2007 Program Chairperson Ching Ching Wu sent attached letter to AAAO President Robert Owen.

Chairman Fulton thanked the membership, executive committee (Wu, Halvorson, Slemons, Kumar and Bryan), the fundraising committee (Lippert, Bryan and Saif),and the awards committee for their input for the 2006 program. Secretary/Treasurer position will remain the same until the 2007 meeting.

After 2006 NCADC expenses, there remained approximately $10,000 in the checking account.

Dr. Fulton introduced 2007 Program Chairperson Ching Ching Wu. Dr. Wu expressed thanks for Dr. Fulton's leadership and presented a plaque of recognition. 2007 symposium topic was briefly discussed followed by adjournment.

At the Awards dinner, Dr. Fulton presented the Ben S. Pomeroy Award to Dr. Hadi Yassine.

Dr. Mahesh Kumar was presented the 2006 Meritorious Service Award *in absentia*.

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ANIMAL DISEASE DIAGNOSTIC LABORATORY

March 31, 2006

Dr. Robert Owen
President
AAAP
953 College Station Road,
Athens, GA 30602-4875

Dear President Owen:

The membership of the 57th North Central Avian Disease Conference urgently and unanimously recommends that the American Association of Avian Pathologists (AAAP) Board of Directors proceed immediately to establish a committee to recommend methods of appropriate response, control and prevention of an high pathogenic Avian Influenza (high path AI) outbreak in commercial, non-commercial poultry and exotic, captive and wild birds. This information should be disseminated to the AAAP membership, appropriate governmental agencies and the public in a timely manner, no later than the fall of 2006.

We appreciate your timely attention on this matter. We believe this is an urgent matter and requires immediate plan and actions from AAAP.

Sincerely,

Ching Ching Wu, DVM, PhD,
Chair,
North Central Avian Disease Conference

406 S. University Street ※ West Lafayette, IN 47907-2065 ※ (765) 494-7440 ※ Fax: (765) 494-9181

Minutes

56th NCADC Business Meeting
March 15, 2005

The 56th NCADC business meeting was called to order by program cochair, Dr. David Halvorson at 3:15pm with 29 members present. Dr. Halvorson called for review of the 2004 minutes and approval. Minutes were approved. Thanks were expressed to program committee, fundraising committee (Lippert, Mills, Bryan) and awards committee (Fulton, Pedersen, Wu), Jan Swanson and Sarah Sommerbelle (University of Minnesota Continuing Education). Executive committee proposal was read as follows: "NCADC extends appreciation to Midwest Poultry Federation for sharing meeting rooms with NCADC at no cost to NCADC. It is proposed that NCADC hold its meetings in coordination with MPF and that NCADC will be willing to share its speakers or members with MPF in educational workshops. NCADC recognizes that the timing of the meeting may present weather unknowns, necessitating air travel for some graduate students presenting papers; therefore, additional revenues will be sought as well as reducing graduate student registration costs 50% without presentation and to no registration cost with presentation." Proposal was seconded by Dr. Kumar. Discussion followed. Proposal passed. Dr. YM Saif agreed to assist fundraising committee on behalf of graduate student travel support.

2007 program chair is Dr. Ching Ching Wu
2008 program chair is Dr. Richard Slemons
2009 program chair is Dr. Mahesh Kumar

2006 Sec/Treasurer election went to Dr. Tom Bryan. Dr. Halvorson introduced 2006 program chair, Dr. Mick Fulton. Dr. Fulton thanked Drs. Halvorson and Njenga (in absentia) for increasing the NCADC attendance with fine symposium and program. Dr. Fulton reminded attendees of the 2006 symposium topic of vaccines and vaccine delivery systems to be held in coordination with the MPF, March 20-21, 2006. Announcement was made of the Poultry Health School at Michigan State University May 9-11, 2005. 2006 Executive Committee consists of Drs. Fulton, Wu, Halvorson, Bryan, two at-large members (Dr. Slemons and Dr. Kumar), fundraising chair, and publications chair. Fundraising committee consists of Drs. Lippert, Mills, Saif and Bryan. Awards committee will be appointed. Dr. Fulton adjourned the meeting at 4pm.

**At the Awards Luncheon, Dr. Nagaraja presented the Ben S Pomeroy Awards to Dr. Binu Velayudham, UMN, "Studies on a recent isolate of avian metapneumovirus type C in Minnesota", and Ms. Pam Olah, UMN, "Analysis of Salmonella sp and Campylobacter sp isolated from freshly processed turkeys". Dr. Ron Lippert was presented the Meritorious Service Award.*

**Amendments to minutes*

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