

## from the president...

### Fighting Avian Influenza

In this issue of Avian Insight, you will find information on Avian Influenza (AI) vaccines. We hope you find it interesting.

LAHI has a long history and great professional depth in the area of AI vaccine production. We are the only US company with nine Conditional Avian Influenza USDA Licenses. This means we have master seeds for each strain and are ready to make vaccine for a particular type when needed by the industry. In addition, we are equipped to produce an auto-genous AI vaccine from a field isolate.

Addressing the needs of the poultry industry in the case of an outbreak of AI is complex. First of all, state and federal authorities must determine the best way to deal with the outbreak.

They will determine the control approach best suited to the situation: quarantine and eradication, vaccination or a combination of both. To make their determination, they must use multiple professional disciplines.

In the LAHI group, we have several professionals who form the "AI Team" including staff members from our veterinary, regulatory, production and diagnostics laboratory divisions. Once an outbreak has occurred in the US poultry industry, we alert the AI team. They are then included in all further communications on this outbreak. This allows us to have, at our disposal, up to date information on the disease outbreak (veterinarian), USDA and state decisions (regulatory), manufacturing (LAHI head of production) and diagnostics (in-house diagnostics experts). Since AI outbreaks are rare, it is critical that

affected producers and companies are given accurate information in response to their inquiries. Through the development of our AI team, LAHI is prepared to service the poultry industry in an efficient and knowledgeable manner when AI vaccines are needed.

If you are involved in an AI outbreak, we hope you will turn to us when the decision to vaccinate has been made.

### Erratum

In the previous edition of Avian Insight, a typographical error occurred in Dr. Peter Holt's article. In the Materials and Methods section, experiment 2, sentence beginning with "For any samples with no growth...", the units on that volume should be in microliters. We apologize for any confusion this error may have caused.

# avian insight

A LOHMANN ANIMAL HEALTH NEWS BRIEF

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## Evaluation of a commercial avian influenza (H7N2) vaccine for protection in turkeys against an avian influenza virus (H7N2) isolated from turkeys in Virginia during 2002

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The following is a written summary of Dr. Tumpey's presentation from the 52nd Western Poultry Diseases Conference, Sacramento, CA entitled "Protective efficacy of an inactivated Avian Influenza vaccine against challenge with a 2002 H7N2 Avian Influenza virus."

with AI virus from the feces of infected wild birds has been a source of infection for domestic turkeys in the U.S.

When AI infects poultry, the virus is excreted from both the respiratory and the digestive tracts. Therefore bird-to-bird transmission is very efficient via aerosol and contaminated feces and droppings or various fomites. It can cause a wide range of disease symptoms of which there are two main pathotypes. Highly pathogenic (HP) AI causes an extremely infectious, multi-organ systemic disease, resulting in high mortality and hemorrhagic or inflammatory lesions. It has been estimated that the next outbreak of a HPAI virus, similar to the 1983 outbreak in Pennsylvania, will have an economic impact of over \$150 million to the U.S poultry industry. AI associated with mild respiratory disease, reductions in egg production and moderate increases in mortality are considered low pathogenic (LP). Surveillance of poultry has resulted in

detection of LPAI virus by isolation or serology among commercial poultry in sixteen states from 1992 through 1998. Major outbreaks involving more than one million turkeys occurred in Minnesota during 1991 through 1995 and in Utah during 1995. In 2000, H6N2 LPAI outbreak among layer chickens occurred in California resulting in a drop in egg production and mild respiratory disease in the uncomplicated form. Also, significant morbidity and mortality was attributed to secondary infections. Many other subtypes of AI have been recovered from poultry and other gallinaceous birds in the last 3 years including H1N2, H5N2, H3N8, H3N4, H6N1, H6N8, and H9N4. Thus, the poultry industry is constantly faced with the potential threat of AI virus infections which can result in substantial economic losses.

More recently, a LPAI virus, type H7N2, was isolated in the Shenandoah Valley of Virginia and portions of North Carolina,

### INTRODUCTION

Avian influenza (AI) is a disease of poultry that has occurred worldwide over the past 100 years. AI is caused by type A Orthomyxoviruses of which there are 15 hemagglutinin (H1-H15) and nine neuraminidase (N1-N9) subtypes. Each AI virus is designated by the specific subtype, for example H5N9. Although chickens and turkeys are not natural host species for AI, the viruses can routinely crossover from the wild-bird reservoir to poultry. It is thought that these transmissions are the result of wild birds shedding large amounts of infectious virus into the environment. For example, water contaminated

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Pennsylvania and West Virginia. This outbreak started in and was largely confined to turkey breeder flocks and meat-type birds; however the broiler and broiler-breeder industries have also been impacted. Before the last positive farm was detected in July of 2002, Virginia's State Veterinarian quarantined 197 Shenandoah Valley poultry farms and ordered the depopulation of 4.7 million turkeys and chickens. The outbreak is estimated to have cost the poultry industry approximately \$130 million.

When flock outbreaks of AI occur, the implementation of control measures and eradication or "stamping-out" policies usually becomes the goal, however vaccination may also be considered as part of the intervention strategy. For example, AI inactivated vaccines were used as part of a control program against sporadic outbreaks of LPAI in Minnesota and Utah. One vaccine strategy in the U.S. for AI includes the use of killed antigen preparations to immunize flocks. These vaccines are full or conditionally licensed for parenteral (subcutaneous or intramuscular) administration and have been successful at providing protection against clinical signs and death. However, controlling AI through vaccination presents a challenge due to continual antigenic changes of viral surface glycoproteins, hemagglutinin (HA) and to a lesser extent neuraminidase (NA). Effective influenza vaccines are largely based on the induction of strain-specific immunoglobulin G (IgG) neutralizing antibodies directed against the HA, which can persist for extended periods in the host. Antibodies to the second major viral surface protein, NA also contributes to recovery from infection. Immunity induced by influenza vaccines provides optimal protection against viruses that are antigenically closely matched with those in the vaccine, but are less effective against antigenic variants within a subtype and provides little, if any, resistance to infection with a different AI virus. Therefore, determination of antigenic relatedness between AI isolates and vaccine efficacy against antigenic variants will provide valuable information to the poultry industry.

The outbreak of H7N2 LPAI in Virginia this past year raised serious questions about the availability of vaccines to provide protection against disease in poultry. A study was undertaken by the U.S. Department of Agriculture at Southeast Poultry Research Laboratory to determine if an existing commercial H7N2 AI vaccine

manufactured by Lohmann Animal Health could provide protection against a recent H7N2 2002 isolate.

### MATERIALS AND METHODS

The commercial AI vaccine was prepared by Lohmann Animal Health. The seed stock, A/Chicken/Pennsylvania/21342/97 (CP/97, H7N2) virus had previous approval by the U.S. Department of Agriculture as an H7 subtype vaccine. The vaccine virus was propagated in embryonated chicken eggs, inactivated with formalin, and emulsified in a proprietary oil-based vaccine. The efficacy of this vaccine was tested in two different age groups of turkeys obtained from the British United Turkeys of America (Lewisburg, WV). Groups of eight 1-day-old and 4-week-old turkeys were immunized with the commercial inactivated vaccine either once or twice. Control turkeys received the same volume of normal allantoic fluid emulsified in the same adjuvant and are identified as sham-vaccinated. Turkeys were vaccinated by subcutaneous inoculation of 0.5 ml vaccine in the nape of the neck. Boosted turkeys received a second inoculation 14 days after initial vaccination. Serum was collected from each bird 14 and 28 days after initial inoculation. Hemagglutination-inhibition (HI) assays were performed in 96-well microtiter plates with 0.5% chicken erythrocytes by standard methods. At 29 days after initial vaccination, turkeys were challenged intranasally with  $10^{7.0}$  EID<sub>50</sub> of A/Turkey/Virginia/15851/02 (TV/02) in a volume of 100  $\mu$ l. An additional group of eight 4-week-old control White Plymouth Rock (WPR) chickens (Southeast Poultry Research Laboratory [SEPRL], Athens, GA) were infected similarly for comparison. Following infection, chickens and turkeys were monitored daily for disease signs for 14 days post-infection. For determination of virus shedding, oropharyngeal and cloacal swabs were taken from 8 birds on days 1-5, 7, 10 and 13 post-infection. Virus isolation and titrations were performed in 10-day-old embryonated chicken eggs and fifty percent egg infectious dose (EID<sub>50</sub>) titers were determined by standard methods.

### RESULTS AND CONCLUSION

Because LPAI H7N2 was detected mostly in turkey flocks, with only a few chicken flocks affected, we first compared susceptibility of the two species to H7N2

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virus infection. **Figure 1** illustrates the mean virus titers recovered from the oropharynx of A/Turkey/Virginia/15851/02 (TV/02) virus-infected chickens and turkeys following an intranasal inoculation with  $10^7$  EID<sub>50</sub> of TV/02 virus. The limit of virus detection was  $10^{1.2}$  EID<sub>50</sub>/ml. In general, TV/02 virus was recovered from the oropharynx of both species during the first week of infection. The level of infectious virus recovered from turkeys was 20 to 158 fold higher than what was detected in the oropharynx of chickens. Further experimentation compared the fifty percent bird infectious dose (BID<sub>50</sub>) in chickens and turkeys by determining the infectious virus titers in the oropharynx at 3 days post-inoculation. It was determined that 100 to 200 times more TV/02 virus was required to infect chickens versus turkeys.

In the vaccine trial, neither sham-vaccinated nor H7N2-vaccinated turkeys developed clinical signs or death following challenge with  $10^7$  EID<sub>50</sub> of TV/02 virus. However, high titers of challenge virus could be detected from swabs collected from the oropharynx on days 1-7 after challenge in the sham-vaccinated group (**Figure 2**). Low or undetectable viral titers were recovered from corresponding cloacal samples from control turkeys or chickens (not shown), indicating that this LPAI virus replicates more efficiently in the respiratory tract versus the gastrointestinal tract. **Figure 2** illustrates that vaccinated (1x and 2x vaccinated) turkeys had a significant reduction in titers of challenge virus shed from the oropharynx in comparison to the sham-vaccinated groups for days 1-5 after challenge. Furthermore, viral shed in vaccinated birds decreased such that only one turkey of 16 was positive for AI virus in the oropharynx, whereas all 8 sham-vaccinated controls still had high viral titers at day 5 post-challenge. A vaccine boost

enhanced the protection from infection as the mean virus titers for days 1-3 after challenge were reduced by 5 to 8 fold in comparison to turkeys that received a single vaccine. Sera were collected from eight turkeys per group and tested for the presence of HI antibody titers against the homologous vaccine virus as well as the 2002 H7N2 virus. HI antibody titers induced by either H7N2 virus were similar as one dose of vaccine induced an HI response of 32 and administration of a second dose of vaccine resulted in an elevated HI antibody of 64.

Figure 2. Vaccine Efficacy

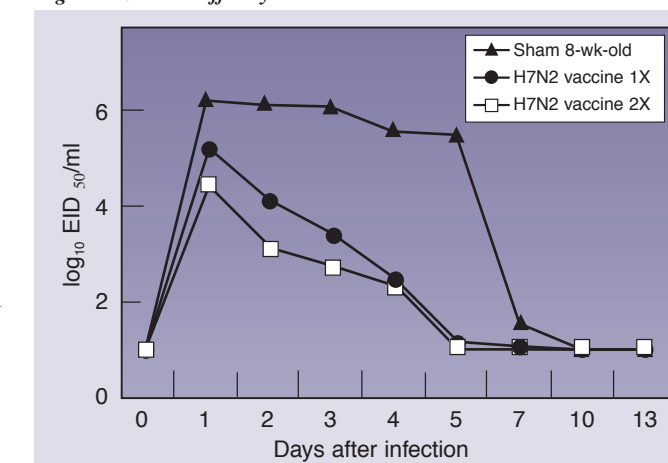
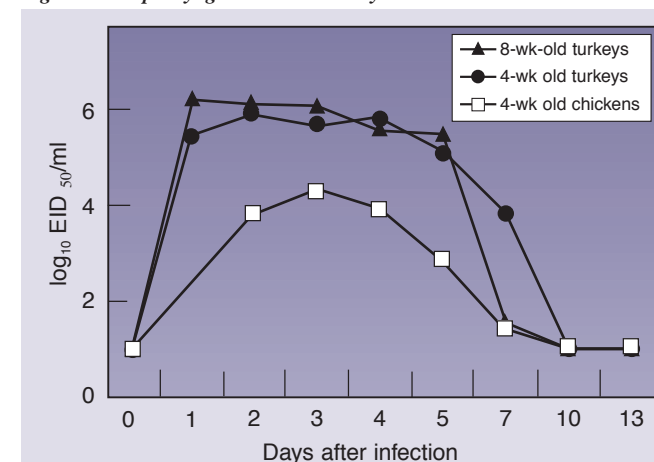


Figure 1. Oropharyngeal Virus Recovery



Once an AI outbreak begins there is insufficient time to prepare a new AI vaccine to control the initial spread of virus. Information regarding the vaccine efficacy of an existing commercial vaccine provides affected states and poultry industries with valuable information when considering vaccination as part of the control or eradication program. This study demonstrated that an AI vaccine prepared from a 1997 seed stock (A/Chicken/Pennsylvania/21342/97) virus provided protection against a recent 2002 AI virus isolated from a turkey in Virginia. The CP/97 vaccine significantly reduced both the number of turkeys shedding the challenge virus and titers shed from vaccinated turkeys as compared to sham vaccinates. The HA protein of the recent 2002 H7N2 Virginia isolate shares a 97% protein sequence identity with the CP/97 vaccine. It has been demonstrated that in poultry the challenge viruses can have as little as 85% HA protein sequence similarity with the vaccine strain and still provide vaccine protection. This broad-based protection against AI isolates observed in poultry provides opportunities to use existing vaccines even in the face of antigenic drift in seasonal field AI viruses.