

ABSTRACTS
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SYMPOSIA AND ORAL SESSIONS

Keynote Symposium

1 Network research and education structures to prevent and control pandemic avian influenza. D. R. Perez*¹ and AICAP Network², ¹*University of Maryland, College Park*, ²*Multiple institutions across the United States*.

During the course of over 2 years since it was initiated, the program on Prevention and Control of Avian Influenza in the US (AICAP) funded by the CSREES-USDA has been strongly committed to building and providing a network research platform in which both new and established investigators could find the necessary synergy to accomplish their respective research objectives in an integrative manner. We are certainly proud of our accomplishments, particularly with respect to the integration of research and education efforts and how the former served as the basis to develop and/or envision new educational efforts. There is no doubt in our minds that our strategy of integration of research and education has paid important, and many times intangible, dividends and that the poultry industry in particular and the public in general have been the greatest beneficiaries of our efforts. Our accomplishments are the result of the unanimous conviction among the AICAP participants that this network research structure is far more than the sum of the parts and that our major strength is that we can work together toward a common goal, which is to prevent and control avian influenza in the United States. Great progress has been made in all the programmatic areas of the project from the molecular basis of interspecies transmission and pathogenesis, to the implementation and training in alternative methods of mass euthanasia and disposal and the development of additional diagnostic tools and vaccination strategies. Our program thus serves as a highly successful experience, which can be applied to many other countries or regions around the world in order to minimize the economic and public health impacts of avian influenza.

Key Words: avian, influenza, network

2 AI infection in animals: A challenge and opportunity for the poultry veterinarian. I. Capua*¹ and D. J. Alexander², ¹*OIE/FAO and National Reference Laboratory for Avian Influenza and Newcastle Disease, Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy*, ²*Virology Department Veterinary Laboratories Agency, Weybridge Addlestone, Surrey, United Kingdom*.

Avian influenza viruses of the H5N1 subtype have become widespread in vast areas of Asia, the Middle East, Europe, and Africa. This opportunity given to the virus has greatly increased its potential to affect the health of wild and domestic animals and humans. Currently, human health is affected both in terms of the reduction of food security and as a result of infection of humans which could be a prelude to the emergence of a new pandemic virus. In the current situation, the persistence of a highly pathogenic H5N1 virus in diverse sectors of the poultry industry has generated a unique set of opportunities for the virus. It has closely encountered avian and mammalian species to which it has been able to adapt and thus enhance its replication efficiency, and in these hosts the virus is probably in a continuous state of evolution. The crucial issue in resolving this situation is to limit the circulation of the virus in the animal reservoir, as this represents a never-ending source of virus. Although specific tools are available, the infrastructure and economic conditions of most of the affected areas are insufficient to react to the emergency. The medical, veterinary, and agricultural scientific communities are challenged with a virus that is moving in a tridimensional fashion, modifying itself as it adapts to different species and reassorting with other influenza viruses of avian and potentially mammalian origin, as it infects new species. A significant collaborative and financial effort in a transparent scientific environment are required to generate data and ideas contributing to the eradication effort. Until the extensive circulation of the virus is limited in the domestic avian reservoir, avian influenza will continue to remain an issue for food security and a global threat for animal and human health.

Key Words: influenza, avian, control

*Author presenting paper.

3 Evaluation of methods for the detection and characterization of avian influenza viruses from wild ducks in the southeastern United States. J. Giambrore*, T. Dormitorio, and K. Guo, *Auburn University, Auburn, AL.*

Wild aquatic birds serve as a natural reservoir for influenza A viruses, which can be transmitted to poultry, and some mammalian species including humans. Many factors are critical for a successful detection and characterization of influenza type A viruses (AIVs) from birds. Egg embryo inoculation, hemagglutination, AC-ELISA, real-time reverse transcription polymerase chain reaction (RRT-PCR), H gene amplification by traditional PCR and sequencing were used to detect, isolate, and characterize AIVs from cloacal swabs of wild ducks from Alabama, Georgia and Florida. Out of 706 swab samples tested so far, only 6 were positive for the matrix gene of AIV by RRT-PCR. None were positive for H5 or H7. One AIV was detected by RRT-PCR (none by AC-ELISA), when cloacal swab samples were used directly for testing. AC-ELISA detected influenza A only when the HA titer of the virus in the allantoic fluid was 256 units or higher. All AIV positive isolates were sent to NVSL for subtyping; however, only one was found to be H10N7 and there was difficulty determining the H subtype of the others. We therefore developed a procedure to amplify the H genes of the isolates for sequencing. After comparing the full H gene sequences of the untyped isolates with other AIV sequences obtained from GenBank by BLAST search, it revealed that the 4 isolates from Alabama (121, 167, 253, and 299) had 94–97% similarity to published H1N1 isolates including one from swine. One isolate (381), which was highly pathogenic to embryos, produced a high HA titer, and was also positive for NDV. Attempts to determine the H gene sequence of this virus has so far been unsuccessful. The incidence of AIVs in wild ducks from the SE USA is extremely low and there has been no report of AIV spread from wild ducks to commercial poultry. The RRT-PCR appeared to be a more sensitive, cost-effective and rapid assay; however, it still needed embryo inoculation, which is time-consuming and labor intensive. The assay needs to be optimized for detection of AIVs directly from cloacal swabs.

Key Words: avian influenza, wild aquatic birds, real-time RT-PCR

4 A gene-based avian influenza vaccine. S. S. Rao*¹, W.-P. Kong¹, C.-J. Wei¹, D. Perez², D. K. Styles³, Z. Z. Yang¹, C. Murray¹, A. Ault¹, C. Andrews¹, and G. J. Nabel¹, ¹*Vaccine Research Center, NIH, Bethesda, MD*, ²*University of Maryland, College Park*, ³*USDA, Maryland*.

Highly pathogenic avian influenza A (HPAI) viruses, specifically H5N1 strains, cause widespread morbidity and mortality in domestic and wild bird populations. While still largely confined to birds, laboratory-confirmed human cases resulting in deaths were reported to the World Health Organization (WHO). These rare human cases are the result of direct virus transmission from infected birds; however a sustained outbreak of HPAI H5N1 in avian species could lead to adaptation to humans. Currently, the US FDA has approved an H5N1 Sanofi vaccine and European regulators have granted preliminary approval of an adjuvanted GlaxoSmithKline vaccine. Both are killed vaccines and are made with egg-based production methods, which could limit manufacturing capacity during a widespread avian outbreak. Challenges to developing HPAI H5N1 vaccines include: developing more efficient manufacturing processes, cross-protection against multiple strains, identifying key viral antigens and identifying immune correlates. While H5N1 vaccines made by reverse genetics allow for rapid generation of safe vaccine strains,

such vaccine viruses are also propagated in eggs. Gene-based vaccines have shown efficacy in animal models, and unlike conventional inactivated vaccines, may induce cellular, in addition to humoral immunity. The benefits of such vaccines include the lack of dependence on eggs in the production process, differentiation from infectious virus, rapid manufacturing, and the capacity to protect against a broad range of viral subtypes. We evaluated protective immunity conferred by DNA vaccines encoding HA from different HPAI H5N1 serotypes in animals. The vaccines elicited antibodies that neutralized multiple serotypes of HPAI H5N1 when given in combinations containing up to 10 HAs. Monovalent and trivalent HA vaccines conferred protection against lethal H5N1 in mice challenged 68 weeks after vaccination. In chickens, protection was observed against heterologous strains of HPAI H5N1 after vaccination with a trivalent vaccine at doses as low as 5 µg. DNA vaccines encoding influenza H5 HA may facilitate the vaccination of poultry and other animal species against newly evolving H5N1 isolates.

Key Words: avian influenza, gene-based vaccine, poultry

5 Protection of chickens against avian influenza with nonreplicating adenovirus-vectored vaccine. H. Toro*¹ and D. C. Tang², ¹*Auburn University, Auburn, AL*, ²*Vaxin Inc., Birmingham, AL*.

Protective immunity against avian influenza (AI) virus has been elicited in chickens by single-dose in ovo or intramuscular vaccination with a replication-competent adenovirus (RCA)-free human adenovirus vector (Ad) encoding the AI virus H5 (AdTW68.H5) or H7 (AdChNY94.H7) hemagglutinins. AdTW68.H5-vaccinated chickens were protected against both H5N1 and H5N2 highly pathogenic (HP) AI virus challenges. AdChNY94.H7-vaccinated chickens were protected against an H7N3 HPAI virus challenge. Chickens vaccinated in ovo with AdTW68.H5 followed by posthatch intramuscular vaccination with AdChNY94.H7 responded to both vectors with robust antibody titers against both the H5 and H7 AI proteins. The use of a synthetic AI H5 gene codon optimized to match the tRNA pool found in chicken cells is more potent than the cognate H5 gene. Mass-administration of this AI vaccine can be streamlined with available robotic in ovo injectors. In addition, Ad5-vectored vaccines can be produced rapidly and the safety margin of the nonreplicating vector is superior to that of a replicating counterpart. Furthermore, this mode of vaccination is compatible with epidemiological surveys of natural AI infections.

Key Words: adenovirus vector, avian influenza, chicken

6 Development and evaluation of a potential universal *Salmonella*-vectored avian influenza vaccine. B. M. Hargis*¹, S. L. Layton¹, D. R. Kapczynski³, K. Cole¹, M. M. Cox¹, Y. M. Kwon¹, L. R. Berghman², K. Liljebjelke³, and W. J. Bottje¹, ¹*Department of Poultry Science, University of Arkansas, Fayetteville*, ²*Department of Poultry Science, Texas A&M University, College Station*, ³*Southeast Poultry Research Laboratory, ARS/USDA, Athens, GA*.

Development of vaccines for effective control of avian influenza (AI) virus in poultry and wild birds is in high demand. Most AI vaccines target the immunodominant antigens such as hemagglutinin (HA) and neuraminidase (NA); however, these vaccines only provide protection against a particular AI serotype. Among the many vaccine strategies, bacterial vectored vaccines expressing an antigenic property of AI are

a promising strategy. We have recently created a *Salmonella*-based AI vaccine, which targets the extracellular domain of the universally conserved ion channel protein M2 or M2e. Antibodies against M2e have previously been shown to provide protection against influenza challenge. Wild type *Salmonella* Enteritidis 13A was attenuated (Δ aroA/ Δ htrA) and the genome manipulated to express M2e protein epitopes (M2e: EVETPIRN, EVETPTRN) as a fusion with the outer membrane protein LamB. In addition, a short 10aa sequence of CD154 (WAEKGYTMS) was inserted to test its potential immune-enhancing function. Following vaccination with the *Salmonella* recombinant vaccine, chickens demonstrated significantly increased M2e-specific IgG antibody titers. Virus neutralization assays in chicken embryos resulted in neutralizing titers in the range of 5.8–6.3 (log₂), indicating the antibodies induced were effective at neutralizing AI. Direct sublethal challenge of vaccinated chicks with A/Turkey/Virginia/158512/2002 (TV/02) H7N2 low pathogenic AI (LPAI) and A/Egret/Hong Kong/757.2/2002 (Eg/02) H5N1 high pathogenic AI (HPAI) viruses showed a significant decrease in morbidity and duration of clinical signs. However, protection from mortality was not observed against direct lethal challenge with HPAI in a subsequent trial. These data suggest that this candidate vaccine may provide broad protection against LPAI; however, additional epitopes might be required for protection against lethal HPAI challenge.

Key Words: avian influenza, vectored vaccine, *Salmonella*

7 The public health impact of avian influenza viruses. J. M. Katz*, K. Hancock, V. Veguilla, J. A. Belsler, T. R. Maines, N. Van Hoeven, C. Pappas, and T. M. Tumpey, *Centers for Disease Control and Prevention, Atlanta, GA.*

Influenza viruses with novel HA and one or more accompanying genes derived from avian influenza viruses, sporadically emerge in humans and have the potential to result in a pandemic if the virus causes disease and spreads efficiently in a population that lacks immunity to the novel HA. Since 1997, multiple avian influenza virus subtypes have been transmitted directly from domestic poultry to humans and have caused a spectrum of human disease, from asymptomatic to severe and fatal. To assess the pandemic risk that avian influenza viruses pose, we have used multiple strategies to better understand the capacity of avian viruses to infect, cause disease and transmit among mammals, including humans. Seroepidemiologic studies that evaluate the frequency and risk of human infection with avian influenza viruses in populations with exposure to domestic or wild birds, can provide a better understanding of the pandemic potential of avian influenza subtypes. Investigations conducted in Hong Kong following the first H5N1 outbreak in humans in 1997 determined that exposure to poultry in live bird markets was a key risk factor for human disease. Among poultry workers, butchering and exposure to sick poultry were risk factors for antibody to H5 virus which provided evidence for infection. A second risk assessment tool, the ferret, can be used to evaluate the level of virulence and potential for host-to-host transmission of avian influenza viruses in this naturally susceptible host. Avian viruses isolated from humans exhibit a level of virulence and transmissibility in ferrets that generally reflects that seen in humans. The ferret model thus provides a means to monitor emerging avian viruses for pandemic risk, as well as evaluate laboratory generated reassortants and mutants to better understand the molecular basis of influenza virus transmissibility. Taken together, such studies provide valuable information with which we can assess the public health risk of avian influenza viruses.

Key Words: pandemic, avian influenza, risk

8 Surveillance efforts through the NIAID Influenza Centers of Excellence (CEIRS) and resources available to the scientific community. D. J. Post*, *DMID/NIAID/NIH, Bethesda, MD.*

The National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH), recently awarded \$23 million per year for 7 years to establish 6 Centers of Excellence for Influenza Research and Surveillance (CEIRS). Collectively, the centers will expand NIAID's influenza surveillance program internationally and in the United States, and will bolster influenza research in key areas, including understanding how the virus causes disease and how the human immune system responds to infection with the virus. The goal of the newly created centers is to provide the federal government with important information to inform public health strategies for controlling and lessening the impact of seasonal influenza as well as an influenza pandemic. Three of the 6 centers work focuses on animal influenza surveillance: St. Jude Children's Research Hospital, University of Minnesota and University of California, Los Angeles. Their work includes determining the prevalence of avian influenza in animals that routinely come into close contact with people; understanding how flu viruses evolve, adapt and transmit infection; and monitor for international and domestic cases of animal and human influenza to rapidly detect and characterize viruses that may have pandemic potential and to create vaccine candidates targeted to those viruses. Ultimately, these studies will lay the groundwork for developing new and improved control measures for emerging and reemerging flu viruses.

Key Words: influenza, surveillance, CEIRS

9 Pandemic influenza planning: Shouldn't swine and poultry workers be included? G. C. Gray*, *University of Iowa College of Public Health, Iowa City.*

Recent research has shown that swine and poultry workers, especially those with intense exposures, are at increased risk of zoonotic influenza virus infections. Multiple studies have found US swine workers to have very strong evidence of previous infections with swine influenza viruses compared to nonexposed controls. Similarly, poultry workers and poultry veterinarians have been shown to be at increased risk of avian influenza virus infections. Mathematical modeling has demonstrated that such workers may accelerate the spread of pandemic viruses in their rural communities. As these workers may contribute to the novel generation of viruses, and serve as a bridging population in the cross-species sharing of influenza viruses, it seems prudent to include swine and poultry workers in influenza preparedness programs. Possible preventive and control interventions include: special education programs to increase workers use of personal protective equipment such as gloves, increased surveillance for influenza viruses among workers and their animals, recommendations that workers seek medical attention should they develop influenza-like illness, workers priority receipt of annual influenza vaccines, and workers priority receipt of pandemic vaccines and antivirals.

Key Words: zoonoses, influenza, pandemic

10 Canadian experiences with AI: A look at regional disease control, past, present, and future. J. P. Vaillancourt*, *University of Montreal, St-Hyacinthe, PQ, Canada.*

Over the past 4 years, the poultry industry in Canada has had 3 outbreaks of reportable avian influenza (2 highly pathogenic H7N3, and one low pathogenic H5N2). In addition to this, several provinces have conducted tabletop and field simulations. From these experiences, it is clear that no one organization has the resource capacity to control avian influenza alone. Hence, both government and industry specialists have been working on improving regional disease control strategies. The poultry industry has the opportunity to act quickly within the first few days of an epidemic to minimize spread of the disease and to establish an effective support structure to assist federal authorities once the disease has been confirmed. To do this, provincial poultry organizations have created teams responsible for decision making, field operations, and communications as soon as an epidemic is suspected. Current efforts include establishing an early reporting system with intervention teams responsible to assist growers with self quarantines, traceback data gathering, poultry traffic control, and even depopulation, disposal, and decontamination procedures. Ontario currently works with insurance institutions to eventually offer coverage to growers to cover expenses not paid by the federal government in case of eradication, such as production losses. All regions have developed training programs for industry personnel regarding biosecurity and regional disease control, including testing these plans with simulations. The future of AI control will require an active surveillance system offering proper compensation to growers in case H5 or H7 strains are identified. Early detection remains the most critical challenge. There are others: confidentiality issues in the context of communications prior to disease confirmation; traceability of people and equipment; quick industry led interventions prior to the federal government being in charge of the control efforts; minimizing the impact of disease control efforts on poultry businesses; and managing the risks associated with smaller poultry operations that are not part of the mainstream industry.

Key Words: avian influenza, disease control, biosecurity

11 AI monitoring plan in Texas, how it was developed and how the plan has worked in the face of outbreaks, with reference to export market access. J. B. Carey*, *Texas A&M University, College Station.*

The Texas AI monitoring and indemnity plan was developed in 1995 in response to industry concerns. The plan is coordinated among responsible state agencies, the poultry industry and the Texas Poultry Federation. Plan elements include sections on purpose and implementation, participating members, funds assessment, initial loss schedule, monitoring program, outbreak response guidelines, vaccination program, flock valuations, biosecurity plan, guidance for carcass burial, and the Texas Animal Health Commission manual of standard operating procedures for Texas LPAI response. The lowest level of monitoring requires 25% of broiler and turkey flocks as they are processed. All breeder flocks are tested every 60 days. Fifty percent of commercial layer flocks are tested every 30 days and all pullet flocks are tested 2 weeks prior to movement. In the event that the risk is increased due to a known infected flock in the area, the level of monitoring is increased within specified zones surrounding the index case(s). The outbreak response guidelines detail reporting and handling of suspected and confirmed flocks including movement restrictions. Provisions for the use of vaccine are also detailed. The monitoring plan has detected 2 infected flocks. Case 1 was identified May 2002 and case 2 was identified May 2004. In both cases, the infection was contained at the index site.

The plan provides the opportunity to educate and inform international trading partners of the level of monitoring and containment procedures that are present in Texas. Reporting of an infected flock of any type has immediate international repercussions. Trade is immediately curtailed following the identification of any AI infection. Trading partners vary widely in their willingness to reopen export markets following such an incident. For example, following the case 2 (May 2004) one important trading partner did not reopen exports until October of 2007. Reasons for such activity in international trade go far beyond the epidemiological aspects of avian influenza.

Key Words: avian Influenza, monitoring, international trade

Symposium: Current and Future Prospects for Natural and Organic Poultry

12 Current and future prospects for organic poultry: Symposium introduction. F. T. Jones* and S. C. Ricke, *University of Arkansas, Fayetteville.*

Recent retail surveys have suggested that over one-half of all Americans have tried organic foods or beverages and nearly 1 in 10 use organic products on a regular basis. Sales of organic foods have nearly tripled since 1997. The fastest growing segment of organic food products is fresh meat and seafood sales and the largest volume of organic meat sales is for poultry. Chicken is the most widely available and most popular organic meat. It is anticipated that organic poultry processing will follow the "logical market development" taken by conventional poultry: first developing a strong production base, then integrating processing and finally moving strongly into higher margin, value-added products. In anticipation of the market trends it is critical that economical, consumer and food safety issues that may be unique to organic and natural poultry be anticipated with focused research on production and processing.

Key Words: organic, natural

13 Organic poultry, consumer perceptions and regulatory issues. P. G. Crandall*¹, E. C. Friedly¹, M. Patton¹, S. Seideman¹, S. C. Ricke¹, C. A. O'Bryan¹, A. F. Fanatico², and R. Rainey¹, ¹*University of Arkansas, Fayetteville,* ²*National Center for Appropriate Technology, Fayetteville, AR.*

Organic poultry is considered to be a gateway food for consumers who are beginning to purchase organic foods. Organic and all natural poultry production, processing and marketing present unique opportunities for poultry companies. However, there are new food safety challenges that must be met because of the restrictions placed by USDA Certified Organic requirements. This paper presents the background on the growing demand for organic foods, definitions for USDA Certified Organic and the reasons behind the growing consumer market in the United States and Europe. Anecdotal sources consider farmers markets to be the preferred sales outlet for pioneers who are just starting to grow organic poultry. There are persistent reports that many of the current 4,400 farmers markets provide a nurturing environment for these new organic growers. Consumers at farmers markets have tradi-