SAES-422 Multistate Research Activity Accomplishments Report

Project/Activity Number: NC1180
Project/Activity Title: Control of Emerging and Re-emerging Poultry Respiratory Diseases in the United States
Period Covered: October 1, 2015 to September 30, 2016
Date of This Report: December 15, 2016
Annual Meeting Date: October 20, 2016

Brief summary of minutes of annual meeting:

The annual meeting was held on Thursday, October 20, 2016, in conjunction with the USAHA annual meeting at the Sheraton Greensboro Hotel, Greensboro, NC, Blue Ashe room.

Dr. Mary Pantin-Jackwood, Chair of NC 1180, opened the meeting at 8:10 am. She welcomed the station representatives and participating scientists. The list below shows the attendees.

Name	Affiliation	State Representative
Mazhar Khan	Connecticut	CT
Chang Won Lee	The Ohio State University (OSU)	OH
Michael Abundo	OSU	
John Ngunjiri	OSU	
Mary Pantin-Jackwood	Southeast Poultry Research Laboratory, ARS, USDA (SEPRL)	USDA
David Suarez	SEPRL	
Patti Miller	SEPRL	
David Swayne	SEPRL	
Haroldo Toro	Auburn University (AU)	AL
Rodrigo Gallardo	University of California, Davis (UC-Davis)	CA
Mazhar Khan	Univ. CT (UCT)	CT
Timothy Johnson	Univ. MN (UM)	MN
Sanjay Reddy	Texas A&M	
Calvin Keeler	University of Delaware (UD)	DE
Tsang Long Lin	Purdue University (PU)	IN
Mark Jackwood	University of Georgia (UGA)	GA
Maricarmen Garcia	UGA	
Brian Jordan	UGA	
Naola Ferguson-Noel	UGA	

Due to scheduling issue, the advisor, Dr. Jeff LeJeune, was unable to attend the meeting. The Chair proposed to approve the meeting minutes of 2015 NC1180 meeting. The minutes were approved by the participating station representatives and members unanimously.

The meeting venue and time for annual NC1180 meeting in 2017 was discussed. The 2017 annual NC1180 meeting will be held in conjunction with USAHA and PRD-CAP again at the Town and Country Hotel, San Diego, CA during the week of October 16, 2017.

The Department of Labor's "Overtime Rule" takes effect on December 1, 2016 and the Rule raises the salary level to over $47,476 per year, such change has a significant impact on research budget and operation, especially on hiring post-doctoral scientists and laboratory technicians for the projects. Station representatives and members raised and discussed the issues and concerns. Nevertheless, the execution of the "Overtime Rule" will be determined by each institution.

Dr. Chang-Won Lee talked about expanding NC1180 by recruiting additional state/station members to join NC1180. The states/stations mentioned and discussed were University of Arkansas/Arkansas, North Carolina State University/North Carolina, University of Maryland/Maryland, University of Missouri/Missouri, Iowa State University/Iowa, Pennsylvania State University and University of Pennsylvania/Pennsylvania, Western University/California, USDA/ARS Poultry Research Unit at Mississippi State, and others.

Dr. Pantin-Jackwood, the Chair, reminded station representatives to send her the station reports, including the impact statements, as directed by the official format, immediately after the annual meeting. Dr. Chang-Won Lee also emphasized the importance for each station to include not only the impact statement but also publications and grants in the report to the Chair for the annual report to be uploaded to NIMSS.

The presentation of annual progress report from each participating station began immediately after the business meeting. Dr. Toro/Alabama was the first to give the report and followed by station representatives and members from California, Connecticut, Delaware, Georgia, Indiana, Minnesota, Ohio, SEPRL-USDA, and Texas, respectively.

All members were actively engaged in the discussions on surveillance, pathogenesis, new diagnostics tools, and vaccine/immunology of various poultry respiratory and immunosuppressive diseases. Research findings and new knowledge were freely communicated among the participating members.

The annual NC1180 meeting adjourned at 12:15 pm, October 20, 2016.

Accomplishments:

Objective I: Identify reservoirs of infectious respiratory disease agents in wild birds and poultry.

- Avian influenza virus (AIV) was not detected in LBM and domestic birds in Connecticut, however was detected in wild birds (CT).

-The survival of avian influenza virus (AIV) in chicken feed and the effectiveness of a commercially available disinfectant (Termin-8) in feed were determined. The disinfectant quickly inactivated the virus (AU).

- Phylogenetic analysis of the HA gene segment from an avian-origin H3H9/N2 AIV 2013 isolate indicated that the virus was related to a wild bird lineage H3 AIV. Avian-origin H3 introductions to turkeys appear to be rare events (UM).

- The origins and within outbreak evolutionary changes of the highly pathogenic (HP) AIV H5N2 that caused the outbreaks in U.S. in 2015 were determined with samples from turkeys and their environment collected at different time-points of the outbreak in poultry (UM).

- Study of the persistence of low (LP) and highly pathogenic (HP) AIV in bedding material and feces obtained from different turkey, broiler and egg layer commercial productive units showed that HPAIV (H5N8) was more persistent than LPAIV (H6N2) (UC-Davis).

- The effectiveness of footbaths using different disinfectants, as a tool to inactivate HPAI and LPAI on rubber boots was determined. Quaternary ammonia and phenol + Glutaraldehide based footbaths were not able to eliminate HP and LPAI live viral particles on boots, while a chlorine based granulated disinfectant was able to destroy the virus at contact. The results make clear that footbaths are only one piece of a biosecurity program and we cannot exclusively rely on them in the prevention of pathogen introduction into commercial flocks (UC-Davis).

- Major histocompatibility complex effect on genetic resistance to infectious bronchitis virus (IBV) was investigated. All tested chicken lines, inbred and commercial, were susceptible to IBV infection; however mortality was detected in only a few lines (UC-Davis).

- Epidemiological analyses of IBV cases in California demonstrated two major IBV outbreak peaks affecting broiler chickens. IBV types CA1737 and Cal 99 were the most prevalent genotypes during the analyzed period (UC-Davis).

-An IBV-like coronavirus was detected from the intestines of a flock of broiler chicks showing typical clinical signs of runting stunting syndrome. Sequencing of the S1 gene showed 93.8% similarity to IBV California 99 and 85.7% to IBV Arkansas. The results suggest a broader tissue tropism of this isolate (UC-Davis).

- Surveillance for viruses in wild birds related to the H5 HPAI virus strains that caused the 2014-2015 outbreak in the U.S. demonstrated that the viruses were present in relatively high levels in wild waterfowl at nearly the same time that outbreaks occurred in domestic poultry in the Pacific flyway. Genetic analysis of the wild bird viruses provided additional epidemiologic data to support the role of wild birds as the source of the poultry outbreaks (SEPRL).

- Phylogenetic analysis of 32 H5 HPAI viruses determined the H5N2 reassortant viruses emerged in November 2014, and the H5N1 and H5N8 reassortant viruses emerged in December 2014 through reassortment with North American low-pathogenicity avian influenza viruses (SEPRL).

- Sequencing the genomes of circulating strains of ILTV from the backyard flock in the US identified novel wild type strains of ILTV (SEPRL).

- Variant and emerging Newcastle disease viruses (NDV) were identified through international collaborations Mexico, Nigeria, Bulgaria, Ukraine, Indonesia, Pakistan, Egypt, Russia and U.S. generating important epidemiological information on the movement and evolution of the NDV strains (SEPRL).

- Repeated isolation of virulent NDV (vNDV) of sub-genotype VIId from backyard chickens in Bulgaria and Ukraine between 2002 and 2013 (SEPRL).

- IBV was frequently detected from sick commercial broiler chickens. Vaccine related genotypes were the most commonly detected. NDV was detected in 47 samples but none were virulent vNDV. ILTV detections from commercial broiler flocks were numerous in 2016. Concurrent infections with IBV and NDV, IBV and ILTV or IBV, NDV and ILTV were detected in 36, 96, and 8 submissions, respectively. Avian influenza was not detected in poultry, commercial or non-commercial, or exhibition/show bird in Delaware in 2016 (UD).

Objective II. Develop improved diagnostic capabilities including real time PCR as well as other rapid on-farm tests for economically important respiratory diseases.

- A duplex reverse transcriptase loop-mediated isothermal amplification (dRT-LAMP) assay has been developed and evaluated for simultaneous detection of H6 and H9 AIV subtypes. The assay exhibited high specificity and sensitivity (CT).

- Based upon the comparative analyses of circulating Ornithobacterium rhinotracheale (ORT) isolates, a clade-specific PCR based was developed. This primer set is being used to screen incoming ORT isolates following implementation of a change in vaccine strategy (UM).

-Two diagnostic platforms, dynamic light scattering (DLS) and surface-enhanced Raman spectroscopy (SERS) are being developed for rapid detection of AIV. The DLS platform has potential for a rapid, simple AIV subtyping assay and the SERS platform has excellent potential to move forward with subtype specific, multiplexed assay development (UI).

- Variation in the level of antibiotic resistance was shown with different Mycoplasma synoviae (MS) isolates. Sequencing of the 23SrRNA of these isolates identified point mutations associated with tylosin resistance. PCR protocols were developed to amplify the domains containing the point mutations and tested on tracheal swabs for MS infected poultry. Determining the antimicrobial resistance profile of MS strains in a timely way provides a therapeutic guide for antibiotic selection and judicious use of antibiotics (UGA).

- Studies validate the use of the choanal cleft as an appropriate sampling site for sensitive detection of avian mycoplasmosis by PCR. This will allow producers and field personnel options for routine sampling that require less skill and personnel, as well as reduce trauma to the poultry during sampling increasing bird welfare (UGA).

- Increased virulence of a Mycoplasma synoviae (MS) outbreak strain from Northeast Georgia indicates that avirulent MS strains may progress into more virulent phenotypes after circulating in the poultry population. These results emphasize the benefit of eradicating MS regardless of immediate clinical impact (UGA).

Objective III. Investigate the pathogenesis and polymicrobial interactions of specific infectious agents associated with poultry respiratory diseases (this includes interactions with underlying immunosuppressive agents).

- Studies indicated the first H5 HPAIV that caused the outbreaks in U.S. in 2014-15 were poorly chicken adapted, and had delayed appearance of lesions, longer mean death times, and reduced replication in endothelial cells as compared to historical Eurasian H5N1 HPAIV (SEPRL).

- Previous infection with virulent strains of Newcastle disease virus reduces highly pathogenic avian influenza virus replication, disease, and mortality in chickens (SEPRL).

- NDV strains with epizootic characteristics that emerged in the Middle East and Asia were confirmed to be highly virulent and behaved as velogenic viscerotropic NDV strains (SEPRL).

- The HN protein of NDV is a crucial determinant of thermostability and the HN gene from a thermostable NDV could be engineered into a thermolabile NDV vaccine strain for developing a novel NDV vaccine (SEPRL).

- Chicken embryo origin (CEO) vaccine strain of ILTV is composed of a mixed population of virus as defined by sequence and pathogenicity. Five unique CEO vaccine derivatives were created which differ in sequence and pathogenicity (UD).

- A first generation Metagenomics protocol has successfully been used to determine the bacterial and DNA virus composition from tracheal swabs collected from poultry (UD).

- Classification of new NDV isolates (taxonomy) was conducted focusing on epidemiological and evolutionary studies. While some genotypes of NDV appear to be globally widespread, others have more regional geographic ranges within continents (SEPRL).

- The phylogenetic network analysis on the full-length S1 sequence of IBV using date-of-isolation information helped determine the genetic trajectory and time-scaled spread of specific IBV types currently circulating in the U.S. (UGA).

Objective IV. Develop new prevention and control strategies for poultry respiratory diseases.

- Current influenza vaccines should be improved by the addition of universal influenza vaccine antigens in order to protect against multiple virus strains. Self-assembling protein nanoparticles (SAPNs) were used to display the two conserved influenza antigens M2e and Helix. The TLR5

agonist flagellin was incorporated into the SAPNs to generate self-adjuvanted SAPNs. The vaccine was tested in chickens and mice (CT).

- Novel IBV vaccines using self-assembled peptide nanoparticle (SAPN) have been generated and tested in chickens (CT).

- Chicken nucleotide-binding oligomerization domain like receptor pyrin domain containing 3 (NLRP3) is ubiquitously expressed in chicken tissues and functions as a cytosolic sensor for LPS and ATP and production and activation of mature chicken IL-1β is chicken NLRP3 dependent (IN).

- Kidney cell-adapted IBV ArkDPI vaccine is stable and confers effective protection against challenge. The spike protein of this virus shows a reduced host cell binding spectrum. These results suggest that replication of the CEK-adapted vaccine strain in chickens might be restricted to fewer tissues than the original vaccine strain. Such restriction might be beneficial as it would reduce adverse reactions but still elicit immunity in the upper respiratory tract (AU).

- Effective protection by vaccination with IBV S ectodomain recombinant protein. All measures indicated more effective protection in the group vaccinated with S1+S2 compared to the group vaccinated with S1 alone. The level of protection after only two applications of a subunit vaccine, without live-virus priming, was remarkable (UA)
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- Infectious bronchitis virus S2 protein expressed from recombinant virus shows that cross-protective capabilities of IBV do not reside in the S2 protein (AU and SEPRL).

- Development of a recombinant vaccine against infectious laryngotracheitis virus (ILTV). (Auburn University & Alabama State University).

- The ectodomain of the influenza virus matrix protein 2 (M2e), which is a highly conserved 24 amino acid protein, has been an attractive target for the development of universal influenza vaccines. Chimeric norovirus p-particle containing consensus M2e sequence of AI viruses (M2e-PP) is highly immunogenic and can provide partial cross protection against challenge with different AIV subtypes. The ability of antibodies induced after M2e-PP vaccination in chickens to bind the M2e and their role in inhibiting AIV replication was dependent on the concentration of M2e-PP antibodies (OSU).

- Application of NS1-truncated variant as live attenuated influenza vaccine (LAIV) for early protection and its complementary use with inactivated vaccine (IV). Priming birds with LAIV at 1 day of age, and boosting with IV three weeks later induced the high antibody titers and provided total protection from heterologous virus challenge (OSU).

- Different humoral immune responses were obtained after one in-ovo vaccination with inactivated NDV and Diatomaceous Earth (DE) used as novel poultry vaccine adjuvant, followed by two subcutaneous boosters on days 21 and 29 of age (UC-Davis).

- Recombinant herpes virus of turkeys (HVT) vaccine expressing an H5 gene against HPAI provided high levels of protection against H5N1 HP virus challenge in chickens. These studies

further our knowledge of the requirements of vaccine formulation during HPAI outbreaks and were used to support inclusion of this vaccine in the U.S. National Veterinary Vaccine Stockpile for AI (SEPRL).

- Poor efficacy of a recombinant herpes virus of turkeys (HVT) vaccine expressing an H5 gene against HPAI in protecting domestic ducks against different H5N1 highly pathogenic avian influenza (HPAI) viruses was demonstrated (SEPRL).

-Novel virus-like particle (VLP) vaccines that contain the antigenic sites of several different viruses protected against AI viruses of HPAIV H5 subtype viruses (SEPRL).

- The negative impact of long-term vaccination on genetic changes in avian influenza field viruses was demonstrated with H9N2 viruses from South Korea. The H9N2 low pathogenic avian influenza (LPAI) virus is common in poultry across North Africa, the Middle East and Asia, and vaccine is commonly used for control. This supports that field viruses must be monitored to detect genetic and antigenic drift and when the field viruses diverge from the vaccine, the vaccines need to be updated to match the emerging field viruses (SEPRL).

-Expression of chicken interleukin-2 by a highly virulent strain of NDV leads to decreased systemic viral load but does not significantly affect mortality in chickens (SEPRL).

- vNDV can replicate in the reproductive tract of hens and contaminate internal components of eggs and eggshell surface, but vaccination was able to prevent internal egg contamination, reducing eggshell surface contamination, and reducing shedding from digestive and respiratory tracts in vNDV challenged hens (SEPRL).

- The ability of a newly developed chicken-induced pluripotent cell line, BA3, to support replication and growth of NDV LaSota vaccine strain was determined to be an excellent candidate for vaccine production due to its highly desirable industrially friendly characteristics of growing to high cell density and capability of growth in serum free medium (SEPRL).

- Mutations in S1 associated with the a nephropathogenic IBV strain does not prevent binding to tracheal tissue, indicating that the initial site of infection for the this strain is likely to be the upper respiratory tract (UGA)

- When applied properly, gel administration of IBV vaccine was not statistically better or worse than conventional spray vaccination (UGA).

- In evaluating hatchery spray IBV vaccination it was found that the process of spray application generated a loss in virus titer. Recommendations were developed for application volume and spray nozzle flow rate and size to achieve effective vaccination of one-day old chicks (UGA).

-Safety and protection efficacy of cell line adapted infectious laryngotracheitis virus (ILTV) BΔORFC strain following in ovo vaccination was demonstrated (UGA).

-Innate immune responses were induced by ILTV virulent strain and chicken embryo origin (CEO) vaccine when administered via the ocular or oral routes. Understanding the timing and effects of innate immune responses in sites of viral replication will help in the development of safer more effective ILTV vaccines (UGA).

- Cold adaptation or heat-treated methods were used to attenuate the variant IBV genotype DMV/1639/11 IBV. Preliminary testing in young chickens showed the experimental vaccines to be safe in laboratory research. the heat-treated vaccine provided better protection based on virus reisolation following challenge (UGA).

- The DMV/1639/11 heat treated vaccine reduced nephropathogenic IBV associated with mortality and airsacculitis condemnations (UD).
- Protection conferred by an AIV subunit vaccine and duration of immunity was superior to the recombinant AIV oil emulsion inactivated vaccine. Modern subunit vaccines may represent efficacious tools for high path avian influenza disease control (UD).

- Application of an ILTV CEO vaccine by drinking water at a dose greater than 1X appeared to limit the back passage of vaccine virus and the associated side effects (UD).

- Opportunistic infections that typically follow an immunosuppressive disease like infectious bursal disease are usually bacterial. Thus, immune suppression has become a significant concern due to the discontinued use of antibiotics in commercial poultry operations. Control of IBD is accomplished using vaccination. Studies showed that amino acids in the hvVP2 projection structures continue to change and contribute to antigenic drift among IBDV strains including the vvIBDV, confounding control efforts.

Impacts:

Objective I

- Genetic analysis of wild bird viruses provided additional epidemiologic data to support the role of wild birds as the source of the poultry H5 HPAI outbreaks.

- Contaminated feed doesn't appear to represent a significant method in which AIV is transmitted in or between houses and farms.

- The high persistence of a HPAI strain in footbaths and the fact that most of the tested footbaths don't eliminate infective viral particles is an alert call to revise, re-evaluate and strategize biosecurity measures in order to prevent the introduction of exotic and also endemic diseases inside poultry premises.

- Understanding the effects of the MHC and innate immunity on IBV virus infection provides substrate for investigation in the control of this endemic pathogen.

- Cal 99 and CA1737 IBV show a moderate level variability compared to the most variable Ark type viruses, which could aid in the development of effective type-specific attenuated vaccines.

Objective II

- A duplex reverse transcriptase loop-mediated isothermal amplification (dRT-LAMP) assay has been developed and evaluated for simultaneous detection of H6 and H9 AIV subtypes.

- A clade-specific PCR based was developed for Ornithobacterium rhinotracheale (ORT) which is being used to screen ORT isolates following implementation of a change in vaccine strategy.

- Two diagnostic platforms, dynamic light scattering (DLS) and surface-enhanced Raman spectroscopy (SERS) have been developed for rapid detection of AIV.

- PCR protocols were developed that help determine the antimicrobial resistance profile of Mycoplasma synoviae (MS) strains in a timely way, providing a therapeutic guide for antibiotic selection and judicious use of antibiotics.

- The use of the choanal cleft as an appropriate sampling site for sensitive detection of avian mycoplasmosis by PCR allows producers and field personnel options for routine sampling that require less skill and personnel, as well as reduce trauma to the poultry during sampling increasing bird welfare.

Objective III

-Successful efforts have been made to determine the bacterial and DNA virus composition of the trachea of birds exhibiting signs of respiratory disease.

- The lack of adaptation observed in chickens with the first H5 HPAIV that caused the outbreaks in U.S. in 2014-15 helped in understanding the unique pathobiology of this virus.

- The HN protein of NDV is a crucial determinant of thermostability and the HN gene from a thermostable NDV could be engineered into a thermolabile NDV vaccine strain for developing a novel NDV vaccine.

- A first generation Metagenomics protocol has successfully been used to determine the bacterial and DNA virus composition from tracheal swabs collected from poultry.

- The phylogenetic network analysis on the full-length S1 sequence of IBV using date-of-isolation information helped determine the genetic trajectory and time-scaled spread of specific IBV types currently circulating in the U.S.

Objective IV

- Self-adjuvanted, self-assembling protein nanoparticles (SAPNs) have been shown to be an promising universal avian influenza vaccine.

- Chicken NLRP3 has the potential to serve as an effective molecular adjuvant to harness the linkage between innate and adaptive immunity and enhance the immunogenicity of vector vaccines for providing protection against poultry respiratory pathogens.

- Ark-DPI vaccine adaptation to CEK improves this type of vaccine as it likely reduces the emergence of vaccine-derived IBV Ark-like strains.

- Inactivation of AIV in AIV-contaminated chicken feed is feasible with approved disinfectants.

- Restricted replication of CEK-adapted Ark IBV vaccine in chickens could be beneficial as it would reduce adverse reactions but still elicit immunity in the upper respiratory tract.

- M2e-PP antibodies are capable of recognizing the native M2e epitopes exposed on the surface of influenza virus-infected MDCK cells and whole virus. It also shows that M2e antibodies have a role in blocking viral replication.

- Studies demonstrate that pc4-LAIV can provide protection in young chickens and the live-priming and IV-boosting can enhance antibody response and protection efficacy.

- Diatomaceous Earth (DE) can serve as a potential adjuvant for vaccines against poultry diseases.

- Application of ILTV CEO vaccine by drinking water at a dose greater than 1X, limited the back passage of vaccine virus and the associated side effects.

Publications:

Costa-Hurtado, M., Afonso, C.L., Miller, P.J., Shepherd, E.M., Cha, R., Smith, D.M., Spackman, E., Kapczynski, D.R., Suarez, D.L., Swayne, D.E., Pantin Jackwood, M.J. 2015. Previous infection with virulent strains of Newcastle disease virus reduces highly pathogenic avian influenza virus replication disease, and mortality in chickens. Veterinary Research. 46:97.

Beltrán, L. G., S. M. Williams, G. Zavala and M. García. Replication patterns of infectious laryngotracheitis virus (ILTV) virulent strain and chicken embryo origin (CEO) vaccine. Submitted Avian Pathology.

Bertran, K., et al. 2016. Lack of chicken adaptation of newly emergent Eurasian H5N8 and reassortant H5N2 high pathogenicity avian influenza viruses in the U.S. is consistent with restricted poultry outbreaks in the Pacific flyway during 2014-2015. Virology. 494:190-197.

DeJesus E, Costa-Hurtado M, Smith D, Lee DH, Spackman E, Kapczynski DR, Torchetti MK, Killian ML, Suarez DL, Swayne DE, Pantin-Jackwood MJ. 2016. Changes in adaptation of H5N2 highly pathogenic avian influenza H5 clade 2.3.4.4 viruses in chickens and mallards. Virology; 499:52-64

Gallardo RA, OA Aleuy, M Pitesky, G Senties-Cue, A Abdelnabi, PR Woolcock, MR Hauck, H Toro (2016). Variability Assessment of California Infectious Bronchitis Virus Variants. Avian Diseases, 60:424-429

Gelb, J. Jr., D. J. Jackwood, E. M. Brannick and B. S. Ladman. Efficacy of Recombinant HVT-IBD Vaccines Administered to Broiler Chicks from a Single Breeder Flock at 30 and 60 Weeks of Age. Avian Dis. 60:603-612, 2016.

Gelb, J., Jr. and M. W. Jackwood. Infectious bronchitis. In: A Laboratory Manual for the Isolation, Identification, and Characterization of Avian Pathogens. S. M. Williams, L. Dufour-Zavala, M. W. Jackwood, M. D. Lee, B. Lupiani, W. M. Reed, E. Spackman, and P. R. Woolcock, eds. 6th edition. 2016.

García, M., S. J. Spatz, Y. Cheng, S. M. Riblet, J. D. Volkening, and G. H. Schneiders. (2016). Attenuation and protection efficacy of open reading frame C (ORF C) gene deleted recombinant of infectious laryngotracheitis virus (ILTV). Accepted J. Gen. Virol.

Ghetas A.M., V.L. van Santen, K. Joiner, and H. Toro (2016) Kidney Cell-Adapted Infectious Bronchitis ArkDPI Vaccine Confers Effective Protection against Challenge. Avian Diseases 60:418-423

Goraichuk I, et al. Complete Genome Sequence of an Avian Paramyxovirus Representative of Putative New Serotype 13. 2016. Genome Announcement; 28;4(4)

Guo X, Flores C, Munoz-Aguayo J, Halvorson DA, Lauer D, Cardona CJ. 2015. Historical and Recent Cases of H3 Influenza A Virus in Turkeys in Minnesota. Avian Dis 59:512-517.

Hauck R., B. Crossley, D. Rejmanek, H. Zhou, R. A. Gallardo. Persistence of High and Low Pathogenic Avian Influenza Viruses in Footbaths and Poultry Manure. Avian Diseases. (Submitted)

Hauck R., R. A. Gallardo, P. R. Woolcock, H. L. Shivaprasad. A Coronavirus Associated with Runting Stunting Syndrome in Broiler Chickens. Avian Diseases. 60:528-534, 2016.

Huang, Y., S. Yang, B. Hu, C. Xu, M. Zhu, Q. Huang, L. Zhang, J. WU; X. Zhang, M. I Khan. Genetic, pathogenic and antigenic diversity of Newcastle disease viruses in Shandong province, China. Infection, Vet Microbiol. 180. 237-244. 2015.

Jackwood, D. J., S. T. Stoute and B. M. Crossley. Pathogenicity of Genome Reassortant Infectious Bursal Disease Viruses in Chickens and Turkeys. Avian Dis. (In Press), 2016.

Jang H, Ngunjiri JM, Lee CW. Association between Interferon Response and Protective Efficacy of NS1-Truncated Mutants as Influenza Vaccine Candidates in Chickens. PLoS One. 11(6):e0156603. 2016

Jordan, B. J., D. A. Hilt, R. Poulson, D. E. Stallknecht, and M. W. Jackwood. Identification of avian coronavirus in wild aquatic birds of the central and eastern USA. J. Wildlife Diseases. 51:218-221, 2015.

Kapczynski, D.R., Tumpey, T.M., Hidajat, R., Zsak, A., Chrzastek, K.,Tretyakova, I., Pushko, P. 2016. Vaccination with virus-like particles containing H5 antigens from three H5N1 clades protects chickens from H5N1 and H5N8 influenza viruses. Vaccine. 34(13):1575-1581

Kapczynski, D.R., Dorsey, K., Chrzastek, K., Moraes, M.,Jackwood, M., Hilt, D., Gardin, Y. 2016. Vaccine protection of turkeys against H5N1 highly pathogenic avian influenza virus with a recombinant HVT expressing the hemagglutinin gene of avian influenza. Avian Diseases. 60(2):413-417.

Karch, C., Li, J., Tan, A., Helal, Z., Fan, Q. and Khan, M. I. and Burkhard, P. Vaccination with self-adjuvanted protein nanoparticles provides protection against lethal influenza challenge. Nanomedicine. S1549-9634(16)30147-2. doi: 10.1016/j.nano.2016.08.030. [Epub ahead of print] 2016.

Kiril M. Dimitrov, Vitaliy Bolotin, Denis Muzyka, Iryna Goraichuk, Olexii Solodiankin, Anton Gerilovych, Borys Stegniy, Gabriela V. Goujgoulova, Mary J. Pantin-Jackwood, Patti J. Miller, Claudio L. Afonso. Repeated isolation of virulent NDVof genotype VIId from backyard chicken in Bulgaria and Ukraine between 2002 and 2013. Archives of Virology; 161(12):3345-3353.

Kwon, J., Lee, D., Swayne, D.E., Yuk, S., Tseren-Ochir, E., Noh, J., Hong, W., Jeong, J., Jeong, S., Gwon, G., Song, C.2016. Highly pathogenic avian influenza A(H5N8) viruses reintroduced into South Korea by migratory waterfowl,2014–2015. Emerging Infectious Diseases. 22(3):507-510.

Leyson, C., M. Franca, M. Jackwood, B. Jordan. Polymorphisms in the S1 spike glycoprotein of Arkansas-type infectious bronchitis virus (IBV) show differential binding to host tissues and altered antigenicity. Virology. 498:218-225, 2016.

Mosley, Y-Y. C., Wu, C.C., and Lin, T.L. 2015. Avian viral vector vaccines for infectious bursal disease. Taiwan Veterinary Journal, 41: 153-163.

Mosley, Y-Y. C., Wu, C.C., and Lin, T.L. 2016. IBDV particles packaged only segment A. Virology, 488: 68-72.

Nazmi A., R. Hauck, A. Davis, M. Hildebrand, L.B. Corbeil, R. A, Gallardo. Diatoms and Diatomaceous Earth as Novel Poultry Vaccine Adjuvants. Poultry Science (In Press)

Nonthabenjawan N, Cardona C, Amonsin A, Sreevatsan S. 2016. Time-space analysis of highly pathogenic avian influenza H5N2 outbreak in the US. Virol J. 13:147.

Pantin Jackwood, M.J., Kapczynski, D.R., Dejesus, E., Costa-Hurtado, M., Dauphin, G., Tripodi, A., Dunn, J.R., Swayne, D.E. 2016. Efficacy of a recombinant turkey herpesvirus H5 vaccine against challenge with H5N1 clades 1.1.2 and 2.3.2.1 highlypathogenic avian influenza viruses in domestic ducks (Anas platyrhynchos domesticus). Avian Diseases. 60:22-23.

Pantin-Jackwood M. J. et al. 2016. Pathogenicity and transmission of H5 and H7 highly pathogenic avian influenza viruses in mallards. J. Virology, 499:52-64.

Preskenis, L. A., B. S. Ladman and J. Gelb, Jr. Identification of Type A Influenza Viruses from Wild Birds on the Delmarva Peninsula, 2007-2010. Avian Diseases In Press. 2016.

Reeves, A.B., Poulson, R.L., Muzyka, D., Ogawa, H., Imai, K., Bui, V., Hall, J.S., Pantin Jackwood, M.J., Stallknecht, D.E., Ramey, A.M. 2016. Limited evidence of intercontinental dispersal of avian paramyxovirus serotype 4 by migratory birds. Infection, Genetics and Evolution. 40(2016):104-108.

Schneiders, G. H. Potential of an infectious laryngotracheitis (ILT) recombinant virus depleted of open reading frame (ORF) C gene as a live attenuated vaccine for in ovo vaccination. Master Thesis. Submitted to University of Georgia May, 2016.

Sá e Silva, M., K. Moresco, K.i Bertran, D. J. Jackwood, D. E. Swayne. Infection with Classical and Very Virulent Infectious Bursal Disease Viruses Produced Virus in Chicken Meat and Vaccination was an Effective Mitigation Strategy. Avian Dis. (In Press), 2016.

Spackman, E. 2016. Avian influenza virus. In: Liu, D. editor. Molecular Detection of Animal Viral Pathogens. Baca Raton, FL: CRC Press. p.377-382.

Stoute S, Chin R, Crossley B, Gabriel Sentíes-Cué C, Bickford A, Pantin-Jackwood M, Breitmeyer R, Jones A, Carnaccini S, Shivaprasad HL. 2016. Highly Pathogenic Eurasian H5N8 Avian Influenza Outbreaks in Two Commercial Poultry Flocks in California. Avian Dis; 60(3):688-93.

Toro H., V. L. van Santen, C. Breedlove Inactivation of Avian Influenza Virus in Non-Pelleted Chicken Feed (2016) Avian Diseases (in press).

van Santen, S. Farjana*, K.S. Joiner, H. Toro (2016). Embryonic Kidney Cell-Adapted Infectious Bronchitis Virus Spike Protein Shows Reduced Host Cell Binding Spectrum. Proceedings of the 9th Int. Symp. Avian Corona- & Pneumoviruses & Complicating Pathogens, Leusden, The Netherlands, 21-24 June (in press).

Valastro, V., E. C. Holmes, P. Britton, A. Fusaro, M. W. Jackwood, G. Cattoli, and I. Monne. S1 gene-based phyogeny of infectious bronchitis virus: An attempt to harmonize virus classification. Infection, Genetics and Evolution, 39, 349-364, 2016.

Zhao, W., Spatz, S., Zsak, L., Yu, Q. Generation of Newcastle Disease Virus (NDV) Recombinants Expressing the Infectious Laryngotracheitis Virus (ILTV) Glycoprotein gB or gD as Dual Vaccines. Methods Mol Biol 1404:89-101. 2016.

Presentations:

Abdelnabi A., R. Hauck, L. Corbeil, R. A. Gallardo (2016). Immune Response Enhancement of Live Attenuated Vaccines Against Poultry Respiratory Viruses. 65th Western Poultry Disease Conference, Vancouver, CA.

Dormitorio T., J. Giambrone and K. Macklin (2016). Litter compositing procedures for inactivation AIV in eggs. Annual AAAP Meeting, San Antonio, TX. Aug 7-10.

Elaish M, Ngunjiri JM, Jang H, Lee CW. Evaluation of in vitro biological role of ectodomain of the influenza virus matrix protein 2 (M2e) specific antibodies in chickens97th CRWAD Meeting. Chicago: Dec. 4–6. 2016.

Figueroa F., R. Hauck, J. Saldias-Rodriguez, R. A. Gallardo (2016). Antiviral effect of hybrid quaternary ammonia - glutaraldehide disinfectant on very virulent infectious bursal disease (vvIBDV) and avian influenza (LPAI) viruses. 65th Western Poultry Disease Conference, Vancouver, CA.

Gallardo R. A., A.P. Da Silva, R. Hauck, H. Zhou. (2016). MHC and innate immune response as factors providing resistance to infectious bronchitis virus. American Veterinary Medical Association / American Association of Avian Pathologists (AVMA/AAAP) Annual Meeting, San Diego, TX.

Gallardo R. A., R. Hauck, D. Rejmanek, B. Crossley. (2016). Highly pathogenic avian influenza viruses affecting the US poultry industry and their persistence. American Veterinary Medical Association / American Association of Avian Pathologists (AVMA/AAAP) Annual Meeting, San Diego, TX.

Gelb, J., Jr., B. Ladman, L. Preskenis, M. Ruano, M. Murphy, and D. Bautista. Recent Trends in IB in Delmarva Including the Nephropathogenic DE/1639 Strain & Its Control. 88th Northeastern Conference on Avian Diseases / Pennsylvania Poultry Sales and Service Meeting. State College, PA. September 28-29, 2016.

Gelb, J., Jr., B. S. Ladman, D. A. Bautista, J. M. Ruano, E. M. Brannick, L. A. Preskenis, and M. M. Murphy. Use of 9CFR Part 107.1 (b) to Control a Variant Strain of Nephropathogenic Infectious Bronchitis Virus in Delmarva Broiler Chickens. Annual Meeting of the American Association of Avian Pathologists. San Antonio, TX. August 6-9, 2016.

Giambrone J., T. Dormitorio, E. Chowdhury, and B. Kaltenboeck (2016). A synthetic vaccine of biodegradable microspheres for controlled release of adjuvant and femtomole-dosed peptide

antigens protects against infectious bursal disease virus. Annual AAAP Meeting, San Antonio, TX. Aug 7-10.

Ghorbani A, Ngunjiri JM, Jang H, Elaish M, Lee CW. Protective efficacy of NS-1 truncated live attenuated influenza vaccine combined with or without M2e subunit vaccine against heterologous and heterosubtypic challenges. 97th CRWAD Meeting. Chicago: Dec. 4–6. 2016.

Jang H, Elaish M,, Ngunjiri JM, Lee CW. Application of NS1-truncated variant as live attenuated influenza vaccine for early protection and its complementary use with inactivated vaccine in chickens 97th CRWAD Meeting. Chicago: Dec. 4–6. 2016.

Jang H, Ngunjiri J, Lee CW. Correlation between Interferon Response and Protective Efficacy of NS1-Truncated Mutants as Influenza Vaccine Candidates in Chickens, 2016 AAAP/AVMA Meeting, San Antonio, TX. August 6-9, 2016.

Khan M. I. Novel universal avian influenza vaccine: its immunogenicity studies in SPF chickens. Guangxi Veterinary Research Institute, Nanning, Guangxi, China. July 7, 2016.

Karch, C., Li, J., Tan, A., Helal, Z., Fan, Q. and Khan, M. I. and Burkhard, P. Peptide Nanoparticles-based vaccine Protect chickens against high pathogenicity Avian Influenza Virus. AVMA, San Antonio, Texas. August 5-9, 2016.

Khan. M. I. Development of nanoparticle based Infectious bronchitis vaccine. 1st Annual Poultry Respiratory Disease Coordinated Agricultural Project. (PRD-CAP) Meeting. Providence RI, October 28, 2015.

Khan. M. I. Development of nanoparticle based Infectious bronchitis vaccine. 2nd Annual Poultry Respiratory Disease Coordinated Agricultural Project. (PRD-CAP) Meeting. Greensboro, NC, October 19, 2016.

Ladman, B. S., J. Gelb, Jr., L. Preskenis, M. Murphy, E. Davis and E. Brannick. Can Three Strains of Infectious Bronchitis Virus Given Simultaneously Induce Protection in Chickens? Annual Meeting of the American Association of Avian Pathologists. San Antonio, TX. August 6-9, 2016.

Lee CW. Multidisciplinary, Multistate Collaborative Project to Control Poultry Respiratory Diseases in the United States. 67th North Central Avian Disease Conference. St. Paul, MN. March 14-15, 2016.

Lee CW. A novel, translational, multidisciplinary approach to control poultry respiratory diseases in the United States. 65th Western Poultry Disease Conference. Vancouver, BC, Canada. April 24-27, 2016.

Lee CW. Poultry Respiratory Diseases Coordinated Agricultural Project (PRD-CAP). 2016 AAAP/AVMA Meeting, San Antonio, TX. August 6-9, 2016.

Li, J., Tan, A., Helal, Z. Karch, C., and Burkhard, P. Khan, M. I. Novel Nanoparticles-based vaccine for infectious bronchitis virus. AVMA, San Antonio, Texas. August 5-9, 2016.

Toro, Haroldo, Vicky van Santen, Cassandra Breedlove (2016) Inactivation of avian influenza virus in chicken feed. Annual Meeting AAAP, August 7-10, San Antonio, TX.

Toro, Haroldo, Vicky van Santen, Cassandra Breedlove (2016). Characterization of Kidney Cell-Adapted IBV ArkDPI Vaccine during Back-Passages in Embryonated Eggs. Annual Meeting AAAP, August 7-10, San Antonio, TX.

V. Santen Vicky L., Fatma E. Eldemery*, Saiada Farjana*, Kellye S. Joiner, Haroldo Toro (2016). Role of spike S2 ectodomain in attachment and selection of ArkDPI IBV vaccine subpopulations in chickens. Annual Meeting AAAP, August 7-10, San Antonio, TX.

V. van Santen, S. Farjana*, K.S. Joiner, H. Toro (2016). Embryonic Kidney Cell-Adapted Infectious Bronchitis Virus Spike Protein Shows Reduced Host Cell Binding Spectrum. 9th Int. Symp. Avian Corona- & Pneumoviruses & Complicating Pathogens, 21-24 June, Leusden, The Netherlands

Zeinab H. Helal, Jianping Li, Christopher Karch, Peter Burkhard and Mazhar I. Khan. Peptide nanoparticle-based vaccine for infectious bronchitis virus. International Vaccine Conference, Boston, US, October2-5, 2016.

Grants:

Giambrone J. “Capacity Building for Detection of Avian Influenza Virus and Safe Disposal of Organic Materials in Case of an Outbreak”. AAES Cuba Grants Program 2016, $11,500.

Toro H., Q. Yu, V.L. van Santen, F.W. van Ginkel, K. Joiner. Infectious Bronchitis Virus S2 Expressed from Recombinant Virus to Confer Protection across Serotypes in Chickens. USDA-AFRI Ohio State University, 2015-2020. $246,158

Toro, H., V.L. van Santen, R. Cattley, K. Joiner. Early Vaccination of Chickens Significantly Decreases Induction of Infectious Bronchitis Virus Specific Immunity. USDA NIFA #2016-67015-24916 2016-2019, $498,988

Toro, H., V.L. van Santen. Termin-8 Inactivation of avian influenza virus in chicken feed. U.S. Poultry & Egg #BRU004, 11/2015-9/2016, $40,000

Chang-Won Lee (U.S. PI), Mahmoud Ibrahim (Egyptian PI). Molecular and Antigenic Evolution of H5N1 Avian Influenza Virus in Egypt and Development of Broadly Effective Vaccine. The National Academies of Sciences, Engineering, and Medicine. 07/01/2016 – 06/31/2019. $331,962