**Period Covered:** October 1, 2008 to September 30, 2009

**Annual Meeting Date(s):** January 26-27, 2010

**Participants:**

**Advisor:** Saif, Yehia (saif.1@osu.edu)

**USDA representative:** Johnson, Peter (PJOHNSON@CSREES.USDA.GOV)

**State Station Representatives:** Giambrone, Joe (giambjj@auburn.edu) – Auburn University; Khan, Mazhar (mazhar.khan@uconn.edu) - University of Connecticut; Glisson, John (jglisson@vet.uga.edu) – University of Georgia; Wu, Ching Ching (wuc@purdue.edu) – Purdue University; Lee, Chang Won (lee.2854@osu.edu) – Ohio State University; Zsak, Laszlo (Laszlo.Zsak@seprl.usda.gov) – USDA, Southeast Poultry Research Lab.

**Other participants:** Keeler, Calvin (ckeeler@udel.edu) – University of Delaware; Johnson, Timothy (joh04207@umn.edu) – University of Minnesota; Toro, Haroldo (torohar@vetmed.auburn.edu) – Auburn University; Garcia, Maricarmen (mcgarcia@uga.edu), Mundt, Egbert (emundt@uga.edu), Jackwood, Mark (mjackwoo@uga.edu), Sellers, Holly (hsellers@uga.edu), Ferguson-Noel, Naola (naolaf@uga.edu) - University of Georgia; Lin, Tsang Long (tllin@purdue.edu) – Purdue University; Pantin-Jackwood, Mary (Mary.Pantin-Jackwood@ars.usda.gov), Yu, Qingzhong (Qingzhong.Yu@ars.usda.gov), Swayne, David (David.Swayne@ARS.USDA.GOV), Miller, Patty (Patti.Miller@ars.usda.gov) - USDA, Southeast Poultry Research Lab.

**Accomplishments:**

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

1. Isolation and characterization of avian influenza viruses (AIV) from wild birds, which include hunter-killed or nesting waterfowl and shorebirds, starlings, and raptors, were accomplished. The enormous data obtained from different states (AL, DE, GA, MN, OH) are being shared.
2. Surveillance activities on the Delmarva Peninsula have yielded infectious laryngotracheitis (LT) virus and infectious bronchitis virus isolates from commercial broiler chickens.
3. In DE, LT incidence down in 2009 due to widespread vaccination. Severity of LT clinical signs and lesions are mild to moderate, very similar to that seen in adverse CEO vaccine reactions.
4. GA isolated and characterized current pathogenic respiratory viruses, bacteria, and mycoplasmas circulating within the poultry industry in Georgia. Identified at least 41 MG genotypes that are distinguishable from live vaccines and unique to individual countries or regions.

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

1. AL developed a rapid, accurate, and economical method for ILTV detection using a molecular technique Loop-mediated isothermal amplification (LAMP). The LAMP could detect viral DNA directly from the tracheae of vaccine virus infected birds as well as ILTV plaques from embryonic eggs.
2. CT designed nine pairs of neuramidinase (NA) subtype-specific primers using Primer Hunter design tool and successfully used in real time RT-PCR with four primer-pool reactions to differentiate nine NA subtypes of AIV.
3. GA developed an indirect N1 and N2 ELISAs which were proven to be effective and rapid assay to identify exposure to challenge virus during a DIVA vaccination strategy. In addition, a species–independent competitive ELISA (cELISA) for the detection of H6, H7, H9 antibodies in several species was developed.
4. MN developed degenerate primer set for full-length amplification of four genes of influenza A viruses in a single reaction.
5. OH established the chloroform-Mag MAXTM method of viral RNA extraction followed by RRT-PCR which can be used as rapid and sensitive test to determine the titer of the viral RNA. Using this method, it was found that different commercial vaccines contain varied antigen contents.
6. SEPRL (USDA) developed two real time RT-PCR assays that allow the differentiation of North American H1N1 from pandemic H1N1. In addition, the current H7 RRT-PCR was improved to detect a broader range of H7 viruses that are found in Western hemisphere.
7. SEPRL demonstrated that NDV Matrix assay failed to detect a virulent NDV. If genotype VII virus was found in North America this assay could be used in the NALHN laboratories.

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

1. DE isolated 5038 IBDV isolate from commercial broiler chickens. Although unique based on VP2 sequencing and monoclonal antibody testing, may not be capable of breaking through maternal immunity in a laboratory designed trial may not be able to break through in real world progeny challenges.
2. GA identifed that temperature plays a pivotal role in the survivability of LPAI virus in feces and in contact with litter. GA also identified that a percentage of chickens receiving recombinant or TCO vaccines carry a significant amount of virulent ILTV in the trachea in the absence of clinical signs after being challenged with virulent ILTV.
3. Comparative genomic analysis of IBV indicates that the replicase protein in addition to the already recognized spike gene of coronaviuses plays a key role in pathogenicity. GA have identified regions in the replicase that likely effects cleavage and assembly of the enzyme.
4. OH identified amino acids contributing to antigenic drift in the Del-E infectious bursal disease virus. The short term implication this has for the poultry industry is that diagnostic assays designed to identify the 254 and 222 amino acids will discover viruses that have antigenically important mutations.
5. OH showed that two virulent infectious bursal disease viruses (vvIBDV) from California are identical and meet all the characteristics of a vvIBDV. Because they have the potential to spread rapidly and cause high mortality in chickens, the impact of these viruses on the U.S. broiler and layer industries could be considerable.
6. OH detected low pathogenic influenza viruses in albumin of eggs using real time RT-PCR and virus isolation in embryonated chicken eggs. Swabs from egg shells were also found positive by RRT-PCR.

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

1. AL developed two recombinant vaccines against H1N1 AIV (one DNA and the other in yeast) and found to induce a measurable immune response in young chickens. The DNA vaccine was given by injection and the yeast vaccine in the drinking water.
2. CT tested in ovo vaccination of recombinant DNA plasmid containing IBV spike gene with interferon-a which showed over 98% of protection rate against M41 field isolate challenge.
3. DE developed a second generation escape resistant RNAi constructs against avian influenza virus and found that avian-specific RNAi constructs against avian influenza virus did not increase the efficiency of RNAi inhibition.
4. IN demonstrated that IBDV large segment gene-based DNA can elicit specific immune response and provide protection of broiler chickens with maternally derived antibody against infection challenge.
5. OH developed NA- and NS-based DIVA vaccine strains using traditional reassortment as well as reverse genetics methods against H3N2 influenza in turkeys. The reassortant DIVA vaccines significantly reduced challenge virus shedding in the oviduct of breeder turkeys as well as trachea and cloaca of both young and old breeder turkeys, suggesting that proper vaccination could effectively prevent egg production drop and potential viral contamination of eggs in infected turkeys.
6. SERPL demonstrated that H7 AI vaccine may not protect against intercontinental H7 field viruses and vaccine may need to be from the same H7 lineage as field viruses to provide protection. In addition, turkeys vaccinated with commercial H1N1 vaccine have a low chance of being protected against swine-origin H1N1 infection.
7. SEPRL developed a model system for NDV vaccination which mimic egg production losses seen in Asia and Mexico in vaccinated poultry were developed and this system will allow the comparison of vaccines.

**Work Planned for Next Year**

1. Continue surveillance and screening of respiratory pathogen from wild and domestic bird populations;
2. Continue development and refinement of diagnostic assays to detect and differentiate poultry pathogens
3. Continue to study polymicrobial infection in poultry using a co-infection model; and continue to study *E. coli* as a primary or secondary pathogen of poultry;
4. Continue development, refinement and testing of vaccine against influenza, ILT, ORT, *E. coli*, and other respiratory pathogens of poutlry.
5. The molecular basis for antigenicity, pathogenicity, and transmission of respiratory pathogens will be studied using naturally occurring viruses and reverse genetically created viruses.
6. Studies will continue on the improved diagnosis and characterization of vvIBDV from California.
7. IBDV Persistence study will be conducted using commercial broiler chicken having different levels of antibodies.
8. Effort will be made to reproduce a mild form of ILTV in SPF birds using given viruses isolated from house dust, litter, beetles, rats, and drinking water from ILT positive farms

**Impacts:**

1. Wild birds are a reservoir of AIVs and some species may serve as potential intermediate host. Viral detection should be done by passage of fecal swab material in embryos first then by RRT-PCR and should exclude AC-ELISA.
2. Composting of AIV infected eggs for as early as 24 hours and late as 52 hours can inactivate AIV. The internal temperature of the pile must reach 560 F for the inactivation to occur. The temperature is a function of the amount of pile turning and moisture. Presently, 7 days are used in the industry to perform this function.
3. Two real time RT-PCR assays that allow the differentiation of North American H1N1 from pandemic H1N1 were developed. The National Animal Health Laboratory Network adopted these tests.
4. Low pathogenic influenza viruses were detected from internal egg contents following experimental infection in turkeys. The possibility of hatchery contamination by egg borne influenza viruses and spread of virus during movement of contaminated cracked eggs and egg flats pose concerns regarding influenza viral dissemination
5. ILTV is present in commercial poultry houses causing mild outbreaks. The viruses were found in the dust, litter, beetles, water, and rats. Heating of the house to 1000 F for 100 hours, composting of the litter for 3 days, improved beetle control, treatment of the drinking water system with commercial biofilm removers, and rodent control will reduce the amount of virus in the house.
6. Factors hindering control of ILT may be suboptimal immunization against ILT resulting from multivalent vaccinations. Reducing the number and diversity of live virus vaccines given concomitantly with ILT vaccines may optimize protection against ILTV and possibly against other viral respiratory diseases.
7. A high titer of ILTV vaccine is required for a prompt neutralizing immune response. Thus, vaccine fractionation would seem counterproductive.
8. Monitoring the ability of infectious bursal disease virus (IBDV) to break through maternal immunity in young broiler chickens is important to assess the immunosupproessive potential of the viruses.
9. IBDV large segment gene-based DNA vaccine has the potential for practical application to confer protection of chickens with maternal antibodies against IBD in the poultry industry.
10. Monitoring infectious bronchitis viruses from commercial broiler chickens is important for monitoring the effectiveness of vaccination programs and to isolate and characterize field viruses that break through vaccine induced immunity.
11. In-ovo DNA immunization may become one of the most important innovation in the DNA vaccination of poultry against IBV, allowing it to be used in commercial in-ovo vaccination as a much safer vaccine than the attenuating live IBV vaccines used currently.
12. Genomic characterization of fowlpox virus and other avianpox viruses for specific virulence markers e.g. full length REV can be done by PCR amplification of the genetic fragments with specific primers. In this regard, DNA isolated from formalin fixed paraffin-embedded tissue sections can be used effectively.

**Publication in Journals:**

1. **Dormitorio, T. V., J. J. Giambrone, K. Guo, and G. R. Hepp.** 2009. Detection and characterization of avian influenza and other avian paramyxoviruses from wild waterfowl in parts of the southeastern United States. Poult Sci. 88:851-855.
2. **Dormitorio, T. V., J. J. Giambrone, K. Guo, and G. R. Hepp.** 2009. Evaluation of field and laboratory protocols to detect avian influenza viruses in wild aquatic birds. Poult. Sci. 88:1852-1831.
3. Babapoor, S., D.A. Almeida, J. J. Fabis, Z. H. Helal, X. Wang, T. Girshick, M. I. Khan. Protective effect of in ovo vaccination with IBV-spike-recombinant DNA and chicken interferon as an adjuvant. Int J. Pout Sci. 8 (11) :1034-1041. 2009.
4. Duitama, J., D. M. Kumar, E. Hemphill, M. Khan, I. I. Mandoiu, and C. E. Nelson. PrimerHunter: a primer design tool for PCR-based virus subtype identification. Nucleic Acid Research.37 (8):2483-2492. 2009.
5. [Huang Y](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=Search&Term=%22Huang%20Y%22%5BAuthor%5D&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_DiscoveryPanel.Pubmed_RVAbstractPlus), B. [Hu](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=Search&Term=%22Hu%20B%22%5BAuthor%5D&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_DiscoveryPanel.Pubmed_RVAbstractPlus), X. [Wen](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=Search&Term=%22Wen%20X%22%5BAuthor%5D&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_DiscoveryPanel.Pubmed_RVAbstractPlus), S. [Cao](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=Search&Term=%22Cao%20S%22%5BAuthor%5D&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_DiscoveryPanel.Pubmed_RVAbstractPlus), D. [Xu](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=Search&Term=%22Xu%20D%22%5BAuthor%5D&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_DiscoveryPanel.Pubmed_RVAbstractPlus), X. [Zhang](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=Search&Term=%22Zhang%20X%22%5BAuthor%5D&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_DiscoveryPanel.Pubmed_RVAbstractPlus), M. I. Khan. Evolution analysis of the matrix (M) protein genes of 17 H9N2 chicken influenza viruses isolated in northern China during 1998-2008. Virus Genes. 38:398-403. 2009.
6. Zhu, W., J. Dong, Z. Xie, Q. Liu, M. I. Khan. Phylogenetic and pathogenetic analysis of Newcastle disease virus isolated from house sparrow *(Passer domesticus)* living around poultry farm in southern China. Virus Genes. DOI 10.1007/s11262-009-0436-0.2009.
7. Warke, A., and Mundt, E. (2008). Prevalence of Antibodies to different Avian Paramyxoviruses in Commercial Poultry in the USA. Avian Dis. 52:694-697.
8. Mundt, E., Gay, L., Jones, L., Saavedra, G., Tompkins, S. M. and Tripp, R. A. (2009). Replication and Pathogenesis Associated with H5N1, H5N2 and H5N3 Low Pathogenic Avian Influenza Virus Infection in Chickens and Ducks. Arch. in Virol. 154:1241-1248.
9. Dlugolenski, D. Hauck, R., Hogan, R. J., Michel, F., and Mundt, E. (2009). Production of H5 specific monoclonal antibodies and the development of a competitive ELISA for detection of H5 antibodies in multiple species. Avian Dis., in press.
10. Gay, Lauren, and Mundt E. (2009). Testing of a New Disinfectant Process for Poultry Viruses. Avian Dis., in press.
11. Avellaneda, G., Mundt, E., Lee, C-W, and Suarez, D. L. (2009). Differentiation of infected and vaccinated animals (DIVA) using the NS1 protein of avian influenza virus. Avian Dis., in press.
12. Liu, Y., Mundt E., Mundt A., Sylte M., Swayne D. and M. García(2009). Development and evaluation of an avian influenza (AI) neuraminidase subtype 1 (N1) based serological ELISA for poultry using the differentiation of infected and vaccinated animals (DIVA) control strategy. Avian Dis., in press.
13. Davidson, I., S. Nagar, I. Ribshtein, I. Shkoda, S. Perk, and M. García.  Detection of wild-and vaccine-type infectious laryngotracheitis virus in clinical samples and feather shafts of commercial chickens.  Avian Dis. In press.
14. Chin, R. P. M. García, C. Corsiglia, S. Riblet, R. Crespo, H. L. Shivaprasad, A. Rodriguez-Avila,P. Woolcock, and M. França. Intervention Strategies for Laryngotracheitis: Impact of Extended Downtime. Avian Dis. 53:574-577. 2009.
15. Williams, S. M., J. A. Smith, M. García, D. Brinson, M. Kiupel, C. Hofacre. Histiolymphocytic and heterophilic bronchopneumonia as a severe reaction to in ovo fowl-pox vaccination. Vet. Pathology. 47(1):176-179.2009.
16. Callison, S. A‡, S. M. Riblet,A. Rodriguez-Avila, and M. García\*. Reverse Restriction Fragment Length Polymorphism (RRFLP) Assay: A novel technique and its application to the rapid genotyping of infectious laryngotracheitis virus (ILTV) live attenuated vaccines. Journal Virol. Methods. 160:119-124. 2009.
17. Waidner, L., R. Morgan, M. García, A. Anderson, E. Bernberg, S. Kamboj, M. Ouyang, G. Isaacs, M. Markis, B. Meyers, P. Green, and J. Burnside. Novel ILTV and HVT microRNAs have conserved genomic locations with those of other Gallid and Meleagrid herpesvirus microRNAs. Virology. 388:128-36. 2009.
18. Oldoni, I‡, A. Rodriguez-Avila, S. M. Riblet, G. Zavala, and M. García\*. Pathogenicity and Growth Characteristics of Infectious Laryngotracheitis Virus (ILTV) Isolates from United States. Avian Pathology. 38:47-53. 2009.
19. Jackwood, M. W., D. A. Hilt, A. W. McCall, C. N. Polizzi, E. T. McKinley, and S. M. Williams. Infectious Bronchitis Virus Field Vaccination Coverage, Vaccine Levels, and Persistence of Arkansas Type Viruses in Commercial Broilers. Avian Dis. 53:175-183. 2009.
20. Morales, A. C., Jr., D. A. Hilt, S. M. Williams, M. J. Pantin-Jackwood, d. L. Suarez, E. Spackman, D. E. Stallknecht, and M. W. Jackwood. Biological Characterization of H4, H6, and H9 type Low Pathogenicity Avian Influenza Viruses from wild Birds in Chickens and Turkeys. Avian Dis. 53:552-562, 2009.
21. Jackwood, M. W., S. Bogoch, E. S. Bogoch, D. Hilt, and S. M. Williams. Efficacy of a Replikin Peptide Vaccine Against Low-Pathogenicity Avian Influenza H5 Virus. Avian Dis. 53:613-617, 2009.
22. Jackwood, M. W., D. L. Suarez, D. Hilt, M. J. Pantin-Jackwood, E. Spackman, P. Woolcock, and C. Cardona. Biological Characterization of Chicken-Derived H6N2 Low Pathogenic Avian Influenza Viruses in Chickens and Ducks. Avian Dis. In press, 2009.
23. Jackwood, M. W., T. O. Boynton, D. A. Hilt, E. T. McKinley, J. C. Kissinger, A. H. Paterson, J. Robertson, c. Lemke, A. W. McCall, S. M. Williams, J. W. Jackwood, and L. A. Byrd. Emergence of a Group 3 Coronavirus Through Recombination. In press. Virology, 2009.
24. Jackwood, M. W., R. Rosenbloom, M. Petteruti, D. A. Hilt, A. W. McCall, and S. M. Williams. Avian Coronavirus Infectious Bronchitis Virus Susceptibility to Botanical Oleoresins and Essential Oils *In Vitro* and *In Vivo*. Accepted: Virus Research, 2009.
25. Shivprasad, H.L., Kim, T., Tripathy, D.N. Woolcock, P.R. and Uzal, F. Unusual pathology of canarypox virus infection associated with high mortality in young and adult breeder canaries (*Serinus canaria*). Avian Pathology, 38: 311-316, 2009.
26. Pillai SPS, SaifYM, LeeCW.Detection of influenza A viruses in eggs laid by infected turkeys. Avian Dis. *In Press.*
27. Pillai SPS & Lee CW. Species and age related differences in the type and distribution of influenza virus receptors in different tissues of chickens, ducks and turkeys. Virol J. In press.
28. Jadhao SJ, Lee CW, Sylte M, Suarez DL. [Comparative efficacy of North American and antigenically matched reverse genetics derived H5N9 DIVA marker vaccines against highly pathogenic Asian H5N1 avian influenza viruses in chickens.](http://www.ncbi.nlm.nih.gov/pubmed/19686695?ordinalpos=1&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_DefaultReportPanel.Pubmed_RVDocSum) Vaccine. 2009 19;27(44):6247-60.
29. Yassine HM, Khatri M, Zhang YJ, Lee CW, Byrum BA, O'Quin J, Smith KA, Saif YM. [Characterization of triple reassortant H1N1 influenza A viruses from swine in Ohio.](http://www.ncbi.nlm.nih.gov/pubmed/19477087?ordinalpos=2&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_DefaultReportPanel.Pubmed_RVDocSum) Vet Microbiol. 2009 139:132-9.
30. L. Wang & Lee CW. Sequencing and Mutational Analysis of the Non-Coding Regions of Influenza A Virus. Veterinary Microbiology. 30;135(3-4):239-247. 2009.
31. SPS Pillai, M Pantin-Jackwood, SJ Jadhao, DL Suarez, L Wang, HM Yassine, YM Saif, CW Lee. Pathobiology of triple reassortant H3N2 influenza viruses in breeder turkeys and its potential implication for vaccine studies in turkeys. Vaccine. 27: 819-824. 2009.
32. Stoute, S. T., D. J. Jackwood, S. E. Sommer-Wagner, G. L. Cooper, M. L. Anderson, P. R. Woolcock, A. A. Bickford, C. G. Senties-Cue, and B. R. Charlton. The diagnosis of very virulent infectious bursal disease in California Pullets. Avian Dis. 53:321-326. 2009.
33. Jackwood, D. J., S. E. Sommer-Wagner, S. T. Stoute, P. R. Woolcock, B. M. Crossley, S. K. Hietala and B. R. Charlton. Characteristics of a very virulent infectious bursal disease virus from California, USA. Avian Dis. 53:592-600. 2009.
34. Lipatov, A. S., Kwon, Y. K., Pantin-Jackwood, M. J. and Swayne, D. E., Pathogenesis of H5N1 influenza virus infections in mice and ferret models differs according to respiratory tract or digestive system exposure. J Infect Dis 199(5):717-25, 2009.
35. Peterson, A. T., Bush, S. E., Spackman, E., Swayne, D. E. and Ip, H. S., Influenza A virus infections in land birds, People's Republic of China. Emerg Infect Dis 14(10):1644-6, 2008.
36. Pfeiffer, J., Pantin-Jackwood, M., To, T. L., Nguyen, T. and Suarez, D. L., Phylogenetic and biological characterization of highly pathogenic H5N1 avian influenza viruses (Vietnam 2005) in chickens and ducks. Virus Res 142(1-2):108-20, 2009.
37. Spackman, E., Ip, H. S., Suarez, D. L., Slemons, R. D. and Stallknecht, D. E., Analytical validation of a real-time reverse transcription polymerase chain reaction test for Pan-American lineage H7 subtype Avian influenza viruses. J Vet Diagn Invest 20(5):612-6, 2008.
38. Spackman, E., Pantin-Jackwood, M., Swayne, D. and Suarez, D. L., An Evalution of Avian Influenza Diagnostics Methods with Domestic Duck Specimens. Avian Dis 53(2):276-280, 2009.
39. Swayne, D. E. and Slemons, R. D., Using mean infectious dose of high- and low-pathogenicity avian influenza viruses originating from wild duck and poultry as one measure of infectivity and adaptation to poultry. Avian Dis 52(3):455-60, 2008.
40. Wasilenko, J. L., Sarmento, L. and Pantin-Jackwood, M. J., A single substitution in amino acid 184 of the NP protein alters the replication and pathogenicity of H5N1 avian influenza viruses in chickens. Arch Virol 154(6):969-79, 2009.
41. Sarmento, L., C.L. Afonso, C. Estevez, Wasilenko, J.L., and M. Pantin-Jackwood. 2008. Differential host gene expression in cells infected with highly pathogenic H5N1 avian influenza viruses. Veterinary Immunology and Immunopathology, 25(3-4):291-302.
42. Sarmento, L., M. Pantin-Jackwood, D.R. Kapczynski, D.E. Swayne, and C.L. Afonso. Immediate early responses of avian tracheal epithelial cells to infection with highly pathogenic avian influenza virus. Dev Biol (Basel).132:175-83, 2008.
43. Kapczynski, D.R., Gonder, E., Liljebjelke, K.A., Lippert, R., Petkov, D., and B. Tilley. 2009. Vaccine induced protection from egg production losses in commercial turkey breeder hens following experimental challenge with a triple reassortant H3N2 avian influenza virus. Avian Dis. 53:7-15.
44. Bogoyavlenskiy, A., Berezin, V.E., Prilipov, A.G., Usachev, E.V., Lyapina, O.V., Korotetskiy, I.S., Zaitceva, I.A., Asanova, S.E., Kydyrmanov, A., Daulbaeva, K., Shakhvorostova, L.M., Sayatov, M.K., King, D.J. 2009. Newcastle disease outbreaks in Kazakhstan and Kyrgystan during 1998, 2000, 2001, 2003, 2004 and 2005 were caused by viruses of the genotypes VIIb and VIId. Virus Genes. 39:94-101.
45. Chakrabarti, S., King, D.J., Cardona, C.J., Gerry, A.C. 2008. Persistence of exotic Newcastle disease virus (ENDV) in laboratory infected *Musca domestica* and *Fannia canicularis*. Avian Diseases. 52:375-379.
46. Kim, L.M., King, D.J., Guzman, H., Tesh, R.B., Bueno, R., Dennett, J.A., Afonso, C.L. 2008. Biological and phylogenetic characterization of pigeon paramyxovirus serotype-1 circulating in North American pigeons.
47. Miller, P.J., Estevez, C., Yu, Q., Suarez, D.L., King, D.J. 2009. Comparison of viral shedding following vaccination with inactivated and live Newcastle disease vaccines formulated with wild-type and recombinant viruses. Avian Diseases. 53:39-49.
48. Miller, P.J., DeCanini, E.L., Afonso, C. L. 2010. Newcastle disease: Evolutionary dynamics of genotypes and the related diagnostic challenges. 10 (1) 26-35.
49. Miller, P.J., Kim, L.M., Afonso, C.L., Ip, H.S. 2009. Evolutionary dynamics of Newcastle disease virus. Virology. 391:64-72.
50. Perozo, F., Merino, R., Afonso, C.L., Villegas, P., Calderon, N. 2008. Biological and phylogenic characterization of virulent Newcastle disease virus circulating in Mexico. Avian Diseases. 52(3):472-479.
51. Perozo, F., Villegas, P., Afonso, C.L. 2008. Genomic comparison of the complete coding and intergenic regions of the VG/GA Newcastle disease virus and its respirotropic clone 5. Virus Genes. 37(2):161-167.
52. Rue,C. A., Susta, L., Brown, C.C., Pasick, J. M., Swafford, S. R., Wolf, P.C., Killian, M.L., Pedersen, J.C., Miller, P.J., Afonso, C.L. Phylogenetic and Pathological Characterization of Newcastle Disease Viruses Isolated from Double-crested Cormorants. 2010. *In press*
53. Jindal N, Patnayak DP, Chander Y, Ziegler AF, Goyal SM. 2010. Detection and molecular characterization of enteric viruses from poult enteritis syndrome in turkeys. Poult Sci. 89:217-26.
54. Khatri M, O'Brien TD, Goyal SM, Sharma JM. 2009. Isolation and characterization of chicken lung mesenchymal stromal cells and their susceptibility to avian influenza virus. Dev Comp Immunol. Dec 28. [Epub ahead of print].
55. Ramakrishnan MA, Gramer MR, Goyal SM, Sreevatsan S. 2009. A Serine12Stop mutation in PB1-F2 of the 2009 pandemic (H1N1) influenza A: a possible reason for its enhanced transmission and pathogenicity to humans. J Vet Sci. 10:349-51.
56. Jindal N, Chander Y, Chockalingam AK, de Abin M, Redig PT, Goyal SM. 2009. Phylogenetic analysis of Newcastle disease viruses isolated from waterfowl in the upper midwest region of the United States. Virol J. 5:191.
57. Ramakrishnan MA, Tu ZJ, Singh S, Chockalingam AK, Gramer MR, Wang P, Goyal SM, Yang M, Halvorson DA, Sreevatsan S. 2009. The feasibility of using high resolution genome sequencing of influenza a viruses to detect mixed infections and quasispecies. PLoS One. 4:e7105.
58. Jindal N, Chander Y, de Abin M, Sreevatsan S, Stallknecht D, Halvorson DA, Goyal SM. 2009. Amplification of four genes of influenza A viruses using a degenerate primer set in a one step RT-PCR method. J Virol Methods. 160:163-6.
59. Johnson TJ, Nolan LK. 2009. Plasmid replicon typing. Methods Mol Biol. 551:27-35.
60. Johnson TJ, Wannemuehler Y, Doetkott C, Johnson SJ, Rosenberger SC, Nolan LK. 2008. Identification of minimal predictors of avian pathogenic Escherichia coli virulence for use as a rapid diagnostic tool. J Clin Microbiol. 46:3987-96.
61. Johnson TJ, Wannemuehler Y, Johnson SJ, Stell AL, Doetkott C, Johnson JR, Kim KS, Spanjaard L, Nolan LK. 2008. Comparison of extraintestinal pathogenic Escherichia coli strains from human and avian sources reveals a mixed subset representing potential zoonotic pathogens. Appl Environ Microbiol. 74:7043-50.
62. Ammayappan, A., C. Upadhyay, J. Gelb, Jr., and V. N. Vakharia. Complete genomic sequence analysis of infectious bronchitis virus Ark DPI strain and its evolution by recombination. *Virology Journal* 5:157. 2008.
63. Ammayappan, A., C. Upadhyay, J. Gelb, Jr., and V. N. Vakharia. Identification of sequence changes responsible for the attenuation of avian infectious bronchitis virus strain Arkansas DPI. *Archives of Virology* 154:495-499. 2009.
64. Arumugaswami, V., P. M. Kumar, V. Konjufca, R. L. Dienglewicz, S. M. Reddy and M. S. Parcells. 2009. Latency of Marek's Disease Virus (MDV) in a Reticuloendotheliosis Virus-Transformed T-cell Line. I: Uptake and Structure of the Latent MDV Genome. Avian Dis. 53:149-155.
65. Arumugaswami, V., P. M. Kumar, V. Konjufca, R. L. Dienglewicz, S. M. Reddy and M. S. Parcells. 2009. Latency of Marek's Disease Virus (MDV) in a Reticuloendotheliosis Virus-Transformed T-cell Line. II: Expression of the Latent MDV Genome. Avian Dis. 53:156-165.
66. Ladman, B. S., S. C. Rosenberger, J. K. Rosenberger, C. R. Pope, and J. Gelb, Jr. Virulence of low pathogenicity H7N2 avian influenza viruses from the Delmarva peninsula for broiler and leghorn chickens and turkeys.  *Avian Diseases* 52:623-631. 2008.
67. Wood, M. K., B. S. Ladman, L. A. Preskenis, C. R. Pope, D. A. Bautista, and J. Gelb, Jr. Massachusetts live vaccination protects against a novel S1 genotype infectious bronchitis virus DMV/5642/06. *Avian Diseases* 53:119-123. 2009.
68. Arusyak Abrahamyan, Éva Nagy, and Serguei P. Golovan. 2009. Human H1 promoter expressed short hairpin RNAs (shRNAs) suppress avian influenza virus replication in chicken CH-SAH and canine MDCK cells. *Antiviral Research* 84: 159-167.
69. Kim, C.-H., H.S. Lillehoj, Y.-H. Hong, C.L. Keeler, Jr. and E.P. Lillehoj. (2010) Comparison of global transcriptional responses to primary and secondary *Eimeris acervulina*  infections in chickens. Developmental and Comparative Immunology 34:344-351.
70. Kim, D.K., Kim, C.H., Lamont, S.J., Keeler, C.L. and H.S. Lillehoj. (2009) Gene expression profiles of two B-complex disparate, genetically inbred Fayoumi chicken lines that differ in susceptibility to *Eimeria maxima.* Poultry Science 88:1565-1579.
71. McCarthy, F. M., T. J. Mahony, M. S. Parcells, and S. C. Burgess. 2009. Understanding Animal Viruses Using the Gene Ontology and AgBase. Trends in Microbiology, 17:328-35
72. Tavlarides-Hontz, P., P. M. Kumar, J. R. Amortegui, N. Osterrieder, and M. S. Parcells. 2009. A deletion within glycoprotein L of Marek's disease virus (MDV) field isolates correlates with a decrease in bivalent MDV vaccine efficacy in contact-exposed chickens. Avian Dis.53:287-29

**Abstracts, Presentations, etc:**

1. [Huang, Yanyan., Xiumei Zhang, Xintian Wen, Sanjie Cao, Xiumei Zhang, and Mazhar I. Khan. Studies of phylogeny and sequence analysis of H9N2 avian influenza viruses](http://avma.omnibooksonline.com/2009/data/papers/7703.pdf). 146th AVMA Annual Convention, Seattle, WA. July 11-14, 2009. CDROM. 7703.2009.
2. Huang, Yanyan., Beixia Hu, Xintian Wen, Sanjie Cao, Xiumei Zhang, and Mazhar I. KhanGenomic analysis of seventeen H9N2 chicken influenza viruses isolated in northern China during 1998–2008. 16th World Veterinary Poutry Association Congress, Marrakesh, Morocco, November 8-12, 2009.P13-AI.p 322.
3. Tripathy, D.N. and Bahaaa, A.F.A. Genetic Characterization of avianpox viruses using DNA isolated from formalin fixed tissue sections. AAAP, AVMA Conference, Seattle, WA, July, 2009.
4. Chen, Y. Y., Wu, C. C., and Lin, T. L. Generation and characterization of reverse-genetic infectious bursal disease virus. The Proceedings of the 60th North Central Avian Disease Conference, Page 16, 2009.
5. Wu, C. C., Chen, Y. Y., and Lin, T. L. Specific humoral immunity elicited by DNA encoding infectious bursal disease virus large segment gene and avian influenza virus hemagglutinin gene. The Proceedings of the 146st Annual Meeting of the American Veterinary Medical Association, Page 23, 2009.
6. Strother M, Cha W, Saif YM, Lee CW. Microsphere-based multiplex branched DNA assay for the detection and differentiation of influenza virus. 7th International Symposium on Avian Influenza. **April 5-8, 2009**. Athens, GA.
7. Wang L, Saif YM, Lee CW. Developing live attenuated influenza in ovo vaccines for poultry. 7th International Symposium on Avian Influenza. **April 5-8, 2009**. Athens, GA.
8. Pillai SPS, Suarez DL, Pantin-Jackwood M, Lee CW. The high susceptibility of turkeys to low pathogenic avian influenza viruses of different origins imply their importance as intermediate hosts. 7th International Symposium on Avian Influenza. **April 5-8, 2009**. Athens, GA.
9. Saif, Y.M. Influenza Vaccines for Turkeys. Proc. 5th Intl. Veterinary Vaccines and Diagnostics Conference, Madison, WI, July 19-23, 2009.
10. Yassine, H.M., M. Khatri, C.W. Lee, and Y.M. Saif: Interspecies Transmission of Triple Reassortant H3N2 Influenza Viruses between Swine and Turkeys: Molecular Studies. Proc. American Association of Avian Pathologists (AAAP-AVMA) Annual Meeting. Poster, Seattle, WA, July, 2009.
11. Khatri, M., H.M. Yassine, Y.M. Saif, and C.W. Lee: Susceptibility of Chicken T Cells to Low Pathogenic H5 Influenza Viruses. Proc. American Association of Avian Pathologists (AAAP-AVMA) Annual Meeting. Abstract, Seattle, WA, July, 2009.
12. **Wang, L., H.M. Yassine, S. Pillai, Y.M. Saif, and C.W. Lee: Development of DIVA Vaccines for the Control of Triple Reassortant H3N2 Influenza in Turkeys.** Proc. American Association of Avian Pathologists (AAAP-AVMA) Annual Meeting. Abstract, Seattle, WA, July, 2009.
13. Yassine, H.M., M. Khatri, C.W. Lee, and Y.M. Saif: Studies on Interspecies Transmission of Triple Reassortant H3N2 influenza A Viruses. Proc. 7th International Symposium on Avian Influenza., Abstract, Athens, GA, April, 2009.
14. Jackwood, D. J. Current status of infectious bursal disease. Proceedings of the XXXIV Annual Asociacion Nacional de Especialistas en Ciencias Avicolas de Mexico, A. C. (ANECA) Convention. Acapulco, Mexico. August 2009.
15. Gelb, J., Jr., D. J. Jackwood, E. Mundt, C. R. Pope, R. Hein, G. Slacum, J. M. Harris, B. S. Ladman, P. Lynch, D. Bautista, M. Ruano, and M. Troeber. Antigenic Characterization and VP2 Analysis of Delmarva IBD Field Viruses. Proc. 146th American Veterinary Medical Assn./ American Assn. Avian Pathologists Ann. Mtg. Seattle, Washington. July 11-14, 2009.
16. Harris, J. M., J. Gelb, Jr., D. J. Jackwood, E. Mundt, B. S. Ladman, C. R. Pope, R. Hein, G. Slacum, P. Lynch, M. RuanoA, M. Troeber, and D. Bautista. Characterization of Infectious Bursal Disease Viruses Isolated from Commercial Chickens. Eighty-first Northeastern Conference on Avian Diseases. Grantville, Pennsylvania. September 17-18, 2009.
17. Preskenis, L. A., J. Gelb, Jr., E. Spackman, and C. R. Pope. Characterization of LPAI H7 Isolates in Three Species of Birds. Eighty-first Northeastern Conference on Avian Diseases. Grantville, Pennsylvania. September 17-18, 2009.
18. Golovan, S. P., Abrahamyan, A., Duvanov, S. Nagy, E., Sharif, S., Bedecarrats, G. Application of RNA Interference Methodology to Inhibit Avian Influenza Virus Replication *in Vitro*. Max Planck Institute for Developmental Biology. Tübingen, Germany. June 12, 2009.
19. Golovan, S. P., Abrahamyan, A., Duvanov, S. Nagy, E., Sharif, S., Bedecarrats, G. Application of RNA Interference Methodology to Inhibit Avian Influenza Virus Replication in Vitro. Poultry Research Institute. Kharkov. Ukraine. June 23, 2009.
20. Golovan, S. P., Abrahamyan, A., Duvanov, S. Nagy, E., Sharif, S., Bedecarrats, G. Application of RNA Interference Methodology to Inhibit Avian Influenza Virus Replication in Vitro. Institute for Experimental and Clinical Veterinary Medicine. Kharkiv. Ukraine. June 26, 2009.
21. Golovan, S. P., Abrahamyan A., E. Nagy, Bedecarrats, G. Design of conservative and highly efficient anti-influenza short hairpin RNAs and microRNAs functional in avian and mammalian cells. *Preliminary submission* 2009. Patent application filed.