

## 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, 2013 to September 30, 2014

**Date of This Report:** December 1, 2014

**Annual Meeting Date:** November 8, 2014

### Participants:

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**Brief summary of minutes of annual meeting:** The annual NC 1180 business meeting was held on Sunday, November 8, 2014 in room M 302, Marriott Marquis Hotel, Atlanta, GA. Dr. Laszlo Zsak, Chair of NC 1180 (term 2013-2014) opened the meeting at 8:00 am. He welcomed the station representatives and participating scientists. The former NC advisor Dr. Mo Saif introduced the new advisor Dr. Jeff LeJeune (The Ohio State University). Dr. Zsak welcomed Dr. LeJeune and thanked Dr. Saif for his efforts in providing encouraging and effective guidance to the group in the past.

Due to schedule conflict, the Secretary (2013-2014 tem) was unable to join the group; therefore, the minutes of 2013 Annual NC1180 Meeting will be approved at a later time. Dr. Ching Ching Wu volunteered to take notes for the 2014 meeting.

The Chair informed the group of the regrets from NIFA-USDA representative Dr. Peter Johnson. The group wished to use electronic means to communicate with Peter when he cannot be present in

the meeting in the future. It is very important to have inputs and updates from NIFA. Dr. Johnson's absence was very much missed by the group.

The meeting venue and time for 2015 was discussed in length and depth. The 2014 meeting was voted to be at Atlanta, GA to accommodate and encouraging attendance. Unfortunately the attendance was not improved. It was decided that the meeting in conjunction with USAHA in Providence, Rhode Island will be the best fit in 2015 because of USDA NIFA NC-CAP project. USAHA is well attended by many stake holders; therefore, it will provide ample opportunity for NC1180 members to interact and seek input from them. Although the 2015 meeting dates and time was tentatively set at October 27, 28, and/or 29, 2015, it will be announced when finalized. The USDA NIFA NC-CAP project submission was discussed and Dr. Chang-Won Lee provided the updates. The official announcement on project funding is pending.

The next item on the agenda was to elect a new Chair and Secretary for NC1180. While Dr. Zsak's term was up, the group appreciated his leadership and elected him for another term (2015-2016). Dr. Zsak accepted. While Dr. Tim Johnson had served the group well, in his absence, Dr. Tsang Long Lin was elected to be the Secretary for 2015-2016 and accepted.

Dr. LeJeune, NC1180 Advisor, reiterated the importance for each station to send impact statement to the Chair for the annual report to be uploaded to NIMSS.

Dr. Zsak, the Chair, reminded station representatives to send him electronic version of the report, including the impact statement as directed by the official format, immediately after the annual meeting.

The presentation of annual progress report from each participating Station began immediately after the business meeting. USDA-ARS-SEPRL was the first to give the report and followed by Georgia, Ohio, Illinois, Delaware, and Indiana. The 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, useful information and new knowledge were freely communicated and exchanged.

The 2014 Annual NC1180 Meeting adjourned at 4:00 pm, November 8, 2014.

Respectfully submitted,

Ching Ching Wu, DVM. PhD  
Sitting in for the Secretary Dr. Tim Johnson, NC1180  
November 20, 2014

### **Accomplishments:**

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

1. Inoculation of chickens, Japanese quail, pigeons, Pekin ducks, Mallard ducks, Muscovy ducks, and Embden geese with the A/Anhui/1/2013 H7N9 virus resulted in infection but no clinical disease

signs. This study provides experimental data to show that quail and chickens are susceptible to infection, shed large amounts of virus, and are likely important in the spread of the virus to humans (SEPRL).

2. Genomic characterization of 45 viruses isolated from blue-winged teal (*Anas discors*) along the Texas and Louisiana Gulf Coast during March of 2012 and 2013 indicated a coincidence with northward migration of this species from Neotropical wintering areas to breeding grounds in the United States and Canada (SEPRL, GA).
3. During a wild bird surveillance in regions of Ukraine suspected of being intercontinental (north to south and east to west) flyways between 2006 and 2011 twenty viruses were isolated and subsequently identified as avian paramyxoviruses (SEPRL). The highest isolation rate occurred during the autumn migration with viruses isolated from mallards, teals, dunlins, and a wigeon.
4. Testing samples from 858 mute swans in the Great Lakes region and Atlantic Coast of the United States indicated that avian paramyxoviruses and avian influenza viruses are common in these birds (SEPRL).
5. Avian coronaviruses were identified in wild aquatic birds of the Central and Eastern United States at a low prevalence (GA).
6. DE determined that in 2013-14, the combination of an extremely cold winter, very high numbers of IBV, ILTV or IBV/ILTV (co-infection) respiratory disease cases made the winter of 2013-14 one of the worst in recent memory for broiler chicken production on the Delmarva peninsula.
7. National Animal Health Laboratory Network Avian influenza surveillance testing at the University of Delaware detected an H7 subtype in non-commercial backyard auction poultry in Delaware in 2014 (DE). No commercial flocks were infected in Delmarva (DE).
8. Avian influenza wild bird surveillance from 2007-2010 in Delaware yielded 39 isolates from waterfowl and shorebird species Ruddy Turnstone This study shows that wild birds are able to harbor subtypes that are important to the poultry industry and reinforces the notion that limiting the interaction between wild birds and commercial poultry is an important aspect of influenza control (DE).

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

1. GA found that the universal IBV primers and probe set to be comparable to challenge virus detection in embryonated eggs; however, for some IBV types the qRT-PCR assay was more sensitive than virus detection in embryonated eggs.
2. IL developed a dynamic light scattering (DLS) assay for simple, rapid, field deployable avian influenza diagnostic tool. Preliminary data demonstrates excellent correlation between ELISA screening and the DLS assay to examine the affinity of these antibodies for different viruses.
3. Primers for real-time RT-PCR for IBV variant DMV/1639/11 were developed and validated and the test was added to our molecular respiratory panel for rapid diagnosis (DE).
4. Approaches used to generate full length sequence of ILTV have been adapted and used to determine the sequence of the ILTV genome from egg material (CAM) and from oral pharyngeal swabs (DE).

**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. Co-infection of chickens or turkeys with lentogenic NDV and LPAIV affected the replication dynamics of these viruses but did not affect clinical signs (SEPRL).
2. Responses to the H7 subtype influenza viruses were intermediate to those elicited by H5N1 and pdm09H1N1 early in infection but that they evolved to resemble the H5N1 response as infection progressed (SEPRL).
3. Complete genome sequence analysis of double reassortant H13N8 influenza A virus from gull in Mongolia indicated the complicated evolutionary history of these viruses (SEPRL).
4. Four diverse AIV isolates for use as vaccines in chickens, including two commercial vaccines and two additional contemporary isolates, against challenge with numerous clade 2.2.1 and clade 2.2.1.1 H5N1 HPAIV Egyptian isolates indicated that there were differences in protection among the vaccines relative to one another based on challenge virus (SEPRL).
5. Co-infection with LPAIV and loNDV does not affect the ability of mallards to be infected with either virus although it may have minimal effects on patterns (source and timing) of viral shedding (SEPRL).
6. OH determined that reassortant IBDV with a vv genome segment A and non-vv segment B were less pathogenic than the vv/vv rB strain but more pathogenic than the cv/cv STC strain and viruses with a serotype 2 genome segment A regardless of the type of genome segment B did not cause clinical disease in SPF chickens or turkeys.
7. OH's observation that vv/serotype 2 reassorted viruses break through maternal immunity suggests that antigenicity of these relatively lower pathogenic viruses may be contributing to their ability to cause immune suppression in commercial broilers.
8. OH studies indicate that the addition of M2e peptide to inactivated influenza vaccine confers improved protection compared to single regime suggesting a possible approach to modify traditional vaccine strategy.

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

1. A highly stable fast-dissolving tablets (FDT) of Newcastle disease virus (NDV) vaccine were produced and provided 100 per cent protection to vaccinated chickens infected with a virulent strain of NDV (SEPRL).
2. Newcastle disease virus recombinants expressing infectious laryngotracheitis virus glycoproteins gB and gD protect chickens against ILTV and NDV challenges (SEPRL, GA).
3. GA found that interference between different types of IBV vaccines was not occurring when combined and administered using a commercial hatchery spray cabinet. UGA also found that detection of IBV vaccine virus early after administration (regardless of strain or route) correlated with protection against homologous challenge.
4. OH observed that a single amino acid substitution (N165H), which removes potential glycosylation site at the HA globular head of two classic H5N1 strains broaden the reactivity to antisera generated against H5N1 viruses from different clusters. The broadened reactivity of the mutant viruses were also confirmed by testing reactivity of antisera prepared from the mutant viruses against reference viruses from both classic and variant clades.
5. IN compared DNA-vaccinated chickens and unvaccinated chickens upon challenge by infectious bursal disease virus (IBDV) by transcriptomic analysis of chicken spleens. the chickens protected from IBDV challenge by DNA vaccination do not have significantly differential and enhanced

expression in the splenic transcripts related to innate immunity, immune cell regulation, and inflammation. On the contrary, the chickens challenged with IBDV only have significantly differential and enhanced expression in the splenic transcripts concerning innate immunity, immune cell regulation, and inflammation.

6. DE determined that vaccination with IBV vaccines Massachusetts serotype Ma5 + Delaware 072 offered no protection against challenge with Arkansas DPI. The lack of protection supports field observations and the high numbers of Arkansas virus isolations from Delmarva broiler chicken respiratory disease cases in Fall of 2013 and Winter of 2014.

7. Drinking water vaccination is the primary method of administration of infectious laryngotracheitis virus (ILTV) chicken embryo origin vaccines. A controlled laboratory study indicated a commercial vaccine given at the recommended 1X dose did not achieve flock coverage on day 5 post vaccination compared to broilers given a 10X dose (DE).

8. Serial passage of infectious laryngotracheitis virus (ILTV) at a reduced incubation temperature of 30C for 32 passages gave encouraging results as a method to attenuate the virus (DE).

9. GA developed two gene deleted ILTV recombinants. The recombinants were completely attenuated and elicited significant protective immune response to virulent ILTV challenge in chickens following in ovo vaccination.

10. GA evaluated the transcriptional status of cytokines in the trachea of birds infected with a virulent strain of the virus and found up-regulation of inflammatory and anti-inflammatory cytokines, up-regulation of IL-2, IL-13, and down-regulation of IL-18 and IL-12.

## **Impacts**

### **Objective I.**

Certain poultry species are reservoirs of the H7N9 influenza virus and that the virus is highly tropic for the upper respiratory tract, so testing of bird species should preferentially be conducted with oropharyngeal swabs for the best sensitivity.

Surveillance for the introduction of IAVs from Central America and northern South America into the United States may be further optimized through genomic characterization of viruses resulting from coordinated, concurrent sampling efforts targeting blue-winged teal and sympatric species throughout the Neotropics and along the United States Gulf Coast.

Wild bird species most likely to be infected with avian paramyxoviruses and data support possible intercontinental transmission of these viruses by wild birds.

Since their introduction to the United States in the late 19th century, mute swans (*Cygnus olor*) can potentially transmit or serve as a reservoir of infectious diseases to humans and poultry.

Aquatic birds may represent a potential reservoir for avian coronaviruses.

Data confirmed the state of extensive geographic mosaicism in AIV from gulls in the Northern Hemisphere.

Infectious bronchitis virus is a leading pathogen of commercial chickens in the U.S. Surveillance testing continues to detect variant strains in Delaware and Delmarva, not to mention other regions

of the U.S. Improved selection of vaccine strains based on surveillance testing is essential, as is developing improved methods of vaccine administration.

### **Objective II.**

Results indicate that qRT-PCR assays can be used to detect IBV challenge virus, but each assay including the assay conditions and thermocycler should be individually evaluated if that data is expected to be comparable to virus detection in embryonated eggs.

DLS platform is highly sensitive, rapid and field deployable for influenza virus subtype detection via screening virus isolates. Rapid field detection and subtyping of AIVs will allow for early intervention, reducing poultry morbidity and mortality.

### **Objective III.**

Infection with a heterologous virus (NDV and AI) may result in temporary competition for cell receptors or competent cells for replication, most likely interferon-mediated, which decreases with time.

Host responses could be targeted to treat severe H7N9 influenza and six FDA-approved drugs could potentially be repurposed as H7N9 influenza therapeutics.

Current breeder vaccination programs may not adequately protect against the reassorted and pathogenic vv/serotype 2 and vv/cv IBDV strains. A change in the breeder vaccination program may be needed to control these viruses.

### **Objective IV.**

Selecting optimal vaccine seed strains for successful HPAIV control is highly important in vaccination against diverse, evolving virus populations.

Fast-dissolving tablet formulation of NDV vaccine for low-cost backyard poultry immunization is a feasible and cost-saving approach.

Recombinant NDV-vectored gB or gD vaccines could be used as bivalent vaccines against NDV and ILTV in chickens.

Key findings can be used to direct the efforts for improving the efficacy of IBV Ark type vaccines given in the hatchery and are an important step in elucidating the factors contributing to the persistence of Ark vaccine in the field.

There is a possibility to develop potential vaccine seed viruses that broadly reacted to different H5N1 clusters using in vitro mutagenesis targeting residues in the antigenic epitopes coupled with reverse genetics.

The addition of M2e peptide to inactivated influenza vaccine is desirable to reduce the vaccine dose.

IBDV large segment gene-based DNA vaccine has the potential for practical application to confer protection of chickens against infectious bursal disease (IBD) in the poultry industry.

Infectious laryngotracheitis virus is a leading pathogen of commercial poultry in the U.S. Vaccines used to control the disease may not be manufactured at a sufficient dose for effective administration via the drinking water. Vaccine development research is warranted.

Genetic engineering ILTV strains can be used to develop safe and efficacious live-attenuated vaccines that can be administered in ovo or at day-old age of chickens.

Findings suggest that ILTV virus may be interfering with the maturation of Th1 cells while favoring the development of robust Th2 response in the trachea and this can improve the development of future vaccines and the potential use of immune-modulants.

## **PUBLICATIONS:**

Amin, O. G. M., and Jackwood, D.J. 2014. Identification and molecular analysis of infectious bursal disease in broiler farms in the Kurdistan Regional Government of Iraq. *Tropical Animal Health and Production*. 46:1297-301.

Bertran, K., Swayne, D.E. 2014. High doses of highly pathogenic avian influenza virus in chicken meat are required to infect ferrets. *Vet. Res.* 45:60.

Cardona, C.J., Halvorson, D.A., Brown, J.D., Pantin-Jackwood, M.J. 2014. Conducting influenza virus pathogenesis studies in avian species. *Methods Mol. Biol.* 1161:169-83.

Chappell, L., Killian, M.L., Spackman, E. 2014. Detection of influenza A antibodies in avian serum samples by ELISA. *Methods Mol. Biol.* 1161:151-67.

Costa-Hurtado, M., Afonso, C.L., Miller, P.J., Spackman, E., Kapczynski, D.R., Swayne, D.E., Shepherd, E., Smith, D., Zsak, A., Pantin-Jackwood, M. 2014. Virus interference between H7N2 low pathogenic avian influenza virus and lentogenic Newcastle disease virus in experimental co-infections in chickens and turkeys. *Vet. Res.* 45:1.

França, M., Howerth, E.W., Carter, D., Byas, A., Poulson, R., Afonso, C.L., Stallknecht, D.E. 2014. Co-infection of mallards with low-virulence Newcastle disease virus and low-pathogenic avian influenza virus. *Avian Pathol.* 43:96-104.

Jordan, B.J., Hilt, D.A., Poulson, R., Stallknecht, D.E., and Jackwood, M.W. 2014. Identification of Avian Coronavirus in Wild Aquatic Birds of the Central and Eastern United States. *J. Wildl. Dis.* 2014. Nov 7.

Kapczynski, D.R., Jiang, H.J., Kogut, M.H. 2014. Characterization of cytokine expression induced by avian influenza virus infection with real-time RT-PCR. *Methods Mol Biol.* 1161:217-33.

Kapczynski, D.R. 2014. Detection of cell-mediated immune response to avian influenza viruses. *Methods Mol. Biol.* 1161:199-215.

Kiss, G., Chen, X., Brindley, M.A., Campbell, P., Afonso, C.L., Ke, Z., Holl, J.M., Guerrero-Ferreira, R.C., Byrd-Leotis, L.A., Steel, J., Steinhauer, D.A., Plemper, R.K., Kelly, D.F., Spearman, P.W., Wright, E.R. 2014. Capturing enveloped viruses on affinity grids for downstream cryo-electron microscopy applications. *Microsc. Microanal.* 20:164-74.

Lal, M., Zhu, C., McClurkan, C., Koelle, D.M., Miller, P., Afonso, C., Donadeu, M., Dungu, B., Chen, D. 2014. Development of a low-dose fast-dissolving tablet formulation of Newcastle disease vaccine for low-cost backyard poultry immunisation. *Vet Rec.* 174:504.

Lee, C.C., Wu, C.C., and Lin, T.L. 2014. Chicken melanoma differentiation-associated gene 5 (MDA5) recognizes infectious bursal disease virus infection and triggers MDA5-related innate immunity. *Archives of Virol.* 159:1671-16686.

Lee, C.C., Kim, B.S., Wu, C.C., and Lin, T.L. 2014. Bursal transcriptome of chickens protected by DNA vaccination versus those challenged with infectious bursal disease virus. *Archives of Virol.* 2014 Oct 1.

Lee, C.W. 2014. Different Approaches Toward Universal Influenza Vaccines. *Asia Pacific Poultry Conference Proceedings.* p.61-63.

Menendez, K.R., García, M., Spatz, S., and Tablante, N.L. 2014. Molecular epidemiology of infectious laryngotracheitis: a review. *Avian Path.* 43:108-117.

Miller, P.J., Torchetti, M.K. 2014. Newcastle disease virus detection and differentiation from avian influenza. *Methods in Molecular Biology.* 1161:235-239.

Morrison, J., Josset, L., Tchitchek, N., Chang, J., Belser, J.A., Swayne, D.E., Pantin-Jackwood, M.J., Tumpey, T.M., Katze, M.G. 2014. H7N9 and other pathogenic avian influenza viruses elicit a three-pronged transcriptomic signature that is reminiscent of 1918 influenza virus and is associated with lethal outcome in mice. *J. Virol.* 88:10556-68.

Muzyka, D., Pantin-Jackwood, M., Stegnyy, B., Rula, O., Bolotin, V., Stegnyy, A., Gerilovych, A., Shutchenko, P., Stegnyy, M., Koshelev, V., Maiorova, K., Tkachenko, S., Muzyka, N., Usova, L., Afonso, C.L. 2014. Wild bird surveillance for avian paramyxoviruses in the Azov-black sea region of Ukraine (2006 to 2011) reveals epidemiological connections with Europe and Africa. *Appl. Environ. Microbiol.* 80:5427-38.

Niu, Z., Bai, F., Sun, T., Tian, H., Yu, D., Yin, J., Li, S., Li, T., Cao, H., Yu, Q., Wu, Y., Ren, G., Li, D. 2014. Recombinant Newcastle Disease virus Expressing IL15 Demonstrates Promising Antitumor Efficiency in Melanoma Model. *Technol Cancer Res Treat.* 2014 Mar 17.



- Pantin-Jackwood, M.J., Miller, P.J., Spackman, E., Swayne, D.E., Susta, L., Costa-Hurtado, M., Suarez, D.L. 2014. Role of poultry in the spread of novel H7N9 influenza virus in China. *J. Virol.* 88:5381-90.
- Pantin-Jackwood, M.J. 2014. Immunohistochemical staining of influenza virus in tissues. *Methods Mol. Biol.* 1161:51-8.
- Pedersen, K., Marks, D.R., Arsnoe, D.M., Afonso, C.L., Bevins, S.N., Miller, P.J., Randall, A.R., DeLiberto, T.J. 2014. Avian paramyxovirus serotype 1 (Newcastle disease virus), avian influenza virus, and *Salmonella* spp. in mute swans (*Cygnus olor*) in the Great Lakes region and Atlantic Coast of the United States. *Avian Dis.* 58:129-36.
- Pepin, K.M., Spackman, E., Brown, J.D., Pabilonia, K.L., Garber, L.P., Weaver, J.T., Kennedy, D.A., Patyk, K.A., Huyvaert, K.P., Miller, R.S., Franklin, A.B., Pedersen, K., Bogich, T.L., Rohani, P., Shriner, S.A., Webb, C.T., Riley, S. 2014. Using quantitative disease dynamics as a tool for guiding response to avian influenza in poultry in the United States of America. *Prev. Vet. Med.* 113:376-97.
- Ramey, A.M., Walther, P., Link, P., Poulson, R.L., Wilcox, B.R., Newsome, G., Spackman, E., Brown, J.D., Stallknecht, D.E. 2014. Optimizing Surveillance for South American Origin Influenza A Viruses Along the United States Gulf Coast Through Genomic Characterization of Isolates from Blue-winged Teal (*Anas discors*). *Transbound Emerg Dis.* 2014 Jul 24.
- Roh, H-J., Hilt, D.A., Williams, S.M., Jackwood, M.W. 2013. Evaluation of Infectious Bronchitis Virus Arkansas-type Vaccine Failure in Commercial Broilers. *Avian Dis.* 57:248-259.
- Roh, H-J., Jordan, B.J., Hilt, D.A., Jackwood, M.W. 2014, Detection of infectious bronchitis virus using real time quantitative RT-PCR and correlation with virus detection in embryonated eggs. In Press. *Avian Dis.* 58:.
- Russell, C.A., Kasson, P.M., Donis, R.O., Riley, S., Dunbar, J., Rambaut, A., Asher, J., Burke, S., Davis, C.T., Garten, R.J., Gnanakaran, S., Hay, S.I., Herfst, S., Lewis, N.S., Lloyd-Smith, J.O., Macken, C.A., Maurer-Stroh, S., Neuhaus, E., Parrish, C.R., Pepin, K.M., Shepard, S.S., Smith, D.L., Suarez, D.L., Trock, S.C., Widdowson, M.A., George, D.B., Lipsitch, M., Bloom, J.D. 2014. Improving pandemic influenza risk assessment. *Elife.* 2014 Oct 16;3.
- Sharshov, K., Sivay, M., Liu, D., Pantin-Jackwood, M., Marchenko, V., Durymanov, A., Alekseev, A., Damdindorj, T., Gao, G.F., Swayne, D.E., Shestopalov, A. 2014. Molecular characterization and phylogenetics of a reassortant H13N8 influenza virus isolated from gulls in Mongolia. *Virus Genes.* 49:237-49.
- Sivay, M.V., Sharshov, K.A., Pantin-Jackwood, M., Muzyka, V.V., Shestopalov, A.M. 2014. Avian Influenza Virus with Hemagglutinin-Neuraminidase Combination H8N8, Isolated in Russia. *Genome Announc.* 2014 Jun 5;2(3).

- Spackman, E., Swayne D.E., Pantin-Jackwood, M.J., Wan, X.F., Torchetti, M.K., Hassan, M., Suarez, D.L., Sá E Silva, M. 2014. Variation in protection of four divergent avian influenza virus vaccine seed strains against eight clade 2.2.1 and 2.2.1.1. Egyptian H5N1 high pathogenicity variants in poultry. *Influenza Other Respir Viruses*. 2014 Oct 3.
- Spackman, E., Pantin-Jackwood, M.J. 2014. Practical aspects of vaccination of poultry against avian influenza virus. *Vet. J.* 2014 Oct 5.
- Spackman, E. 2014. Preface. *Animal influenza virus. Methods Mol. Biol.* 2014;1161.
- Spackman, E., Killian, M.L. 2014. Avian influenza virus isolation, propagation, and titration in embryonated chicken eggs. *Methods Mol. Biol.* 1161:125-40.
- Spackman, E. 2014. Influenza subtype identification with molecular methods. *Methods Mol. Biol.* 1161:119-23.
- Spackman, E. 2014. Avian influenza virus detection and quantitation by real-time RT-PCR. *Methods Mol. Biol.* 1161:105-18.
- Spackman, E., Lee, S.A. 2014. Avian influenza virus RNA extraction. *Methods Mol. Biol.* 1161:93-104.
- Spackman, E. 2014. A brief introduction to avian influenza virus. *Methods Mol. Biol.* 1161:61-8.
- Suarez, D.L., Chester, N., Hatfield, J. 2014. Sequencing artifacts in the type A influenza databases and attempts to correct them. *Influenza Other Respir. Viruses*. 8:499-505.
- Susta, L., Hamal, K.R., Miller, P.J., Cardenas-Garcia, S., Brown, C.C., Pedersen, J.C., Gongora, V., Afonso, C.L. 2014. Separate evolution of virulent newcastle disease viruses from Mexico and Central America. *J. Clin. Microbiol.* 52:1382-90.
- Susta, L., Jones, M.E., Cattoli, G., Cardenas-Garcia, S., Miller, P.J., Brown, C.C., Afonso, C.L. 2014. Pathologic Characterization of Genotypes XIV and XVII Newcastle Disease Viruses and Efficacy of Classical Vaccination on Specific Pathogen-Free Birds. *Vet. Pathol.* 2014 Feb 7.
- Swayne, D.E. 2014. Laboratory methods for assessing and licensing influenza vaccines for poultry. *Methods Mol. Biol.* 1161:185-98.
- Swayne, D.E., Spackman, E., Pantin-Jackwood, M. 2014. Success factors for avian influenza vaccine use in poultry and potential impact at the wild bird-agricultural interface. 2014. *Ecohealth*. 11:94-108.
- Volkova, M.A., Irza, A.V., Chvala, I.A., Frolov, S.F., Drygin, V.V., Kapczynski, D.R. 2014. Adjuvant effects of chitosan and calcium phosphate particles in an inactivated Newcastle disease vaccine. *Avian Dis.* 58:46-52.

Wu, Y., Yan, S., Lv, Z., Chen, L., Geng, J., He, J., Yu, Q., Yin, J., Ren, G., Li, D. 2014. Recombinant Newcastle disease virus Anhinga Strain (NDV/Anh-EGFP) for Hepatoma Therapy. *Technology in Cancer Research and Treatment*. 13:169-175.

Yen, L, Oh, S.H., Driskell, E.A., and Driskell, J.D. 2014. Screening Antibodies with Labeled Gold Nanoparticle DLS Assays. In *Proceedings of Central Regional American Chemical Society*, Pittsburg, PA October 29, 2014.

Zhao, W., Hu, H., Zsak, L., Yu, Q., Yang, Z. 2013. HN gene c-terminal extension of Newcastle disease virus is not the determinant of the enteric tropism. *Virus Genes*. 47:27-33.

Zhao, W., Zhang, Z., Zsak, L., Yu, Q. 2014. P and M gene junction is the optimal insertion site in Newcastle disease virus vaccine vector for foreign gene expression. *J. Gen. Virol.* 2014 Oct.

Zhao, W., Spatz, S., Zhang, Z., Wen, G., Garcia, M., Zsak, L., Yu, Q. 2014. Newcastle disease virus (NDV) recombinants expressing infectious laryngotracheitis virus (ILTV) glycoproteins gB and gD protect chickens against ILTV and NDV challenges. *J. Virol.* 88:8397-406.

#### **PATENTS:**

1. Novel immunogenic composition; Docket 20.14; Yu, Q., Zsak, L., Spatz, S. (SEPRL) 2014.
2. Compositions, vectors, kits, and methods for immunizing against avian infectious bronchitis virus; Toro, H. (Auburn University, AL) and Yu, Q. (SEPRL) 2014.