

NC1180 MULTISTATE PROJECT ANNUAL REPORT 2020

Multistate Project NC-1180

Control of endemic, emerging and re-emerging poultry respiratory diseases

Period the Report Covers: (September 30th 2019 – October 1st, 2020)

Reporting stations: AL, CA, GA, NE, OH, IL, DE, NY, IA, IN and SEPRL (USDA)

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Summary of 2020 meeting minutes

Meeting started at 6:00am PST

-Meeting minutes 2019 approved

-Next year's meeting venue: Chicago Dec 7-8th with CRAWD.

-A motion by Dr. Gallardo was accepted to appoint Dr. Maricarmen Garcia as Secretary for this and next year (2020-2021) and then Chair for 2022-2023. Dr Gallardo will act as Chair for 2020 and 2021 since Dr. Johnson will be on sabbatical leave working for Cargill.

-Dr. Velleman emphasized that we need to focus on collaborative efforts. Individual results should be tied to collaborative efforts, this is key for project renewal. In preparation of the annual report impact statements are important in addition, highlight publications that show collaborations between stations is required. We have a mid-term review in 2022 and in Fall 2024 we are due to renewal.

-Dr. Siewert our new USDA/NIFA representative had time to present himself. Dr. Siewert invited our members to participate in USDA/NIFA review panels. At least two members volunteered.

-Report presentations started at 6:33am with Alabama. Interesting discussions occurred in IBV, AI and other topics. Several members agreed to collaborate on common research studies. Presentations from stations ended at 2:45 PST.

-New prospect members were introduced (Monique Franca UGA, Alejandro Banda MSU) they showed interest in joining the project.

-Six colleagues from Minnesota are members of the project and none of them assisted to the meeting. In addition, no report was received this year. It was advised to contact them through the admin Advisor Dr. Velleman. We also discussed that nobody from NC State is represented in this multistate. We will contact Dr. Rocio Crespo.

-Finally, Dr. Garcia reminded everybody that collaborations are really important to keep this project alive. Meeting adjourned at 3:00pm PST.

Accomplishments

OBJECTIVE 1 - INVESTIGATE THE ECOLOGY OF POULTRY RESPIRATORY DISEASES AND THEIR ROLE IN POULTRY FLOCKS.

-Surveillance and genotyping efforts of circulating Infectious bronchitis virus (IBV) strains were conducted in Auburn (Alabama) and Davis (California). In Alabama the prevalent circulating molecular type was ARK/DPI and in California a 15 years assessment revealed that the prevalent IBV molecular types fluctuates, between 2019 to 2020 the most prevalent molecular types were Cal 3099 (Cal ent.) and Mass type strains. From Delaware it was informed that ILTV, IBV and NDV mix infections were

prevalent in 2019 but genotyping of these viruses are pending. This information was shared in the meeting.

- Surveillance and genotyping efforts of circulating avian reovirus (ARV) in California indicated that the use of autogenous vaccines favors the emergence of new variants for which effective vaccination control does not exist.

- Field studies were conducted to determine the persistence of IBV and ILTV vaccines in broiler and layer flocks and found that these vaccines prevail for longer periods of time during the lifespan of the flock.

- A survey among Poultry professional was conducted to assess their perception on participating in voluntary disease mapping and monitoring programs. This survey clearly identified two groups of poultry professionals one that perceives the benefits of such a program and a group who perceives that sharing disease information is a risk to high to take.

- Prevalence of *Mycoplasma gallisepticum* (MG) in Purple Finches was higher than in House Finches indicated that both species can serve as reservoir of the bacteria. The virulence and persistence of poultry originated MG was compared to finch originated MGs in House Finches. No differences in tissue tropism and replication were observed between poultry and finch MGs.

- The incidence of *Avibacterium paragallinarum* (AP) infections have increased in the United States. In a flock with not sign of infectious coryza (IC) the bacteria genome was detected by real-time PCR but not by conventional PCR. Full genome indicated that this AP isolate lacks the *HMTp210 gene*.

- Influenza viruses: Full genome sequencing of the low and highly pathogenic H7N3 viruses that emerged in 2020 in domestic poultry from North and South Carolina revealed that these viruses originated in wild birds.

- In a collaboration between SEPRL, University of Georgia and the University of Connecticut found that agricultural and geographical factors greatly influenced the spread of the highly pathogenic H5 AIV in the Midwestern United States during the 2015 outbreak.

OBJECTIVE 2- DEVELOP NEW AND IMPROVED DIAGNOSTIC TOOLS FOR POULTRY RESPIRATORY DISEASES.

- Phylogenetic analysis of Reoviruses using target genes L2, M2, S1, S3 and M3 genes was a successful approach that led to study distinct regions of the genome that represent potential determinants of antigenicity and pathogenicity. This approach was established as a diagnostic-genotyping assay for ARV samples from California and Alabama.

- The reemergence of infectious coryza (IC) caused by *Avibacterium paragallinarum* has underscore the need for a rapid genotyping assay to improve the diagnosis of the bacteria and better understand the epidemiology of the disease. Two target genes (HMTp210 and HagA) were identified as potential candidates for such assay.

- Current Infectious laryngotracheitis virus (ILTV) sequence typing methods are not optimized to perform directly from clinical samples. Given the large size of the targeted segments, ILT sequence typing performs best in isolated viral strains and in clinical samples with high viral copy numbers. Using previously identified target genes, smaller target sites were amplified. Amplification and sequencing of these smaller targets was successful from clinical samples with lower copy number.

- New and improved Taqman real time PCR assays were developed for detection of *Ornithobacterium rhinotracheale* (ORT), *Bordetella avium* (BA), and detection of Newcastle disease virus of the Class II.

- Multiple full length or close to full length genomes infectious bronchitis viruses (IBV) were obtained from archived samples using next generation sequencing.

OBJECTIVE 3 - ELUCIDATE THE PATHOGENESIS OF POULTRY RESPIRATORY DISEASES

- We demonstrated strong correlations between the levels of innate and adaptive responses to virus infection and changes in the relative abundances of specific microbiota in the respiratory and digestive tracts. We also demonstrated that TLR3 and MDA5 are the key functional dsRNA receptors in quail cells and highlighted their coordinated roles in the induction of innate immune responses upon virus infection.

- Work is focusing on detecting MDV genes essential for transmission could have major implications in the design of vaccines that could target MDV spread in a chicken house. Blocking infection and subsequent immune suppression would be highly beneficial and understanding the conserved gC protein during transmission will help in this endeavor.

OBJECTIVE 4. DEVELOP CONTROL AND PREVENTION STRATEGIES FOR POULTRY RESPIRATORY DISEASES

- This multistate program has generated several biosecurity and disease prevention programs across the United States.
- Several methods were tested that effectively inactivate poultry respiratory pathogens allowing better management and disease prevention in commercial poultry flocks.
- Underappreciated subpopulations in avian influenza vaccines are capable of enhancing interferon production. This strategy can be used to further improve the efficacy of current live vaccines.
- In addition, novel small molecule antimicrobials effective against E. Coli and Mycoplasma were discovered.

Impacts

OBJECTIVE 1 - INVESTIGATE THE ECOLOGY OF POULTRY RESPIRATORY DISEASES AND THEIR ROLE IN POULTRY FLOCKS.

- Surveillance and genotyping efforts for economically important infectious diseases of poultry (IBV, ILTV, MG, ARV) were fundamental for the industry to craft and modify prevention strategies through vaccination programs, management, and biosecurity.
- Proper administration of IBV and ILTV live attenuated vaccines is critical to reduce vaccine persistence and to improve vaccination efficacy.
- The identification of two finch species (House and Purple) as reservoirs of *Mycoplasma gallisepticum* (MG).
- Identification of wild birds as the origin of both the 2015 H5 and 2020 H7N3 avian influenza viruses that caused poultry outbreaks is a reminder for continued need for regulation of wild birds near poultry operations.
- The survey conducted among Poultry Professional revealed a specific group with significant negative perception on the use of disease monitoring platforms. The outcome of the survey underscores the need for a tailored approach to achieve maximum participation in collaborative disease monitoring.

OBJECTIVE 2- DEVELOP NEW AND IMPROVED DIAGNOSTIC TOOLS FOR POULTRY RESPIRATORY DISEASES.

- Improvement of ILTV genotyping assays
- Selection of target genes for phylogeny analysis of *Avibacterium paragallinarum* and Avian Reovirus.
- Development of improved Taqman Real-time PCRs for detection of *Ornithobacterium rhinotracheale* (ORT), *Bordetella avium* (BA), and Newcastle disease virus of the Class II.
- Complete genomes for several different lineages of IBV by next generation sequencing.

OBJECTIVE 3 - ELUCIDATE THE PATHOGENESIS OF POULTRY RESPIRATORY DISEASES

- Microbiome studies established the basis for understanding the causal relationships between specific virus-induced immune responses and changes in respiratory microbiota structure. Our immune gene knockout avian model for infectious disease research will enhance our understanding of key genes related to antiviral immunity in poultry.
- A limited influx of inflammatory cells was detected in the trachea after ocular exposure with ILTV while most leukocyte population shifts were identified in the CALT. This work underscores that mucosal inoculation of ILTV into lymphoid rich tissues is relevant to elicit a local cellular immune response that will reduce damage to the trachea.
- The recall of a population of activated CTLs to the trachea after challenge was associated with both CEO, TCO and rHVT-LT induced protection. On the contrary, the recall of an activated NK cells population to the trachea was associated with absence or partial TCO and rHVT- LT induced protection.
- Several viruses including AIV, NDV, coronaviruses including IBV, SARS-CoV-2 and MERS and bacteria e.g *Avibacterium paragallinarum* were characterized molecularly and biologically through poultry challenges.
- Using MHC-B haplotype chicken lines a model resistant-susceptible to IBV infections was established

and used to understand the basis of the resistance. Amino acid bound Zn and Mn proved to increase resistance to IBV infections in resistant lines.

-Collaborative efforts between stations have made progress in elucidating the pathobiology of IBV associated false layer syndrome and finally progress have been made in understanding the pathobiology of avian reovirus variants affecting broiler chickens.

OBJECTIVE 4. DEVELOP CONTROL AND PREVENTION STRATEGIES FOR POULTRY RESPIRATORY DISEASES

-Using infectious bursal disease virus (IBDV) and reverse genetics this virus is being tested as a vector in vaccines to generate protective immunity against infectious challenge in turkeys

-A novel *in vitro* interferon-based pipeline was developed for live vaccine discovery.

-The novel small molecule antimicrobials studies have resulted in a patent application and has the potential for commercial application

-Commercially available HVT-NDV recombinant vaccine protection in chickens was tested against the 2019 velogenic NDV detected in California. Other vaccine platforms were tested too i.e. NDV LaSota as a vector for IBV and ILTV. Finally, the duration of the immunity generated by AIV vaccines was investigated.

-Biosecurity programs targeted to commercial, small flocks and gamefowl were generated to prevent the introduction to flocks of endemic and foreign animal diseases in the U.S.

-Important information was discovered and will help future vaccine development. One example is that the sub-population structure and immune pressure generated by IBV vaccines impact innate immune response, antibody avidity, and protection is essential for vaccine development.

Publications (Highlighted denote collaboration between stations)

Abundo MC, Ngunjiri JM, Taylor KJM, Ji H, Ghorbani A, KC M, Weber BP, Johnson TJ, Lee CW. Assessment of two DNA extraction kits for profiling poultry respiratory microbiota from multiple sample types. Submitted.

Abundo MC, Ngunjiri JM, Taylor KJM, Ji H, Ghorbani A, KC M, Elaish M, Jang H, Weber BP, Johnson TJ, Lee CW. Evaluation of Sampling Methods for the Study of Avian Respiratory Microbiota. *Avian Diseases*. 64(3):277-285. 2020.

Apopo, A.A., Kariithi, H.M., Ateya, L.O., Binopal, Y.S., Sirya, J.H., Dulu, T.D., Welch, C.N., Hernandez, S.M., Afonso, C.L. 2019. A retrospective study of Newcastle disease in Kenya. *Tropical Animal Health and Production*. 52:699-710.

Ayala, A.J., Hernandez, S.M., Olivier, T.L., Welch, C.N., Dimitrov, K., Goraichuk, I.V., Afonso, C.L., Miller, P.J. 2019. Experimental Infection and Transmission of Newcastle Disease Vaccine Virus in Four Wild Passerines. *Avian Diseases*. 63(3):389-399. <https://doi.org/10.1637/11980-092918-Reg.1>.

Bertran, K., Criado, M., Lee, D., Killmaster, L.F., Sa-E-Silva, M., Lucio, E., Widener, J., Pritchard, N., Atkins, E., Mebatsion, T., Swayne Leghorn chickens by recombinant fowlpox vector vaccine with updated H5 insert against Mexican H5N2 highly avian influenza viruses. *Vaccine*. 38(6):1526-1534. <https://doi.org/10.1016/j.vaccine.2019.11.072>.

Bertran, K., Pantin Jackwood, M.J., Criado, M., Lee, D., Balzli, C.L., Spackman, E. Suarez, D.L., Swayne, D.E. 2019. Pathobiology and innate immune responses of gallinaceous poultry to clade 2.3.4.4A H5Nx highly pathogenic avian influenza virus infection. *Veterinary Research*. 50:89. <https://doi.org/10.1186/s13567-019-0704-5>.

Beyene TJ, Lee CW, Lossie G, El-Gazzar MM, Arruda AG. Poultry professionals' perception of participation in voluntary disease mapping and monitoring programs in the United States: a cluster analysis. *Avian Diseases*. *In press*.

J. Blakey, B. Crossley, A.P. Da Silva, D. Rejmanek, C. Jerry, **R.A. Gallardo**, S. Stoute. Infectious Bronchitis Virus Associated with Nephropathy in Diagnostic Cases from Commercial Broiler Chickens in California. *Avian Diseases*. In press.

Butt, S.L., Moura, V., Susta, L., Miller, P.J., Hutcheson, J.M., Cardenas, G., Brown, C., West, F., Afonso, C.L., Stanton, J. 2019. Tropism of Newcastle disease virus strains for chicken neurons, astrocytes, oligodendrocytes, and microglia. *BMC Veterinary Research*. 15:317. <https://doi.org/10.1186/s12917-019-2053-z>.

Chaudhry, M., Webby, R., Swayne, D.E., Rashid, H., Debeauchamp, J., Killmaster, L.F., Criado, M., Lee, D., Webb, A., Yousaf, S., Asif, M., Ain, Q., Khan, M., Khan, M., Hasan, S., Yousaf, A., Mustaque, A., Bokhari, S., Sajid Hasni, M. 2020. Avian influenza at animal-human interface: One-health challenge in live poultry retail stalls on Chakwal, Pakistan. *Influenza and Other Respiratory Viruses*. 14(3):257-265. <https://doi.org/10.1111/irv.12718>.

Chen, L., Donis, R.O., Suarez, D.L., Wentworth, D.E., Webby, R., Engelhardt, O.G., Swayne, D.E. 2019. Biosafety risk assessment for production of candidate vaccine viruses to protect humans from zoonotic highly pathogenic avian influenza viruses. *Influenza and Other Respiratory Viruses*. 14:215-225. <https://doi.org/10.1111/irv.12698>.

Chanthavixay, G., Kern, C., Wang, Y., Saelao, P., Lamont, S.J., **Gallardo, R.A.**, Rincon, G. and **Zhou, H.**, 2020. Integrated Transcriptome and Histone Modification Analysis Reveals NDV Infection Under Heat Stress Affects Bursa Development and Proliferation in Susceptible Chicken Line. *Frontiers in Genetics*, 11, p.1176.

Da Silva, **R.A. Gallardo**. Review: The Chicken MHC: Insights on genetic resistance, immunity and inflammation following infectious bronchitis virus infections. *Viruses* (2020) Accepted <https://www.mdpi.com/2076-393X/8/4/637>

Da Silva, A.P., Aston, E.J., Chiwanga, G.H., Birakos, A., Muhairwa, A.P., Kayang, B.B., Kelly, T., **Zhou, H.** and **Gallardo, R.A.**, 2020. Molecular Characterization of Newcastle Disease Viruses Isolated from Chickens in Tanzania and Ghana. *Viruses*, 12(9), p.916.

Da Silva, A., Schat, K. A., & **Gallardo, R. A.** (2019). Cytokine responses in tracheas from MHC congenic chicken lines with distinct susceptibilities to infectious bronchitis virus. *Avian Diseases*.

Deist, M. S., **Gallardo, R. A.**, Dekkers, J., **Zhou, H.**, & Lamont, S. J. (2020). Novel combined tissue transcriptome analysis after lentogenic Newcastle disease virus challenge in inbred chicken lines of differential resistance. *Frontiers in Genetics*, 11, <http://review.frontiersin.org/review/506097/18/453015#/tab/History>

Helmy YA, Kathayat D, Ghanem M, Jung K, Closs G, Jr., Deblais L, Srivastava V, El-Gazzar M, Rajashekara G. 2020. Identification and characterization of novel small molecule inhibitors to control *Mycoplasma gallisepticum* infection in chickens. *Vet Microbiol*. 247:108799. 2020.

Ferreira, H.L., Reilley, A.M., Goldenberg, D., Ortiz, I.R., **Gallardo, R.A.**, **Suarez, D.L.** 2020. Protection conferred by commercial NDV live attenuated and double recombinant HVT vaccines against virulent California 2018 Newcastle disease virus (NDV) in chickens. *Vaccine*. 38(34):5507-5515. <https://doi.org/10.1016/j.vaccine.2020.06.004>.

Ferreira, H., Suarez, D.L. 2019. SNP analysis used to select conserved regions for an improved real-time RT-PCR test specific to Newcastle Disease Virus. *Avian Diseases*. 63:625-633. <https://doi.org/10.1637/aviandiseases-D-19-00071>.

Figuroa, T. Derksen, S. Biswas, A. Nazmi, D. Rejmanek, B. Crossley, P. Pandey, **R.A. Gallardo**. Persistence of LPAI and HPAI in Reused Poultry Litter, Effects of Litter Amendment Use and Composting Temperatures. *J Appl. Poult. Res*. 2020. Accepted.

Gallardo, R. A., da Silva, A. P., & Rebollo, M. A. (2020). Effects of Amino acid-bound Zinc and Manganese Feed Additives on MHC Haplotype Chickens Challenged with Infectious Bronchitis Coronavirus. *Avian Diseases*. Accepted for publication May 21st, 2020.

Gallardo, R. A., Da Silva, A. P., Egaña-Labrin, S., Stoute, S., Kern, C., **Zhou, H.**, Corsiglia, C. (2020). Infectious Coryza: Persistence, Genotyping, and Vaccine Testing. *Avian diseases*, 64(2), 157-165. <https://www.aaapjournals.info/doi/abs/10.1637/aviandiseases-D-19-00184>

R. A. Gallardo. Invited Review: Infectious bronchitis virus variants: Generation, Surveillance, Control and Prevention. *Austral Journal of Veterinary Sciences*. 2020. In press.

Goraichuk, I.V., Davis, J.F., Kulkarni, A.B., Afonso, C.L., Suarez, D.L. 2020. A 25-year-old sample contributes the first complete genome sequence of avian coronavirus vaccine strain ArkDPI, reisolated from commercial broilers in the United States. *Microbiology Resource Announcements*. 9:1-4. <https://doi.org/10.1128/MRA.00067-20>.

Goraichuk, I.V., Williams Coplin, T.D., Wibowo, M.H., Durr, P.A., Asmara, W., Artanto, S., Dimitrov, K.M., Afonso, C.L., Suarez, D.L. 2020. Complete Genome Sequences of 11 Newcastle Disease Virus Isolates of Sub-genotype VII.2 from Indonesia. *Microbiology Resource Announcements*. 09(5):e01519-19. <https://doi.org/10.1128/MRA.01519-19>.

Goraichuk, I.V., Kulkarni, A.B., Williams Coplin, T.D., Suarez, D.L., Afonso, C.L. 2019. First Complete Genome Sequence of Currently Circulating Infectious Bronchitis Virus Strain DMV/1639 of the GI-17 Lineage. *Microbiology Resource Announcements*. 8(34):e00840-19. <https://doi.org/10.1128/MRA.00840-19>.

Goraichuk, I.V., David, J.F., Kulkarni, A.B., Afonso, C.L., Suarez, D.L. 2020. Complete Genome Sequence of Avian Coronavirus Strain GA08 (GI-27 Lineage). *Microbiology Resource Announcements*. 9(9):e00068-20. <https://doi.org/10.1128/MRA.00068-20>.

Goraichuk, I.V., Kapczynski, D.R., Seal, B., Suarez, D.L. 2020. First complete genome sequence of an avian metapneumovirus subtype B strain from Hungary. *Microbiology Resource Announcements*. 9:e00177-20. <https://doi.org/10.1128/MRA.00177-20>.

Hicks, J.T., Duvuuri, V.R., Lee, D., Torchetti, M., Swayne, D.E., Bahl, J. 2020. Agricultural and geographic factors shaped the 2015 highly pathogenic avian influenza H5N2 outbreak within the midwestern United States poultry industries. *PLoS Pathogens*. 16(1):e1007857. <https://doi.org/10.1371/journal.ppat.1007857>.

Kariithi, H.M., Welch, C.N., Ferreira, H.L., Pusch, E.A., Ateya, L.O., Binopal, Y.S., Lichoti, J.K., Apopo, A.A., Afonso, C.L., Suarez, D.L., Dulu, T.D. 2019. Genetic characterization and pathogenesis of the first H9N2 low pathogenic avian influenza viruses isolated from chickens in Kenyan live bird markets. *Infection, Genetics and Evolution*. 78:104074. <https://doi.org/10.1016/j.meegid.2019.104074>.

Kariithi, H.M., Welch, C.N., Ferreira, H.L., Pusch, E.A., Ateya, L.O., Binopal, Y.S., Lichoti, J.K., Apopo, A.A., Afonso, C.L., Suarez, D.L., Dulu, T.D. 2019. Genetic characterization and pathogenesis of the first H9N2 low pathogenic avian influenza viruses isolated from chickens in Kenyan live bird markets. *Infection, Genetics and Evolution*. 78:1040 <https://doi.org/10.1016/j.meegid.2019.104074>.

Krieter, A., N.P. Ponnuraj, and **K.W. Jarosinski.** 2020. Expression of the conserved herpesvirus protein kinase (CHPK) of Marek's disease alphaherpesvirus in the skin reveals a mechanistic importance for CHPK during interindividual spread in chickens. *J Virol*. 94(5):e01522-19. <https://doi.org/10.1128/JVI.01522-19>

Krunkosky, M., **M. García,** G. Beltran, S.M. Williams, D.J. Hurley, R. M. Gogal Jr. Ocular Exposure to Infectious Laryngotracheitis Virus Alters Leukocyte Subsets in the Head-associated Lymphoid Tissues and Trachea of 6-week-old White Leghorn Chickens. *Avian Pathology*.49: 4, 404-417. 2020.

Kwon, J., Bahl, J., Swayne, D.E., Lee, Y., Song, C., Lee, D. 2019. Domestic ducks play a major role in maintenance and spread of H5N8 highly pathogenic avian influenza viruses in South Korea. *Transboundary and Emerging Diseases*. 14:257–265. <https://doi.org/10.1111/tbed.13406>.

Lee, D., Swayne, D.E., Criado, M.F., Killmaster, L.F., Iqbal, S., Rashid, H., Chaudhry, M. 2019. Genome sequences of a H9N2 avian influenza virus strain found in Pakistan in 2017. *Microbiology Resource Announcements*. 8(28):e00433-19. <https://doi.org/10.1128/MRA.00433-19>.

Leyson, C., Youk, S., Smith, D.M., Dimitrov, K., Lee, D., Larsen, L., Swayne, D.E., Pantin Jackwood, M.J. 2019. Pathogenicity and genomic changes of a 2016 European H5N8 highly pathogenic avian influenza virus (clade 2.3.4.4) in experimentally infected mallards and chickens. *Virology*. 537:172- 185. <https://doi.org/10.1016/j.virol.2019.08.020>.

Msoffe, P.L., Chiwangad, G.H., Cardona, C.J., Miller, P.J., Suarez, D.L. 2019. Isolation and Characterization of Newcastle Disease Virus from Live Bird Markets in Tanzania. *Avian Diseases*. 63(4):634-640. <https://doi.org/10.1637/aviandiseases-D-19-00089>.

Reinoso-Perez, M. T., K. V. Dhondt, A. V. Sydenstricker, D. Heylen, and A. A. Dhondt. 2020. Complex interactions between bacteria and haemosporidia in coinfecting hosts: An experiment. *Ecology and Evolution* **10**:5801-5814.

Pantin-Jackwood, M.J. 2020. Immunohistochemical staining of influenza virus in tissues. In: Spackman, E., editor. *Animal Influenza Virus Methods and Protocols*. 3rd edition. *Methods in Molecular Biology*, volume 2123. New York, NY: Humana. p. 29-36. https://doi.org/10.1007/978-1-0716-0346-8_3.

Swayne, D.E., Suarez, D.L., Sims, L. 2020. Influenza. In: Swayne, D.E., Boulianne, M., Logue, C. M., McDougald, L.R., Venugopal, N., Suarez, D.L., de Wit, S.D., Grimes, T., Johnson, D., Kromm, M., Prajitno, T. Y., Rubinoff, I. Zavala, G., editors. *Diseases of Poultry*. 14th edition. Hoboken, NJ: John Wiley & Sons Inc. p. 219-256. <https://doi.org/10.1002/9781119371199>.

Swayne, D.E. 2019. Avian influenza. In: Allen, D.G., Carter, K.K., Constable, P.D., Dart, A., Davies, P.R., Davies, J.L., Quesenberry, K.E., Swayne D.E., editors. *Merck Veterinary Manual*. 12th edition. Kenilworth, NJ: Merck & Co, Inc. p. 1-8. Available: <https://www.merckvetmanual.com/poultry/avian-influenza/avian-influenza>.

Swayne, D.E. 2020. Laboratory methods for assessing and licensing influenza vaccines for poultry. In: Spackman, E., Editor. *Animal Influenza Virus Methods and Protocols*. 3rd Edition. *Methods in Molecular Biology*, volume 2123. New York, NY: editors. *Perspective In Medicine*. Woodbury, NY: Cold Spring Harbor Laboratory Press. 10(6):a038679. <https://doi.org/10.1101/cshperspect.a038679>.

Stephens, C.B., Spackman, E., Pantin Jackwood, M.J. 2020. Effects of an H7 highly pathogenic and related low pathogenic avian influenza virus on chicken egg production, viability, and virus contamination of egg contents and surfaces. *Avian Diseases*. 64(2):143 [rg/10.1637/0005-2086.64.2.143](https://doi.org/10.1637/0005-2086.64.2.143).

Spackman E, Sitaras I. 2020. Hemagglutination Inhibition Assay. In: Spackman, E., Editor. *Animal Influenza Virus Methods and Protocols*. 3rd Edition. *Methods in Molecular Biology*, volume 2123. New York, NY: Humana. p. 11-28. doi: https://doi.org/10.1007/978-1-0716-0346-8_2.

Spackman, E. 2020. A brief introduction to avian influenza virus. In: Spackman, E., editor. *Animal Influenza Virus Methods and Protocols*. 3rd Edition. *Methods in Molecular Biology*, volume 2123. New York, NY: Humana. p. 83-92. doi: https://doi.org/10.1007/978-1-0716-0346-8_7.

Spackman, E., Lee, S.A. 2020. Avian influenza virus RNA extraction. In: Spackman, E., Editor. *Animal Influenza Virus Methods and Protocols*. 3rd Edition. *Methods in Molecular Biology*, volume 2123. New York, NY: Humana. p. 123-135. doi:https://doi.org/10.1007/978-1-0716-0346-8_10.

Spackman, E. 2020. Avian influenza virus detection and quantitation by real-time RTPCR. In: Spackman, E., Editor. *Animal Influenza Virus Methods and Protocols*. 3rd Edition. *Methods in Molecular Biology*, volume 2123. New York, NY: Humana. p. 137-148. https://doi.org/10.1007/978-1-0716-0346-8_11.

Spackman, E., Killian, M. 2020. Avian influenza virus isolation, propagation and titration in embryonated chicken eggs. In: Spackman, E., editor. *Animal Influenza Virus Method and Protocols*. 3rd Edition. *Methods in Molecular Biology*, volume 2123. New York, NY: Humana. p.149-164. https://doi.org/10.1007/978-1-0716-0346-8_11.

Spackman, E., Chappell, L., Killian, M. 2020. Detection of influenza A antibodies in avian serum samples by ELISA. In: Spackman, E., Editor. *Animal Influenza Virus Methods and Protocols*. 3rd Edition. *Methods in Molecular Biology*, vol 2123. New York, NY: Humana. p.151-167.

https://doi.org/10.1007/978-1-4939-0758-8_14

Taylor KJM, Ngunjiri JM, Abundo MC, Jang H, Elaish M, Ghorbani A, Kc M, Weber BP, Johnson TJ, Lee CW. Respiratory and Gut Microbiota in Commercial Turkey Flocks with Disparate Weight Gain Trajectories Display Differential Compositional Dynamics. *Appl Environ Microbiol*. 86(12):e00431-20. 2020.

Taylor, T.L., Volkening, J.D., Dejesus, E., Simmons, M., Dimitrov, K.M., Glenn, T.E., Suarez, D.L., Afonso, C.L. 2019. Rapid, multiplexed, whole genome and plasmid sequencing of foodborne pathogens using long-read nanopore technology. *Scientific Reports*. 9:16350. <https://doi.org/10.1038/s41598-019-52424-x>.

Yamaji, R., Saad, M.D., Davis, C.T., Swayne, D.E., Wang, D., Wong, F.Y., Mccauley, J.W., Peiris, J., Webby, R.J., Fouchier, R.A., Kawaoka, Y., Zhang, W. 2020. Pandemic potential of highly pathogenic avian influenza clade 2.3.4.4 A (H5) viruses. *Reviews in Medical Virology*. 30:1-16. <https://doi.org/10.1002/rmv.2099>.

Yu, Q., Li, Y., Dimitrov, K., Afonso, C.L., Spatz, S.J., Zsak, L. 2020. Genetic stability of a Newcastle disease virus vectored infectious laryngotracheitis virus vaccine after serial passages in chicken embryos. *Vaccine*. 38(4):925-932. <https://doi.org/10.1016/j.vaccine.2019.10.074>.

Youk, S., Lee, D., Ferreira, H.L., Afonso, C.L., Absalon, A.E., Swayne, D.E., Suarez, D.L., Pantin Jackwood, M.J. 2019. Rapid evolution of Mexican H7N3 highly pathogenic avian influenza viruses in poultry. *PLoS One*. 14(9):e0222457. <https://doi.org/10.1371/journal.pone.0222457>.

Youk, S., Lee, D., Jung, J., Pantin Jackwood, M.J., Song, C., Swayne, D.E. 2020. Live markets as evolutionary epicentres of H9N2 low pathogenicity avian influenza viruses in Korea. *Emerging Microbes & Infections*. 9(1):616-627. <https://doi.org/10.1080/22221751.2020.1738903>.

Wang, Y., Saelao, P., Kern, C., Jin, S., **Gallardo, R.A.**, Kelly, T., Dekkers, J.M., Lamont, S.J. and **Zhou, H.**, 2020. Liver Transcriptome Responses to Heat Stress and Newcastle Disease Virus Infection in Genetically Distinct Chicken Inbred Lines. *Genes*, 11(9), p.1067.

J. Mushi, G. Chiwanga, E. Amuzu-Aweh, M. Walugembe, R. Max, S.J. Lamont, T. Kelly, E. Mollel, P. Msoffe, J. Dekkers, **R. A. Gallardo, H. Zhou**, A. Muhairwa. Phenotypic variability and population structure analysis of Tanzanian free-range local chickens. *BMC Veterinary Research*. Accepted

Walugembe, M., Amuzu-Aweh, E.N., Botchway, P.K., Naazie, A., Aning, G., Wang, Y., Saelao, P., Kelly, T., **Gallardo, R.A., Zhou, H.** and Lamont, S.J., 2020. Genetic Basis of Response of Ghanaian Local Chickens to Infection With a Lentogenic Newcastle Disease Virus. *Frontiers in Genetics*, 11.

Zegpi R. A.*, K. S. Joiner, V. L. van Santen, H. Toro (2020). Infectious bronchitis virus population structure defines immune response and protection. *Avian Diseases* 64:60-68.

Zegpi R.A.*, L. He, Q. Yu, K. S. Joiner, V. L. van Santen, H. Toro (2020). Limited protection conferred by recombinant Newcastle disease virus expressing infectious bronchitis spike protein. *Avian Diseases* 64: 53-59.

Zegpi R. A.*, C. Breedlove, S. Gulley, H. Toro (2020). Infectious bronchitis virus immune responses in the Harderian gland upon initial vaccination. *Avian Diseases* 64:92-95.

Zhang, J., Kaiser, M. G., **Gallardo, R. A.**, Kelly, T. R., Dekkers, J., **Zhou, H.**, & Lamont, S. J. (2020). Transcriptome analysis reveals inhibitory effects of lentogenic Newcastle disease virus on cell survival and immune function in spleen of commercial layer chicks. *Genes*, 11(9), 1003.

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