

The University
of Georgia



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Department of Population Health
Poultry Diagnostic and Research Center
Athens GA

Objective 2. Develop improved diagnostic capabilities including real time PCR as well as other rapid on-farm tests for economically important respiratory diseases.

- **Title:** Development of Real-Time Quantitative RT-PCR Assays to Detect GA07, GA08 and GA13 Infectious Bronchitis Virus Types. Eric M. Shepherd, Brian J. Jordan, Deborah A. Hilt, and Mark W. Jackwood
- **Objective:** To develop a rapid type specific diagnostic test for commonly isolated infectious bronchitis viruses.
- **Impact/Summary:** Having a specific assay to detect the newest serotypes circulating in the field is important and will provide timely information that can be used to make informed decisions on IBV vaccination programs.

Obj. 4. Develop new prevention and control strategies for poultry respiratory diseases.

- **Title:** Infectious Dose of Infectious Bronchitis Virus Arkansas-DPI Vaccine given by a Hatchery Spray Cabinet. Mark W. Jackwood, Christina M. Leyson, and Brian J. Jordan
- **Objective:** Determine the infectious dose for Ark-DPI vaccine given by spray.
- **Impact/Summary:** We found that a 100 X dose of Ark-DPI vaccine given by spray was required to obtain the same level of infection and immunity obtained when the vaccine is given by eyedrop. A critical dose of a minor subpopulation in the vaccine is needed to suitably vaccinate the birds.

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- **Title:** Evaluating protection against infectious bronchitis virus by clinical signs, ciliostasis, challenge virus detection and histopathology Mark W. Jackwood, Brian J. Jordan, Ha-Jung Roh, Deborah A. Hilt and Susan M. Williams
- **Objective:** To determine the relationship between different parameters used to evaluate vaccine protection against challenge in broilers.
- **Impact/Summary:** Obviously, decreasing IBV infection and replication in the upper-respiratory tract will decrease transmission and mutations leading to variant viruses, and herein we demonstrate that protection of the cilia will decrease secondary bacterial infections, which have been shown to lead to condemnations and increased mortality. Thus, it appears that examining both criteria would be important when evaluating IBV vaccine efficacy.

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- **Title:** Development of a DNA Vaccine Against a Variant strain of Infectious Bronchitis Virus. Frances G. Ashby, Deborah A. Hilt, Mark W. Jackwood, Brian J. Jordan
- **Objective:** To evaluate a DNA vaccine utilizing the entire spike gene for efficacy in ovo.
- **Impact/Summary:** Being able to respond quickly to new variants of infectious bronchitis virus is important for control. DNA vaccines can be made easily and inexpensively and if efficacious in ovo could be applied to IBV control in broilers.

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- **Title:** Modification of a Hatchery Vaccine Spray Cabinet to Improve Infectious Bronchitis Virus Application Problems. Brian J. Jordan, Eric M. Shepherd, Frances G. Ashby, Deborah A. Hilt, Mark W. Jackwood
- **Objective:** To optimize vaccination of infectious bronchitis virus in a hatchery spray cabinet.
- **Impact/Summary:** This novel spray cabinet assembly holds many advantages over the traditional syringe based design, and provides innovation for the application of any mass sprayed vaccine

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- **Title:** Polymorphisms in the S1 Spike Glycoprotein of Arkansas-type Infectious Bronchitis Virus Show Differential Binding to Various Chicken Tissues. Christina M. Leyson, Brian J. Jordan, Mark W. Jackwood
- **Objective:** To identify specific amino acid changes in the spike gene of IBV that affect host cell binding.
- **Impact/Summary:** The differences in binding to chicken tracheal tissue and embryonic tissues were observed, which have implications for vaccine development particularly when subpopulations of vaccine virus are considered.

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- **Title:** Optimal Sample Processing for Diagnostic Avian Mycoplasma Real-time PCR. R. Jude, and N. Ferguson-Noel
- **Objective:** To determine the influence of commonly used sample transport and preparation media on the sensitivity of real time PCR for avian mycoplasma.
- **Impact/Summary:** This information allows the poultry industry to maximize the benefits of these costly diagnostic assays and set scientifically based standards in different laboratories for sample submission and handling for MG and MS real-time PCR.

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- **Title:** Evaluation of the Egg Transmission of ts-11 and ts-11-like *Mycoplasma gallisepticum* (MG) isolates. N. Armour, N. Ferguson-Noel
- **Objective:** To investigate the rate of egg transmission and virulence of the live MG ts-11 vaccine and two ts-11-like isolates (one from vaccinated broiler breeders one from their broiler progeny) suspected of having reverted to virulence and transmitted vertically.
- **Impact/Summary:** These results provide the first conclusive evidence of transovarian transmission of an isolate genotyped as ts-11, and indicate that ts-11-like isolates vary in their virulence and ability to transmit via the egg.

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- **Title:** Determining the Genetic Basis of Attenuation in *Mycoplasma gallisepticum* (MG) Vaccine Strains N. Ferguson-Noel, M. Garcia, C. Ricketts, S. Ayyampalayam, J. Maurer.
- **Objective:** To use whole genome sequence analysis to compare and contrast ts-11 vaccine, ts-11-like field isolates, and reference strains to identify putative virulence factors and unique genome targets for future strain typing.
- **Impact/Summary:** The PCRs can be used to detect ts-11 without the time, expense and specialized equipment needed for DNA sequencing.

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- **Title:** Protection efficiency of gene deleted infectious laryngotracheitis virus (ILTV) strains. Maricarmen García
- **Objective:** The main objective of US Poultry & Egg project # 683 was to develop and evaluate the potential of an ILTV strain attenuated by deletion of the open reading frame (ORF) C gene as an ILTV vaccine for *in ovo* administration.
- **Impact/Summary:** This is the first completed study on the potential use of ILTV gene deleted strains for *in ovo* vaccination and provides the framework for further study the development of ILTV strain suitable for *in ovo* vaccination. The B Δ ORFC strain in its present form still causes increase mortalities in MAb- chickens during the first week of age. Ongoing experiments are been performed to further attenuate the B Δ ORFC and determine if it is suitable for *in ovo* vaccination. Two methods for attenuation will be utilize 1) continuous passages in cell culture, 2) generation of a second deletion using B Δ ORFC as the parental strain.

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- **Title:** Patterns of replication and local immune responses elicited by infectious laryngotracheitis virus (ILTV). Maricarmen García.
- **Objective:** The objective of this study was to determine how the route of viral entry influences the pattern of replication of virulent field isolate 63140 and CEO vaccine strain.
- **Impact/Summary:** These experiments will increase our understanding of ILTV interaction with mucosal tissues that first come in contact with the virus during vaccination or natural infection. How the route of inoculation influences viral replication and local immune responses is relevant to improve the delivery and efficacy of ILTV vaccines. Ongoing experiments include determining the innate immune responses elicited by CEO vaccination when administered via natural routes of viral entry (oral, ocular and intranasal).

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- **Title:** Genotyping analysis of archived and circulating ILTV strains. Maricarmen García and Steve Spatz.
- **Objective:** The objective of this study is to compare full genomes sequences of wild-type strains of ILTV in backyard flocks, strains from commercial flocks and vaccine strains to determine the role of vaccine recombination in the emergence newly circulating ILTV isolates.
- **Impact/Summary:** This study failed to detect any ILTV recombinants that were the progeny of two licensed US ILTV vaccine strains. This results are relevant as indicate that the compartmentalized use of CEO and TCO in US reduce the risk of vaccine recombination. A PCR sequencing rapid single locus ILTV genotyping assay was developed that can be implemented in diagnostic laboratories. Ongoing experiments include pathotyping experiments of newly genotype isolates will be pursued to compared virulence, persistence, and transmission of these isolates with previously pathotyped ILTV strains (USDA, 63140), and further development of genotype specific real time PCR assays.

Publications

- Armour, N.K. and Ferguson-Noel, N. Evaluation of the egg transmission and pathogenicity of *Mycoplasma gallisepticum* isolates genotyped as ts-11. Avian Pathol. doi:10.1080/03079457.2015.1044890, 2015.
- García, M., A. Vagnozzi, G. Zavala, L. Read, and S. Sharif. Cytokine genes expression during lytic infection with virulent infectious laryngotracheitis virus (ILTV) strain. Submitted Avian Diseases.
- Jackwood, M. W., B. J. Jordan, H-J. Roh, D. A. Hilt and S. M. Williams. Evaluating protection against infectious bronchitis virus by clinical signs, ciliostasis, challenge virus detection and histopathology. Avian Dis. 59:368-374, 2015.
- Roh, H-J., B. J. Jordan, D. A. Hilt, M. B. Ard, and M. W. Jackwood. Hatchery Spray Cabinet Administration Does Not Damage Avian Coronavirus Infectious Bronchitis Virus Vaccine Based on Analysis by Electron Microscopy and Virus Titration. Avian Dis. 59:149-152, 2015.
- Schneiders, G. H., S. M. Riblet, Y. Cheng, S. J. Spatz, and M. García. Characterization and immunogenicity of open reading frame C (*ORF C*) gene-deleted mutant of Infectious laryngotracheitis virus (ILTV). Submitted to Vaccine.
- Spatz, S. J, S. M. Riblet, T. A. Ross, and M. García. Comparative genomic studies of five sequences of infectious laryngotracheitis virus strains from the US: development of a single locus PCR-based diagnostic assay to differentiate (ILTV) strains from commercial and backyard flocks. Submitted Journal of General Virology.
- Vagnozzi, A., S. M. Riblet, S. M. Williams, G. Zavala, and M. García. Infection of broilers with two virulent strains of Infectious laryngotracheitis virus: Criteria for evaluation of experimental infections. Avian Dis. 59:394–399. 2015
- Vagnozzi, A., S. M. Riblet, G. Zavala, R. Ecco, C. L. Afonso and M. García. Evaluation of the transcriptional status of host cytokines and viral genes in the trachea of vaccinated and non-vaccinated chickens after challenge with infectious laryngotracheitis virus (ILTV). Accepted Avian Pathology.