Antibiotic treatments in both studies improved the growth and feed efficiency of broilers when compared with infected, untreated controls. In study 1, supplemental AvL at 1.0 g/kg produced similar or improved growth and feed efficiency compared with bacitracin. Avi-Lution® and bacitracin similarly suppressed a cecal bacterial subset, defined by 16S ribosomal gene sequencing, that was found to be positively associated with C. perfringens infection and with the molar proportion of cecal butyric acid. In study 2, avilamycin significantly improved broiler body weights and feed conversion by d 21 before peak challenge symptoms were observed. Through d 42, however, AvL, avilamycin, and AvL2 treatments similarly improved mortality-adjusted feed efficiency, whereas AvL2 significantly improved broiler body weights. These results demonstrate that Avi-Lution® ameliorated performance losses of necrotic enteritis in broilers.

**Key Words:** necrotic enteritis, bacitracin, avilamycin, broiler, Clostridium perfringens

### T136 Effect of synbiotic supplementation on intestinal integrity of broilers

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**1Zoetis; 2Rose Acre Farms**

As researchers and industry leaders search for an alternative to the use of sub-therapeutic antibiotics to help alleviate concerns of antibiotic resistance and satisfy consumer and regulatory demands, probiotics have received increased attention for their ability to improve enteric health in poultry. The objective of this experiment was to evaluate the effects of synbiotic supplementation on broiler intestinal integrity. A total of 300, day-old Cobb 500 broiler chicks were randomly assigned to one of two treatment groups, each consisting of 3 replicate pens (50 birds/pen). Treatment groups consisted of a non-treated control and a treated group supplemented with a synbiotic (PoultryStar®; 500 g/ton). On days 7, 14, 21, and 35, eight birds per treatment were euthanized and ileal samples were collected. Intestinal integrity parameters included transepithelial electrical resistance (TEER) before and after lipopolysaccharide (LPS) inclusion and apical to basolateral flux of fluorescein isothiocyanate-LPS (FITC-LPS) as an indicator of paracellular permeability. Data were analyzed using the Proc Glimmix of SAS (SAS Institute Inc.) and significance is set at P ≤ 0.05. Intestinal integrity increased over the course of the study and had a significant treatment by time interaction. At day 35, birds provided treated feed exhibited significantly higher TEER than birds on non-treated feed. While endotoxin permeability was not found to be significant, exposure to FITC-LPS significantly increased TEER in the treated group as compared to the control group at day 21. Interestingly, this was when birds subject to the synbiotic treatment tended to have lower initial TEER than the control birds, suggesting the synbiotic supplementation may increase the resilience of the intestinal tract in the face of a potential pathogen. Overall, these results suggest that synbiotic supplementation may be an effective means of improving intestinal integrity and allow the intestinal tract to adapt to pathogenic stressors, thus helping to prevent bacterial translocation and enteric diseases.

**Key Words:** gut integrity, permeability, probiotic, barrier function, broilers

### T137 Analysis of five clinical cases of runtng-stunting syndrome in broiler chickens caused by avian astrovirus and avian reovirus

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Five clinical cases of broilers of 5 to 12 days of age were analyzed. The history as reported by submitter was running birds and high mortality. Clinical evaluation showed moderate depression, poor body condition, lack of uniformity in size among each group of birds, lameness, poor feathering, dehydration, and whitish diarrhea. At necropsy the most remarkable lesion was pale, dilated thick-walled intestines with watery contents and undigested food (4/5). The pancreas showed whitish discoloration (1/5). In 3/5 groups mild aerosaculitis and pericarditis were observed. Samples for bacteriology, histopathology, and virus isolation were collected. Histopathologic findings for 1/5 cases showed abnormality of intestinal villi and pancreatitis. This case was positive for avian reovirus. 4/5 cases showed remarkable micro changes in intestine and pancreas. Collected samples of these four groups of birds were positive to Avian Astrovirus. All isolated viruses were confirmed by PCR. Apparently, avian astrovirus is capable of causing running stunting syndrome without compromising the villi integrity.

**Key Words:** Avian Astrovirus, Avian reovirus, intestinal integrity

### T138 A comparison of mid-lay serology and IBV Arkansas protection in flocks receiving different commercial Salmonella enteridis (SE) bacterins

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SE vaccines help minimize the risk of food borne illness in the layer industry. The purpose of this study was to compare Arkansas (Ark) infectious bronchitis virus (IBV) protection of two SE bacterins—one containing Ark antigen and the other containing only Mass antigen. **Study Design:** Hy-Line W-36 pullets received a single live Ark vaccination at 2 weeks of age. At 13 weeks of age, pullets in adjacent rows received either SE-ND-IB Vaccine A (0.25ml; no Ark) or SE-ND-IB Vaccine B (0.25ml; contains Ark). Three weeks later the birds were moved to a layer farm where they remained in adjacent rows. At 45 weeks of age, 40 birds from each group were moved to isolators in Durham, NC, along with 30 2-week old SPF leghorns. A week later, 24 birds from each vaccine treatment and 20 SPF controls were challenged with 4.5 log10 Ark IBV. 16 birds from each vaccine treatment and 10 SPF controls remained un-challenged; vaccines were bled for serology comparison. Five days later, all birds were examined for clinical signs and airsac lesions; tracheas were submitted for histopathology; choana and trachea swabs were tested for IBV PCR. **Results:** There were no significant clinical signs or airsac lesions in any challenged groups. 63% of challenge controls had 3-score tracheal lesions (necrosis) compared to none of the commercial birds. There were also no differences in tracheal lesions (most had modest infiltrates and epithelial hyperplasia) between challenged and non-challenged commercial birds. Challenge controls were strongly positive on Ark PCR of both choana and trachea samples, indicating a good “take”. Challenge of Vaccine A birds yielded 79% and 83% protection of choana and trachea (PCR negative) compared to 67% and 96% protection, respectively, in Vaccine B birds. There were no significant differences in IBV (including Ark HI), NDV and SE serology. **Discussion:** A mid-lay Ark challenge of commercial layers did not cause an increase in tracheal lesions and resulted in low Ark recovery levels, indicating solid immunity in both vaccine treatments. This study demonstrates that an SE bacterin containing Ark is not necessary to achieve solid Ark IBV immunity—probably because of live Ark priming and the boosting influence of field challenge on the layer premise at housing time.

**Key Words:** IBV, Arkansas, SE, bacterin, protection

### T139 Replication of chicken embryonic kidney cell culture-adapted Ark DPI infectious bronchitis virus vaccine strain in chickens

**Saida Farajana**1, Ramon Zegpi, Haroldo Toro, Vicky Santen

**Auburn University**

ArkDPI-like infectious bronchitis virus (IBV) is frequently isolated from respiratory disease cases in intensive broiler-producing areas of the U.S. despite vaccination with live-attenuated ArkDPI vaccines. Commercial ArkDPI vaccines contain subpopulations that are rapidly selected in vaccinated chickens and are a likely source of many of the ArkDPI-like viruses isolated. We previously eliminated these vaccine subpopulations selected in chickens by adaptation of a commercial ArkDPI-derived vaccine to chicken embryonic kidney (CEK) cell culture, and demonstrated protection against virulent Ark-type IBV challenge following vaccination.
with our CEK-adapted Ark DPI vaccine strain. However, we also observed three amino acid changes in the spike protein of the CEK-adapted Ark-DPI vaccine strain compared to the major population of the vaccine from which it was derived, and found that these changes eliminated detectable binding of recombinant spike protein to chicken tissues in vitro. Both the lack of detectable binding of recombinant spike protein to chicken tissues and lack of the subpopulations that are selected in chickens predict that the CEK-adapted vaccine strain replicates to lower levels in chickens than the commercial vaccine strain from which it was derived. To characterize replication of our CEK-adapted Ark DPI vaccine strain in chickens and compare it to replication of the commercial ArkDPI vaccine strain, we orally inoculated three groups of ten SPF leghorn chickens with 10⁴ or 10⁵ EID₅₀ CEK-adapted Ark DPI, or 10⁴ EID₅₀ commercial Ark DPI vaccine. Replication of the vaccine virus in infants of chickens of each group was monitored by Taqman qRT-PCR to determine the relative levels of viral RNA in tears 3, 5 and 8 days post-vaccination (DPV) and choanal and tracheal swabs 5 and 8 DPV. Data was analyzed by ANOVA and Tukey’s post-test. As expected, vaccine viral RNA was consistently statistically significantly lower in all three sample types in chickens inoculated with CEK-adapted vaccine compared to the commercial vaccine strain when the same dose (10⁴ EID₅₀) was used and in tracheal swabs even when ten times the dose was used. In spite of its lower ability to replicate in chickens, the CEK-adapted vaccine strain provides effective protection.

Key Words: Infectious bronchitis virus, Ark DPI, Vaccine, qRT-PCR, Attenuation

T140 Kidney cell-adapted Infectious Bronchitis Ark DPI vaccine is stable and protective Ramon Alejandro Zegpi Lagoste⁴,⁵, Cassandra Breedlove, Vicky van Santen, Haroldo Toro Auburn University

We previously demonstrated that adaptation of an embryo-attenuated infectious bronchitis virus (IBV) Arkansas (Ark) Delmarva Poultry Industry (DPI)-derived vaccine to chicken embryo kidney (CEKp7) cells shifted the virus population towards homogeneity in spike (S) and non-structural protein (NSP) genes. Moreover, typical Ark vaccine subpopulations emerging in chickens vaccinated with commercial Ark vaccines were not detected in chickens vaccinated with CEKp7. In the present study, both conventional and next generation sequencing results show that the changes observed during CEK adaptation remained after five back-passages in ECE indicating that kidney-cell adaptation drastically increased the stability of this vaccine virus population. In a first protection study one-day-old chickens were vaccinated with 10⁴ or 10⁵ EID₅₀/chicken of the 2nd ECE back-passage of CEKp7 (CEKp7e2) and demonstrated to be protected against Ark virulent (10⁹ EID₅₀) challenge. In a 2nd protection trial, CEKp7e2 was compared against protection conferred by an attenuated commercial ArkDPI-derived vaccine, different from which the CEK-adapted virus originated. All vaccinated chicken groups showed a significant reduction of respiratory signs and viral load after Ark virulent challenge compared to unvaccinated-challenged controls. In CEKp7e2 vaccinated chickens, viral subpopulations different from the challenge virus were detected in a marginal number (6-7%) of chickens after challenge. In contrast, IBV S1 sequences differing from the predominant population in the challenge virus were detected after challenge in a large number (77%) of chickens vaccinated with the commercial Ark attenuated vaccine. The CEK-adapted IBV ArkDPI-derived vaccine is a stable and effective vaccine, which drastically reduces the emergence of Ark-like viruses both at vaccination and after challenge.

Key Words: Infectious bronchitis virus, Coronavirus, virus adaptation, genetic vaccine, vaccine

T141 Development of a potential IBV Arkansas serotype vaccine candidate: Arkansas GA Grace Albanese⁶,⁷, Deborah Hilt, Mark Jackwood, Brian Jordan University of Georgia

Avian infectious bronchitis virus (IBV) is a gammacoronavirus that causes infectious bronchitis (IB), an economically significant upper-respiratory disease of chickens. Almost all commercial poultry are vaccinated against IBV using live-attenuated, serotype-specific vaccines applied by spray in the hatchery. Previous work by our laboratory and others has shown that the Arkansas DPI (Ark DPI) serotype vaccine does not infect and replicate like a typical (Mass) IBV vaccine after spray application, is constantly isolated in the field, and does not provide adequate protection from challenge. A previous Ark serotype vaccine, Arkansas 99 (Ark99), was the first Ark-type virus to be attenuated and used as a vaccine in the field. The original Ark99 protected from challenge, but caused severe reactions post-vaccination, leading to its discontinued use when ArkDPI was produced. This study proposed to revive Ark99 and further evaluate its potential as a vaccine. The Ark99 vaccine seed stock was first passaged 60 times in embryonating eggs to produce a more attenuated vaccine, Arkansas GA (ArkGA). Following attenuation, the replication efficiency and protection from challenge of ArkGA was evaluated. ArkGA was administered to 100 one-day-old broiler chicks using a spray cabinet at a dose of 1 x 10⁴ EID₅₀ per chick. Choanal swabs were taken from each chick at multiple time points post-vaccination for qRT-PCR to verify that ArkGA was infecting and replicating in a pattern typical for IBV, and for sequencing to confirm that the virus population replicating in the birds was the same as the ArkGA vaccine. Twenty-eight days post-vaccination, birds were challenged with pathogenic Ark DPI and a necropsy was performed five days later during which choanal swabs were taken for virus detection by qRT-PCR and virus isolation, clinical signs were recorded, and tracheas were collected for ciliostasis scoring. The results from this trial indicate that ArkGA is a suitable Ark-type vaccine candidate, showing efficient infection and replication post-vaccination and consistent protection from challenge.

Key Words: Broiler, Vaccine, Infectious Bronchitis Virus, Arkansas

T142 Rapid and specific identification of infectious bronchitis virus (IBV) by real time reverse transcriptase-polymerase chain reaction (RT-PCR) with synthetic DNA templates and clinical specimens Jongseo Mo⁴,⁵, Michael Angelichio⁶, Lisa Gow⁷, Valerie Leathers⁷, Mark Jackwood⁴ Poultry Diagnostic and Research Center, University of Georgia; ²IDEXX Laboratories

Rapid identification of infectious bronchitis virus (IBV) is not only important for control of the disease, but also to rule out other important respiratory diseases including infectious laryngotracheitis, Newcastle disease, and avian influenza. Real-time PCR has nowadays become one of the most important methods of gene quantitation due to its large dynamic range, high sensitivity, and specificity. Since vaccine control strategies are only effective when designed around the currently circulating IBV types, it is imperative to develop and characterize these serotypes in an accurate and rapid fashion. Pan-real time RT-PCR test for IBV as well as specific tests for common types was developed. We also utilized synthetic DNA templates that resemble the hypervariable region in the S1 gene subunit of IBV to generate an standard curve to quantify the presence of the target gene. Real-time PCR was then performed on clinical specimens obtained from bird studies conducted by our laboratory. The results of these assays indicate that the test and its effect on sensitivity and specificity was accessed. Lastly, clinical specimens obtained from bird studies conducted by our laboratory were processed for validating the authenticity of these assays.

Key Words: Infectious Bronchitis, Real-time PCR

T143 Improved protection by including infectious bronchitis virus S2 ectodomain Fatima Elderny⁴,⁵, Kellye Joiner, Haroldo Toro, Vicky van Santen Department of Pathobiology, College of Veterinary Medicine, Auburn University

Infectious bronchitis virus (IBV) quickly evolves by point mutations, recombination, and selection processes that result in continuous emergence of new serotypes, which prevents effective vaccination. The spike (S) protein is the most variable protein of IBV and the major inducer of neutralizing antibodies. While the spike S1 subunit mediates viral attachment to host cells, the S2 subunit, which is more conserved among IBV strains, is responsible for membrane fusion and likely assists in virus attachment. Based on our previous results showing increased binding to chicken tissues...
of S1+S2 ectodomain compared to S1 alone, we hypothesized that immunization with S1+S2 ectodomain protein confers more effective protection against challenge than immunization with S1 protein. We produced IBV Arkansas-type (Ark) strep-tagged glycosylated soluble trimeric recombinant S1 and S1+S2 proteins from codon-optimized constructs in HEK293T cells. Specific-pathogen-free chickens in groups A and B were primed at 12 days of age with 10 µg of S1 or 20 µg of S1+S2 protein emulsified in Sep-pic Montanide™ ISA 71 VG adjuvant respectively, then boosted 21 days later. Groups C and D were unimmunized (adjuvant only) challenged and unimmunized/unchallenged control groups. Chickens in groups A, B and C were challenged with virulent Ark IBV 21 days after boost. Chickens immunized with recombinant S1+S2 protein showed statistically significantly (P < 0.05) reduced viral loads as determined by qRT-PCR 5 days post-challenge in both tears and tracheas compared to chickens immunized with recombinant S1 protein. Consistent with viral loads, significantly reduced (P < 0.05) tracheal mucosal thickness, lymphocyte infiltration, deciliation and necrosis revealed that immunization with recombinant S1+S2 protein provided improved protection of tracheal integrity 5 days post-challenge compared to immunization with recombinant S1 protein alone. These results indicate that recombinant S1+S2 ectodomain protein confers better protection against challenge than recombinant S1 protein, suggesting that the S2 domain has an important role in inducing protective immunity. Thus, including the S2 domain with S1 might be promising for better viral vectored and/or subunit vaccine strategies.

**Key Words:** infectious bronchitis, coronavirus, recombinant spike protein, S2, immunization

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**SCAD III**

**T144 Effect of pullet vaccination on development and longevity of immunity** Emily Aston*, Brian Jordan, Maricarmen Garcia, Mark Jackwood University of Georgia

Avian respiratory disease causes significant economic losses in commercial poultry operations. To protect against these respiratory diseases, standard vaccination protocols commonly target viral diseases caused by infectious bronchitis virus (IBV), Newcastle disease virus (NDV), and infectious laryngotracheitis virus (ILTV). Typically, these practices involve serial vaccinations administered at certain time intervals in ovo, at the hatchery, or in the poultry house. Most of these vaccines are live attenuated and offer good protection against homologous challenge when they are individually administered. However, little research has been performed to investigate how the timing of immunization with multiple live attenuated vaccines affects the development of antibodies and protection against challenge in poultry. The aim of this study was to examine the effect of serially administered, live attenuated vaccines against IBV, NDV, and ILTV on the development of mucosal and systemic immunity and protection against challenge in long-lived birds. SPF White Leghorns were administered a combination of multiple live attenuated vaccines against IBV, NDV, and ILTV until sixteen weeks of age, after which certain groups were challenged with IBV, NDV, or ILTV at 20, 24, 28, 32, and 36 weeks of age. Post-challenge, clinical signs were recorded and samples were collected to determine antibody levels, ciliostasis, and viral load in respiratory tissues. Immunized birds were protected from challenge. Compared to vaccinated birds in their respective challenge groups, nonvaccinated birds challenged with IBV displayed clear differences in ciliostasis and significantly increased clinical signs, and nonvaccinated birds challenged with ILTV exhibited significantly more severe clinical signs. Compared to vaccinated birds challenged with NDV, clear differences in ciliostasis and clinical signs of nonvaccinated birds challenged with NDV were not observed. Understanding how the administration of multiple live attenuated vaccines impacts the development of immunity and protection from challenge will contribute key information leading to improved vaccination programs that achieve maximum immune protection from field challenge in long-lived birds.

**Key Words:** Infectious bronchitis, Newcastle, Infectious laryngotracheitis, Vaccination, Immunity

**T145 Cytokine expression patterns in conjunctiva, Harderian gland and trachea after ocular or oral inoculation with a virulent strain of infectious laryngotracheitis virus (ILTV)** Gabriela Beltrán*, Sylva M. Riblet1, Wanderley Moreno Quinteiro2, Leah Read1, Shayan Sharif2, Maricarmen Garcia1 The University of Georgia; 2University of Guelph

Infectious laryngotracheitis virus primarily infects the upper respiratory tract of chickens. The main sites of ILTV lytic replication are the conjunctiva, nasal cavity and the trachea mucosa. We have previously shown that the route of inoculation greatly alters the replication patterns of virulent ILTV strain 63140. When strain 63140 was administered via the ocular route viral replication was detected in trachea, conjunctiva and nasal cavity. In contrast when administered via the nasal or oral routes replication was limited to the nasal cavity. The nasal cavity, conjunctiva and the Harderian gland are structures that although not anatomically connected to the respiratory system are the first to come in contact with the virus and contain associated lymphoid tissues which play essential roles in induction of local immune responses. The specific objective of this study was to determine how the route of inoculation of virulent ILTV strain 63140 influenced cytokine gene expression in conjunctiva, Harderian gland and trachea tissues after ocular or oral inoculation. Relative quantification of host gene expression for type 1 interferon (IFN alpha and beta), type I interferon (IFN gamma), interleukines IL1b, IL6, and inducible nitric oxide (iNOS) was performed by reverse transcriptase real-time PCR. Preliminary analysis shows that three days post ocular inoculation significant up regulation of IFN alpha, beta, and gamma gene expression was detected in conjunctiva of infected chickens. While in conjunctiva of chickens inoculated via the oral route five days post-inoculation significant up regulation of IFN-gamma, IL6, and iNOS gene expression was detected. Further analysis of cytokine gene expression in Harderian gland and trachea will be presented.

**Key Words:** Infectious laryngotracheitis, ILTV replication patterns, Host cytokine gene expression, ILTV lytic replication, Routes of inoculation

**T146 The role of litter beetles, water, and rodents in avian influenza virus transmission** Isabelle Kallenberg*, Joe Giambrone, Ken Macklin, Teresa Dormitorio Auburn University

Avian Influenza (AI) is an infectious respiratory disease of birds caused by Avian Influenza Virus (AIV). AIVs commonly infect poultry and wild birds; however, some strains, e.g., H5N1, H1N1, H7N9, have infected and caused mortality in a variety of mammals including humans. Wild waterfowl are the natural reservoir of influenza A viruses and serve as a continuous source of virus for domestic poultry and other animal species including humans. Waterfowl are typically asymptomatic whereas poultry commonly exhibit extreme respiratory distress, discolored wattles, high morbidity, and even mortality. Despite the implementation of biosecurity measures, the United States suffered from an H5N2 highly pathogenic AIV (HPAIV) outbreak from late 2014 to mid-2015 that affected almost 50 million birds and cost about $3.3 billion for depopulation and indemnification of farmers. Possible means of AIV transmission onto poultry farms include wild waterfowl, contaminated shoes, equipment, and trucks, but not much is currently known about other modes of AIV transmission. Darkling beetles, biofilm-containing waterlines, and rodents have been shown to transmit infectious laryngotracheitis virus (ILTV), and therefore our current studies will determine if AIV can also be transmitted via these vectors. Preliminary results showed that AIV was detectable by RRT-PCR in feed, beetles, and water lines up to 5 days, 3 days, and 24 hours, respectively. Cp values ranged from 23 to 40. Experiments will be replicated to confirm results and