

S-M91 The use of a defined probiotic product (Biomin® Poultrystar) and organic acids to control salmonella enteritidis in broiler chickens. A. Berchieri¹, E. Sterzo¹, J. Paiva¹, C. Luckstadt³, and R. Beltran*², ¹*Faculty of Agricultural and Veterinarian Science, Jaboticabal, Brazil*, ²*Biomin USA, San Antonio, Texas*, ³*Biomin Deutschland GMBH, Germany*.

Background: Contaminated poultry meat and eggs from *Salmonella* sp. continues to be a major concern for consumers. As a result finding ways to reduce or prevent *Salmonella* contamination at all stages of the supply chain has been a major focus for many research organizations worldwide. Producing a low pH (acidic) environment in the gut has been known to promote an adverse effect on the growth of pathogenic bacteria in the gut. By supplementing the diet with probiotics or acids these strategies have also shown to promote growth.

Objective: To test the efficacy of a defined probiotic product, an acidifier as well as the combination to control *Salmonella enteritidis* (SE) in broiler chickens.

Design and Setting: 5 groups, 18 birds per group were weighed, randomly allocated and reared in cages at isolation units. The groups consisted of the following:

A: ACIDIFIER (3 kg / t of feed)
 B: ACIDIFIER (3 kg / t of feed) + Biomin® PoultryStar (20 g/1000 birds)
 C: Biomin® PoultryStar (20 g/1000 birds)
 D: NEG CONTROL
 E: POS CONTROL (0.1 ml SE culture containing 1.0 x 10⁶ CFU /ml)

All broiler chickens were kept on dry litter and water and feed was given ad libitum. The recommended manufactured dose of the probiotic was 20g/1000 heads (via drinking water) for the first three days and the acidifier was mixed into the diet at 3 kGS per ton of feed. Seventy-two hours after oral application of tested products the chicks were challenged with SE, orally inoculated with 0.1 ml of SE per bird, which contained 1x10⁶ CFU/ml. At days 3, 5, 7, and day 10, three birds were sacrificed from each group to measure for cecal colonization of *Salmonella enteritidis*.

Results: Birds that received the probiotic alone as well as the group that received the combination of the probiotic and the acidifier differed significantly then the control group.

Conclusions: Our findings suggest that by combining the use of an acidifier and Biomin® PoultryStar in the diet, the risk of *Salmonella* sp. infection in broilers could potentially be reduced and could help safeguard the health of consumers.

Key Words: *Salmonella enteritidis*, probiotic, organic acids, nutrition

Tuesday, January 24 SCAD (Avian Diseases) II Room: B312

S-T92 Evidence for immunodeficiency affecting outcome of avian infectious bronchitis virus infection. H. Toro*¹, V. van Santen¹, and F. Hoerr^{1,2}, ¹*Auburn University, Auburn*, ²*Alabama State Veterinary Diagnostic Laboratory, Auburn*.

This study was aimed at understanding the causes for the failure of adequate protection against infectious bronchitis virus (IBV) in vaccinated chickens. We investigated genetic, phenotypic, and pathogenicity characteristics of two locally obtained IBV isolates (98/4614 and 00/7149), which exhibited Ark-type restriction fragment length polymorphism. In addition we evaluated the effects of viral immunodeficiency on IBV outcome of infection. The S-1 gene sequence analysis confirmed that both IBV isolates were similar to Ark serotype strains at both the nucleotide and amino acid levels. Heterologous neutralization indices obtained from cross neutralization tests between IBV field isolates and the reference ArkDPI reference strain reached values similar to homologous reactions, demonstrating a high antigenic relatedness between them and with the ArkDPI vaccine strain. The inoculation of isolates 98/4614 or 00/7149 into SPF chickens caused mild respiratory signs. The histopathological findings in the trachea and larynx were consistent with the clinical signs, with birds showing mild hyperplasia and lymphocytic infiltration in the mucosa. Assessment of the influence of immunodeficiency was performed using chicken anemia virus (CAV) and infectious bursal disease virus (IBDV) in SPF chicks. One group was infected with CAV and IBDV at day 7 of age and subsequently inoculated with IBV isolate 98/4614 at day 15 of age. Another group was inoculated at day

15 with IBV 98/4614 only. Tracheal and nasal swabs lasted longer in the immunodeficient group. Samples of trachea showed higher IBV genome concentrations in the immunodeficient chickens. In lachrymal fluid IBV genomes were detected until day 28 after IBV inoculation while in immunocompetent birds IBV genomes were no longer detectable after day 14 post IBV inoculation. The local specific IgA response in lachrymal fluid was earlier and higher in immunocompetent chicks. These experimental results corroborate epidemiological evidence indicating that immunosuppression is most likely playing a role in the outcome of IBV infection in the field.

Key Words: infectious bronchitis, immunosuppression, chickens

S-T93 The decay of maternal antibody to infectious bursa disease virus in cockerel chick. F.O Ajasin*¹, A.M Raji¹, and C.O Aiki-Raji², ¹*Federal College of Animal Health and Production Technology, IAR&T, Moor Plantation, Ibadan, Nigeria*, ²*University of Ibadan, Ibadan, Nigeria*.

Infectious bursa disease (IBD), was first reported in Nigeria in 1973, also known as Gumboro disease is a viral infection that causes lymphoid degeneration of the bursa of fabricius and subsequent suppression of the humoral immune response of immature birds causing substantial economic losses. The major method of prevention is by vaccina-

tion. However, outbreak of IBD virus infections have been reported in vaccinated flocks. An investigation into the decay of maternal antibody to infectious Bursa disease virus was conducted on total of 500 day old cockerel chicks to determine the time required for maternal antibodies to infectious bursa disease (IBD) to decay. The chicks were only vaccinated against Newcastle disease at day old with B1 and Lasota strain at 22 day old. The sera samples were collected between April and July 2005, Jugular vein-puncture at 2, 7, 15 and 30 days of age bled each chick. A total of 250 blood samples were collected for each period and 10 were randomly selected for the test. The blood samples collected were poured into a bottle and allowed to settle down for 4-5 hours. The separated sera were decanted into Eppendorf tube, placed inside the fridge and stored at -20o C deep freezer until tested. The agar gel precipitation test was used as described by Cullen and Wyth (1975) on individual sera. Bursa was removed from chicken that died of infectious bursa virus (IBDV) and homogenized in normal saline. The homogenate was then used as the virus antigen. 1 gram of agar (agar rose) was weighed, 99ml of deionized water was added to it. 0.01 gram sodium oxide was added to prevent mycotic growth. It was boiled for 35 minutes to melt the agar. 6mls of the agar was dispensed into 5cm diameter Petri dishes and allowed to solidify. The results indicate that maternal antibody waned in the test cockerel chicks from day 15; although by day 22 only 20% of the chicks showed some evidence of maternal antibody. Vaccination could be advised from 15th day after hatching.

Key Words: cockerel chicks, maternal antibody, infectious bursa disease virus

S-T94 Production of a polypeptide representing the hypervariable region of infectious bursal disease virus VP2 and the whole VP2 in *Pichia pastoris*. M. Hamoud* and P. Villegas, *University of Georgia, Athens.*

Genomic material for IBDV was extracted from formalin fixed paraffin embedded tissue yields fragmented dsRNA. An extracted fragment representing the hypervariable region was cloned and transformed into *Pichia pastoris* to produce a polypeptide of that region. The whole VP2 gene of IBDV was also expressed in *Pichia pastoris* for production of the complete protein. Production of VP2 and its partial protein was done to investigate the role of the hydrophilic peak peptides in the cell mediated immune response to IBDV and to develop recombinant vaccines for different IBDV strains.

Key Words: IBDV, *Pichia pastoris*, recombinant protein, chicken, VP2

S-T95 Very virulent infectious bursal disease in Columbia, South America. J. Giambrone*¹ and D. Jackwood², ¹*Auburn University, Auburn,* ²*Ohio State University, Wooster.*

Broiler chickens from the largest integrator in Columbia experienced severe morbidity and mortality from 20 to 30 days of age. The condition affected other nearby broiler and layer companies in the area around Barranquilla. Affected birds were listless, their feathers were roughened up, and they were stunted and had urate or bloody diarrhea. From 25 to 75% of the birds had clinical signs and mortality typically peaked near 50%. On post-mortem exam, affected chickens had focal to confluent

muscular hemorrhages, and the bursae of Fabricius were enlarged, edematous, and/or hemorrhagic. Some birds had hemorrhages in the glands of the proventriculus and in the kidneys. Microscopic examination showed lesions suggestive of very virulent infectious bursal disease (vvIBD). This conditioned occurred in flocks that received from 2 to 3 intermediate IBD vaccines during the first 3 weeks of age. Bursae were placed in phenol and chloroform and were sent to the laboratory of Dr. Jackwood at OSU. RNA was extracted from the tissues and subjected to RT and PCR. Sequencing done directly from the amplified cDNA fragments revealed a high degree of complementarity with very virulent IBD virus isolates from Europe, Venezuela, and Brazil. Management and IBD vaccination changes reduced the incidence and severity of vvIBDV.

Key Words: vvIBDV, Columbia, poultry

S-T96 Comparison of FTA® paper and Phenol for storage, extraction and molecular characterization of infectious Bursal disease virus. L. Purvis*, P. Villegas, and F. Perozo, *University of Georgia, Athens.*

Sample collection and retrieval of nucleic acids is an important part in characterizing Infectious Bursal Disease Virus (IBDV) samples. The recently introduced FTA card inactivates viruses, and maintains the nucleic acid, making it a great asset to use for IBDV sample transportation. The use of phenol as an inactivation method has been widely used for many years to import and ship samples safely internationally. This sampling method for IBDV was compared to samples collected on the FTA card. RT-PCR and nucleotide sequences were performed using standard methods and analyzed to determine the differences between the collection methods. The FTA card proved to be an adequate and useful tool in sample collection for IBDV and could eliminate phenol usage for inactivation and storage of viruses shipped internationally.

Key Words: IBDV, FTA

S-T97 Sequence analysis of the complete proviral genome of reticuloendotheliosis virus APC strain. T. Barbosa*, G. Zavala, S. Cheng, and P. Villegas, *University of Georgia, Athens.*

Reticuloendotheliosis (RE) is an oncogenic and immunosuppressive disease in poultry. RE virus (REV) can be a contaminant of vaccines being inserted on large genome virus like MDV and Fowlpox. Attwater's Prairie Chicken (APC) is an endangered species, in part due to enzootic REV infection. Our laboratory has several isolates from APC showing or not tumors. Full length sequence analysis of REV may help to identify regions involved in pathogenesis. In this study we have used an oncogenic isolate designated APC-566. The DNA provirus integrated in DF-1 cells and/or tumor cells was used as template for sequencing. PCR reactions were performed using proofreading DNA polymerase and sequencing by the dideoxy chain termination method. Nucleotide sequences were aligned with previously published sequences followed by phylogenetic analysis. The gag and pol genes were highly conserved. The ENV gene, first thought to be highly variable as in other avian retroviruses, was very similar to available REV sequences. The LTR gene had significant differences among the virus strains analyzed. These differences consisted primarily of deletion and insertions or even short

repeat regions. The role of those changes on LTR in REV pathogenicity is under study. This is the first American clinical REV isolate to be fully sequenced.

Key Words: reticuloendotheliosis virus, REV, sequence, proviral DNA, genomic analysis

S-T98 Molecular characterization of chicken and turkey astroviruses. M. Pantin-Jackwood* and E. Spackman, *Southeast Poultry Research Laboratory, ARS, USDA.*

This study investigated the genetic diversity of astroviruses circulating in poultry. Intestinal samples collected from commercial turkey and broiler flocks from different regions of the United States during 2005 were examined for the presence of Astroviruses by RT-PCR. Astroviruses were found in samples collected from flocks with enteritis and stunting as well as from healthy flocks. Phylogenetic analysis performed by partial sequence analysis of the polymerase gene (ORF-1b) indicates the presence of four clearly different astroviruses circulating in poultry: Turkey Astrovirus 1 (TAsTV-1), Turkey Astrovirus 2 (TAsTV-2), Avian Nephritis Virus (ANV) and a newly identified Chicken Astrovirus (CAsTV). Nucleotide identity between these groups was between 52 and 60%. A high level of genetic variation was also observed among the isolates in each group. Turkey astroviruses (TAsTV-1 and TAsTV-2) and ANV were detected in turkeys, TAsTV-2 being by far the most frequently detected. Avian nephritis virus and CAsTV were both detected in broiler chickens, with some regional distributions observed. In conclusion, we found four genetically distinct avian astroviruses which are widespread in turkeys and broilers. Since the viruses were detected from sick and healthy flocks, the significance of their presence needs to be further investigated.

Key Words: astrovirus, chicken, turkey, sequence

S-T99 Laboratory evaluation of commercial disinfectants on the survivability of H7N2 avian influenza and Newcastle disease virus. E. Benson*, R. Alphin, B. Ladman, G. Malone, and M. Lombardi, *University of Delaware, Newark.*

Current methods of avian influenza (AI) control in poultry require euthanasia of flocks known or suspected to be infected. Environmental concerns have made disposal of AIV infected carcasses a major issue in many poultry-producing areas. Having a rapid, practical, cost-effective and biosecure means of inactivating AIV within the facility, litter, carcasses and on support equipment, is essential in containing disease outbreaks. The decontamination of poultry houses and equipment will be evaluated in a four-phase process.

The objective of phase one of the study (the portion presented herein) was to test the efficacy of five commercially available disinfectants on low pathology avian influenza (LPAI) and Newcastle disease virus (NDV). The viruses were tested on four materials typically found in poultry houses (glass, plastic, wood, and galvanized steel). The samples were separated and inoculated individually with A/chicken/MD/MINH MA/03(H7N2) and LaSota strain of NDV virus and allowed to dry at room temperature. Separate samples were exposed to liquid applied Virkon S, foam applied Virkon S, foam applied DF200, liquid applied BioSentry 904 and fog applied Virocid. Solution containing the decontaminated virus was then collected and inoculated into 10-11 day-old

SPF embryos that were incubated and candled daily for five days. The embryos were refrigerated and fluid was tested for hemagglutination activity (HA). HA and positive control activity were used to indicate successful disinfection. Most disinfectants had similar neutralization activity with the viral agents tested on both glass and plastic. Differences in neutralization activity were observed on both wood and metal surfaces, both of which are typically found in poultry houses. The results will be used to select the agents for the second phase, which will include disinfecting colony houses.

Key Words: avian influenza, disinfectant, decontamination, Newcastle disease, H7N2

S-T100 Evaluation of the use of fire fighting foam for large scale emergency euthanasia of floor reared meat type birds. M. Dawson*¹, E. Benson¹, R. Alphin¹, G. Malone¹, G. Van Wicklen¹, and I. Estevez², ¹*University of Delaware, Newark,* ²*University of Maryland, College Park.*

Preliminary studies have shown that fire fighting foam enriched with carbon dioxide (CO₂) is an effective means of euthanasia for floor raised broilers. Subsequent trials were conducted where the fire fighting foam procedure was tested side by side against the poly euthanasia tent procedure used in recent avian influenza outbreaks (Delmarva, 2004). The results from the comparative trials suggest that the fire fighting foam procedure requires equal or less time from application to cessation of movement than the poly tent method.

In the portion of the project presented, two trials were conducted. In the first trial, the humaneness of both the foaming and poly tent euthanasia procedures were compared. Birds were instrumented with electrocardiogram (ECG) and accelerometers to measure cardiac activity and the movements that the birds experienced during the euthanasia process. Data collected during the two euthanasia procedures will be used to quantitatively determine stress levels and duration of the stress levels that the birds may experience. To insure that the fire fighting foam procedure was not drowning birds, post-mortem analysis on the experimental birds was conducted to evaluate cause of death and possible drowning. No evidence of drowning was discovered in the tested birds.

In the second trial we simulated field type conditions. One thousand and two hundred birds were divided into six pens and were euthanized using the foam and poly tent procedures (three replications of each procedure). One bird per each pen was instrumented with ECG and accelerometers as for trial one above. Blood samples were drawn from ten birds per test for pre-test and post-test steroid analysis.

Key Words: broiler, euthanasia, avian influenza, poly tent, foam

S-T101 National training program on euthanasia and disposal procedures for catastrophic poultry disease events. G. Malone*¹ and N. Tablante², ¹*University of Delaware, Georgetown,* ²*University of Maryland, College Park.*

Rapid response to an emergency disease situation such as Avian Influenza (AI) is critical in the control and eradication of an outbreak. A comprehensive training program is being offered to prepare responders on the options for euthanasia and disposal of infected meat-type poultry flocks. The half-day comprehensive training program covers human

health considerations when responding to an AI outbreak and the options for euthanasia and carcass disposal of infected breeder, broiler and turkey flocks. Material, equipment and labor needs; and detailed step-by-step procedures on how to implement each option is discussed. Much of the instructional information and pictorials used in this training were obtained from lessons-learned from actual AI events. A total of 16 sessions in 14 different poultry producing states have been conducted to date. Participants include 50% poultry industry personnel (poultry company health, production and environmental managers) 40% agencies (department of agriculture, USDA, and emergency management) and 10% university service and cooperative extension staff. Response to this training has been highly favorable and very timely. Based on pre- and post-training questionnaires, participants identified euthanasia procedures and the in-depth instructions on in-house composting for carcass disposal and virus containment as being the most helpful. However, to become confident in implementing these procedures there may be a need for additional simulation and hands-on training. These free-of-charge training sessions will continue into 2006 or until such time that all segments of the poultry industry in the USA have been trained.

Key Words: poultry, avian influenza, catastrophic disease, mass euthanasia, mass disposal

S-T102 An optical interferometer for detection of avian influenza virus. J. Xu¹, D. Suarez², and D. Gottfried*¹, ¹Georgia Tech Research Institute, Atlanta, ²Southeast Poultry Research Lab, Athens.

There exists a need for a rapid and sensitive penside diagnostic test to identify poultry flocks infected with avian influenza virus (AIV). The availability of a simple and economical diagnostic test for on-site analysis would greatly aid in the control of avian influenza at the outset and during an outbreak. An interferometric waveguide biosensor developed at Georgia Tech provides rapid, sensitive, and cost-effective detection of biomolecular interactions. A surface-sensitive evanescent field, propagating through the planar waveguide, is used to measure a refractive index change caused by molecular binding events on the waveguide surface. Biorecognition elements, such as antibodies, aptamers, and nucleic acids, are covalently bound to the thiol-modified waveguide using a bifunctional crosslinker. This biosensor has been used for direct detection of proteins, oligonucleotides, viruses, and bacteria. Near real-time measurement can be achieved without the need for rinsing, incubation, or reporter labeling. Recent results will be presented on the development of a biosensor assay for AIV. Using monoclonal and polyclonal antibodies against the H7 hemagglutinin (HA) antigen, both direct and sandwich immunoassays were tested. Analytical sensitivity of less than 0.1 HA units/ml was obtained. This biosensor was found to be more sensitive than a commercially available influenza immunochromatographic assay by at least 2 orders of magnitude and possesses HA antigen specificity.

Key Words: biosensor, avian influenza, immunoassay, hemagglutinin, optical waveguide

S-T103 A replication-defective adenovirus vaccine carrying the H5 gene of avian influenza virus induced high antibody levels after in ovo delivery. H. Toro*¹, D. Tang², K. van Kampen², D. Suarez³, and P. Gao², ¹Auburn University, Auburn, ²Vaxin, Birmingham,

Alabama, ³Southeast Poultry Research Laboratory, Athens, Georgia.

A novel replication-defective human adenovirus serotype 5 vector allowing introduction of expression cassettes for a variety of antigens, including the hemagglutinin (HA) gene of influenza virus, was tested in chickens. In previous work we demonstrated that chickens inoculated by different routes (intramuscularly, intranasally, and in ovo) with this vector carrying the H1 or the H3 gene of human influenza strains A/PR/8/34 or A/Panama/2007/99 developed hemagglutination inhibition (HI) antibody titers against these strains. In this study we developed a new construct containing the H5 gene of avian influenza. Recombinant vector AdH5 (1.5 x 10⁸ pfu/ml) was applied in ovo at days 10 or 18 of incubation into fertile eggs. Hatched chicks of each group were further divided into two groups: Half of the birds were boosted via the nasal route with the same dose at day 15 of age and the remaining birds were not further inoculated. Sera obtained at day 29 from birds vaccinated in ovo at day 10 showed HI titers varying between 2 and 7 log₂ with a median of 4 log₂. Chicken vaccinated at day 10 and boosted at day 15 showed HI titers varying between 2 and 9 log₂ with a median of 6 log₂. Chickens vaccinated at day 18 showed titers varying between 2 and 9 log₂ with a median of 5 log₂. Chickens vaccinated at day 18 and boosted at day 15 showed values between 2 and 8 log₂ with a median of 6 log₂. These results demonstrate that chickens successfully respond to this adenovirus recombinant vaccine when delivered by the in ovo route.

Key Words: avian influenza, recombinant vaccine, adenovirus, chicken

S-T104 S-Pecificity of an avian influenza (AI) North America Lineage Neuraminidase-2 (N2) based ELISA and its application for differentiating vaccinated from infected animals (DIVA). M. Garcia*¹, E. Winkelmann², M. Jackwood¹, A. Das³, and D. Suarez³, ¹The University of Georgia, Athens, ²Federal University of Santa Maria, Rio Grande do Sur, Brazil, ³Southeast Poultry Research Laboratory, ARS, USDA, Athens.

The control of avian influenza in poultry gains unprecedented importance under the increasing threat of an avian "flu" pandemic. Eradication and control of highly pathogenic AI viruses in poultry has been commonly achieved by culling large populations of birds in the affected regions. Vaccination against AI has also been utilized as a control strategy to protect remaining poultry populations after a highly pathogenic outbreak, and to control the spread of low pathogenic AI viruses in poultry. As part of a vaccination control strategy the differentiation of infected and vaccinated animals (DIVA) is essential. One of the DIVA strategies that have been developed uses inactivated vaccine containing the same hemagglutinin (HA) subtype as the challenge virus with a different neuraminidase (NA) subtype. The success of the NA-DIVA strategy relies in the specificity of the serological assay to detect NA specific antibodies induced by exposure to the challenge virus.

The objective of this study was to evaluate the specificity of a baculovirus expressed N2 protein from a North America lineage virus for future use on a NA-DIVA strategy. The recombinant N2 antigen reactivity was tested against two anti-sera panels from birds inoculated with live and inactivated N1 to N9 virus subtypes. In addition, serum samples collected from birds infected with recent North American N2 viruses were tested by indirect immunofluorescence and N2 ELISA. The application of the N2 ELISA in a NA-DIVA vaccination control strategy will be discussed.

Key Words: avian influenza, neuraminidase, ELISA, DIVA