to have significantly higher overall hatch of normal live embryos than that of the other system (p≤.05). In a direct correlation to the higher hatch of normal live embryos, data revealed that eggs vaccinated with the Inovoject had significantly less live pips (in shell) than the other system regarding injections done on day 18, day 19, and overall.

Key Words: application trial, commercial hatchery, egg injection

T127 Consumers' preference for egg shell and yolk colour in Nigeria: A case study of Iddo and Akinyele local government area of Oyo State, Nigeria.

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This study determined the consumer preferences for egg shell and yolk colour in the study area. One hundred and eighty respondents that consume eggs were randomly selected in the study area. The result revealed that the respondents age range between 20 to 60 years and 160(88.9%) were males while 20(11.1%) were females. All the respondents had formal education with 10(5.6%) primary education, 50(27.8%) HND, 50(27.8%) BSc, while 60(33.3%) MSc qualifications. Majority 140(77.8%) of the respondents rear birds while 40(22.2%) do not rear birds. Among the respondents that rear birds 100(55.6%) rear layers, 40(22.2%) rear broilers and cockerels respectively. Majority 130(72.2%) of the respondents agreed that brown eggs are more preferable to other colour of eggs and 100(55.6%) of the respondents agreed that white shell eggs cracks faster than brown eggs. The result shows that majority 107(59.5%) of the respondents agreed that yellow yolk eggs has better aroma than white yolk.
In conclusion, most of the respondents prefer brown egg shell due to its attractiveness and hardness while yellow yolk is preferred due to its aroma. It is recommended that feed ingredients that would produce brown egg shell and yellow yolk should be used in layers mash to increase market for the eggs and enhance high profit.

Key Words: consumers, preference, egg shell color, egg yolk color, Nigeria

T128 Consumer preference for different meats of chicken in Nigeria: A case study of University of Ibadan employees.

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This study was designed to ascertain the consumers' preference for different meats of chicken in Nigeria and University of Ibadan was purposively selected for the study.
One hundred and seventy employees that indicated interest were randomly selected and interviewed for this study. The results revealed that majority of the respondents were in their middle aged of 31 and 40 years and 155 (91.2%) were males while 15 (8.8%) were females. Few (29.4%) of the respondents rear poultry while 120(70.6%) do not rear poultry. Also, the result revealed that 60 (35.3%) of the respondents prefer layer meat for consumption, 85(50%) preferred broiler meat and 25 (14.7%) preferred cock meat which indicates that majority of the consumers prefer broiler meat. Most of the respondents indicated that they consume chicken mostly in the festival period which could be due to the high cost of chicken meat. 25(14.7%) of the respondents consume chicken meat because it is tasty, 55(32.4%) tender while 35(20.6%) consume chicken meat because it is soft and meaty. The responses to attitudinal statements revealed that most of the respondents consume chicken occasionally as 85(50%) agreed that chicken is too expensive. Chi-square result revealed that there is a significant relationship between consumers' preferences for broiler to layers and cockerels (X^2=8.40, P≤0.05)
In conclusion, consumers of chicken meat consume chicken because of the tenderness and occasionally because it is too expensive. It is recommended that cost of rearing poultry should be reduced to enable consumers to afford it.

Key Words: consumers, preference, chicken, meat, university employees

SCAD II

T129 Experimental reproduction of runting and stunting syndrome in broilers.

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Runting-stunting syndrome (RSS) is a transmissible, infectious disease affecting young broilers between 1-2 weeks of age. Clinical features of RSS include severe weight suppression lack of flock uniformity, diarrhea, and a significant increase in the rate of feed conversion. Cystic enteropathy is consistently observed by histopathological examination of small intestine samples from affected flocks. Although descriptions of RSS date back to the 1970s, the etiologic agent(s) has yet to be identified. In previous studies, we isolated several viruses from RSS-affected birds, but no single virus, to date, has reproduced the clinical disease. All evidence suggests this is a multifactorial disease. To examine factors associated with disease, it is imperative to experimentally reproduce the clinical disease. In these studies, intestines from RSS positive broilers were collected, homogenized and stored at -80C. Numerous infection studies were performed in day-old commercial broilers in Horsfall Bauer isolation units. First, oral infection of day-old commercial broilers with homogenized RSS stock or a 0.2 micron filtrate of homogenized stock resulted in the reproduction of the clinical disease in a dose dependent response by 12 days post challenge as assessed by significant body weight suppression and cystic enteropathy in the intestinal villus. These results confirm that the disease can be reproduced from intestinal contents and that viruses are capable of reproducing RSS. In subsequent studies, day-old broilers were challenged with chloroform (CHCl3)-treated filtrate to further characterize the viral populations that contribute to RSS. By 12 days post infection, body weights of the CHCl3-treated group were significantly lower than the negative controls. In addition, cystic enteropathy was observed histologically in the CHCl3-treated group implying that nonenveloped viruses can cause clinical RSS. The challenge model described can be reliably used to reproduce the clinical signs of RSS and is

an important tool for evaluating treatments or vaccines used for control of the disease.

Key Words: runting and stunting syndrome, viral enteric disease, infectious stunting syndrome, RSS, enteric disease of poultry

T130 Development of a recombinant vaccine against runting and stunting syndrome in chickens. H. Sellers, G. Zavala, and E. Mundt*, *Poultry Diagnostic and Research Center, College of Veterinary Medicine, University of Georgia*, *Athens, GA*.

Runting and stunting syndrome (RSS) has been recognized since the late 1970s in broiler chickens. Although non-infectious factors may influence the severity of RSS, several viruses have been associated with this syndrome. Most of these viruses are not cultivable in vitro. To overcome this problem, a cloning approach was chosen to target small round viruses, Picornaviridae. The nucleotide sequence of the RNA dependent RNA polymerases (RdRp) is the most conserved sequence within this virus family. Using degenerated primers based on RdRp sequences, RT-PCR fragments were amplified and sequenced. However, the sequences were not present in the NCBI gene bank. Using several approaches, a 1.8 kbp fragment was amplified. While a genbank search did not result in any similar sequences, the in silico translated amino acid sequence showed a 34% similarity to a turkey astrovirus capsid protein. The open reading frame of the new capsid protein was cloned and a recombinant protein was expressed in a baculovirus system. Subsequently, the purified protein was used as an oil-emulsion vaccine in commercial broiler breeders along with a group of non-vaccinated broiler breeders. The development of antibody titer in the hens and the maternal derived antibodies in the offspring was monitored by an ELISA using the recombinant protein. A low ELISA titer was observed in the broiler breeders prior to vaccination, probably due to the fact that this or a similar astrovirus is circulating in the field. After the second vaccination the vaccinated broiler breeders showed a significant increase in titer compared to the control birds. The same increase response was observed in the offspring. In challenge experiments using the offspring from hens receiving three vaccinations, a level of protection was observed against RSS. Progeny from vaccinated hens gained significantly more weight compared to offspring from the nonvaccinted/challenged controls. In addition, the number and size of enteropathic cystic lesions in the small intestine was significantly reduced in the offspring from vaccinated hens. Thus the recombinant vaccine may be a potential vaccine candidate in efforts to control RSS.

Key Words: chicken, RSS, vaccine

T131 Survival of avian influenza virus in eggs during litter composting. T. V. Dormitorio*[SC], J. J. Giambrone, and K. S. Macklin, *Auburn University*, *Auburn, AL*.

In-house composting of litter has been shown to be effective in eliminating avian pathogens. Infectious laryngotracheitis virus (ILT), avian influenza virus (AIV), and Newcastle disease virus (NDV) have been inactivated at composting temperatures of 40°C and above.
Survival of AIV in embryonated eggs during an experimental in-house composting was determined. Ten-day old embryonated eggs were inoculated with a virus isolate (H10N7), and then buried into the compost pile. Eggs were withdrawn from the pile at various time intervals and allantoic fluids were tested for the presence of the virus by hemagglutination (HA) test. Results showed that 100% of the eggs inoculated with fluids from H10N7-inoculated and composted eggs contained residual live virus for up to 36 hours of composting, but at 54 hours and onwards, the virus was eliminated. On the other hand, H10N7 survived 72 hours at room temperature (27-30°C). The compost pile temperature (at the site where the eggs were buried) reached 60°C. Humidity (%RH) and dew point (°C) were 100% and 50°C, respectively. These results can be used to develop management strategies that can inactivate AIV in eggs laid by infected hens.

Key Words: avian influenza, composting, litter, virus inactivation, hemagglutination

T132 Development of an H5 monoclonal antibody for establishment of an H5 species independent cELISA system. D. Dlugolenski*[SC], R. Hauck, R. J. Hogan, and E. Mundt, *University of Georgia*, *Athens*.

Since the emergence of high pathogenic avian influenza virus (HPAIV) H5N1 in Asian countries, HPAIV H5N1 has become a major concern for human health. Waterfowl are the natural host reservoir for AIV, but cross species transmission can readily occur. The determination of the hemaglutinin (HA) subtype is restricted to inhibition and virus neutralization assays. Both assays are labor intensive and not suitable for automation. The presence of HA5 and HA7 antibodies are of major interest since these subtypes of AIV can develop a highly pathogenic phenotype. To develop a test which can be used in automatic settings the HA5 protein of a wild bird isolate [A/duck/NC/674964/07 (H5N2)] was cloned and subsequently expressed in a baculovirus system. In addition, a monoclonal antibody specific for the HA5 antigen (H5-mAb) was generated. Both reagents were used to establish a competitive ELISA (cELISA) system. The cELISA performed with influenza antibody free sera or sera of animals infected with AIV HA suptypes other than H5 showed no significant inhibition of H5-mAb binding, indicating a high specificity of the test. In contrast, sera of animals (chickens, turkeys, mallards, redheads, wood ducks, and cats) experimentally infected with H5-encoding AIV or vaccinated with inactivated vaccines were able to significantly inhibit the binding of the H5-mAb. The cELISA showed a significant inhibition (> 25%) of mAb binding in hemaglutination inhibition (HI) positive serum samples in chicken, duck, and turkey sera with a reproducibility of >95%. This test provides a platform for further development of this type of assay.

Key Words: avian influenza, cELISA, H5, recombinant protein, monoclonal antibody

T133 Co-localization of the cellular protein p68 with a viral protein of infectious bursal disease virus. R. Stricker*[SC] and E. Mundt, *University of Georgia, Department of Population Health*, *Athens*.

A better understanding of viral replication is the basis for the discovery of new ways to interfere. Thus, there is an increasing interest in dissecting the molecular pathways of viral replication. It is known that cellular proteins operate as functional elements during viral replication. In our investigations we focused on the cellular dsRNA binding protein p68,

an established RNA helicase, which seems to have a regulatory function during replication of hepatitis C virus, HIV, and colon cancer. To examine a possible viral-host-interaction we investigated the role of p68 during replication of infectious bursal disease virus (IBDV). RT-PCR using chicken mRNA was performed and the p68 gene was cloned and sequenced. By using a recombinant baculovirus technology p68 was expressed and subsequently purified by affinity chromatography. The identity of the purified protein was confirmed by MALDI/TOF. An anti p68 serum was generated in a rabbit and used for the determination of the localization of the protein in IBDV infected cells. Interestingly, in IBDV infected cells p68 was present in the cytoplasm. In non-infected cells the protein was observed in the cell nucleus. Double-labeling studies using monoclonal antibodies raised against IBDV proteins and the anti-p68 rabbit serum were performed using different anti-species conjugates. Surprisingly a co-localization of p68 was observed only with the viral protease VP4. This indicates that the investigated cellular protein p68 interacts with the viral protease. Subsequent experiments showed that this phenotype was independent from the used IBDV strain and independent from interferon alpha. Several transfection experiments in DF1 cells revealed that only the presence of the viral protein VP4 was necessary and sufficient for a presence of p68 in the cytoplasm. This phenotype was also observed when an inactive viral protease was expressed in the cytoplasm of transfected cells. Thus the proteolytic activity of VP4 was not necessary for the phenotype of p68. The interaction of VP4 with p68 might play an important role during the regulation of the translation process of the IBDV polyprotein.

Key Words: IBDV, replication, cellular protein, p68, VP4

T134 Pathogenicity of infectious bursal disease virus variant AL2 in chickens. H. Toro*, J. C. Effler, F. J. Hoerr, V. L. van Santen, and F. W. van Ginkel, *Auburn University*, *Auburn, AL*.

We evaluated the effects of infectious bursal disease virus (IBDV) variant AL2 in 4-day-old SPF chickens and in broiler chickens of commercial origin (with specific maternal immunity and vaccinated in ovo against IBDV). SPF chickens showed reduced weight gain compared to uninoculated controls and bursa lymphocytic depletion as determined by bursal index (BI) and bursa histomorphometry. Bursa histomorphometry and BI results differed during the early stages of the infection. Because bursa histomorphometry results were consistent with viral RNA detection, such values seem to be more appropriate for the assessment of AL2 viral infectivity in chickens. Infected SPF chickens showed a significant increase of splenic IgM+ cells at 5 and at 8 days post-inoculation (PI). On day 8 PI the number of total IgM+ cells in the spleen was inversely related to the viral dose inoculated. Birds receiving higher virus concentrations showed a significant increase ($P<0.05$) of total spleen CD4+ cell counts on day 8 PI suggesting a role for these cells in protective immunity. Broiler chickens were exposed to IBDV AL2 via the drinking water on days 3 and 14 of age. No clinical signs were observed in these birds. Compared to uninfected controls, AL2 infected broilers showed significant bursa lymphocyte depletion on day 30 of age as assessed both by BI and histomorphometry. IBDV RNA detection in the bursae and seroconversion to IBDV after day 30 of age confirmed that bursal lymphocyte depletion was due to IBDV resuming replication.

Key Words: infectious bursal disease, AL2, pathogenicity

T135 Detection of infectious laryngotracheitis virus in the water lines with real-time PCR and viral isolation. S. C. Ou*[SC], J. J. Giambrone, and K. S. Macklin, *Auburn University*, *Auburn, AL*.

Infectious laryngotracheitis virus (ILTV) causes respiratory disease in chickens and can result in severe economic loss to the poultry industry world-wide. Sources of ILTV transmission are clinically infected chickens, latent infected carrier chickens, and litter and fomites that are contaminated with ILTV. ILTV can persist for months in chicken carcasses, litter, and fomites. Rigorous biosecurity and vaccination are critical for ILT control. Biofilms in water lines are a source of pathogen transmission. Biofilms can protect microorganisms from environmental influences and sanitizers. In this study, a commercial CEO ILT was placed in isolation unit water lines and allowed to maintain overnight. The next day, the water lines were flushed with tap water three times. Three-week-old SPF chicks were then placed in these isolation units. At 7, 14 and 21 days later, swab samples were obtained from the nipple drinkers, the internal surface of the water lines, and chicken trachea. Swabs were checked with real-time PCR and then incubated in eggs for viral isolation. Results showed that all swabs were positive for ILTV DNA and contained live virus. Water lines in chicken houses may be an important source for ILTV transmission to subsequent flocks.

Key Words: infectious laryngotracheitis virus, ILTV, water line, real time PCR

T136 The evolution rate of avian coronavirus infectious bronchitis virus in the presence and absence of vaccination. E. T. McKinley*[SC], D. A. Hilt, and M. W. Jackwood, *University of Georgia*, *Athens*.

Coronaviruses are positive sense RNA viruses that have a high mutation and evolution rate due to the lack of proofreading activity of the RNA-dependent RNA polymerase, its copy-choice replication mechanism and rapid generation time. Infectious bronchitis virus (IBV) was first identified in the 1930s and since then, many different serotypes have been identified and shown to persist in the field. We sequenced and analyzed the entire genome of eleven IBV field viruses of different serotypes, Mass, Conn and CAL, isolated over a 41, 25 and 8 year period respectively. Poultry producers routinely vaccinate for Mass and Conn whereas no vaccine exists for CAL. Our data showed that Mass and Conn did not accumulate changes while CAL accumulated changes and had a high rate of evolution. This indicates that the Mass and Conn viruses isolated from the field were possibly re-isolated vaccine viruses that were circulating in birds for only a short time while the CAL viruses were true field isolates that had been in circulation for years.

Key Words: coronavirus, evolution, vaccination

T137 Full-length genomic comparisons of emerging coronaviruses. S. W. Thor*[SC], D. A. Hilt, C. Polizzi, and M. W. Jackwood, *University of Georgia*, *Athens*.

Viruses in the Family Coronaviridae are of global economic importance. Because coronaviruses have high rates of both mutation and recombination, it is not surprising that they have proven to be a major source of emerging zoonotic diseases. Although the spike region for many of the coronaviruses has been sequenced, there are very few full genomic

sequences available. This is especially true for the coronaviruses that infect animals that are in close contact with humans; including dogs, cats, rats, mice, chickens, and turkeys. Therefore, we were interested in examining the complete genomic sequence of selected animal coronaviruses from groups I, II, and III. Mutation rates, recombination events, and phlyogenetic analysis will be presented.

Key Words: coronavirus, genomic sequencing, mutation rate, recombination, zoonoses

T138 Assessment of the polymerase genes in pathogenic and attenuated strains of infectious bronchitis virus. J. E. Phillips*[SC], D. A. Hilt, S. W. Thor, E. T. McKinley, and M. W. Jackwood, *University of Georgia*, *Athens*.

Infectious Bronchitis Virus (IBV) is a positive sense RNA virus that causes an upper respiratory disease in chickens. The disease is mostly controlled by vaccination with live attenuated viruses. Pathogenicity of IBV has traditionally been attributed to the spike glycoprotein, which is involved in attachment and entry into the host cell. However, evidence in literature indicates that other genes may also be involved. We were interested in examining the viral RNA-dependent RNA-polymerase genes because replication is critical to virus survivability and production of lesions in the host. In this study the 1a and 1b genes of the attenuated Arkansas vaccine and pathogenic Ark-DPI parent strain of IBV were sequenced and compared. There were 344 nucleotide differences in the polymerase genes. Differences in individual proteins will be presented and the possible effects of the amino acid changes will be discussed. This work provides critical information needed to examine mutations individually and in combination to verify their role in coronavirus pathogenicity. With that knowledge, it is hoped that safer more efficacious vaccines can be produced.

Key Words: infectious bronchitis virus, coronaviridae, antigenic variants, pathogenicity, polymerase

T139 Uncommon tumor type associated with ALV-A infection in fancy breed chickens. S. M. Williams*, G. Zavala, and T. Barbosa, *Univesity of Georgia*, *Athens*.

Multiple sections of formalin fixed feathered skin, bones and heads were submitted to the Poultry Diagnostic and Research center for microscopic examination. Samples were taken from adult fancy breed chickens with a history of tumor formation over several months. Both roosters and hens were affected and submitted. Microscopically the tumors were consistent with sarcomas, both undifferentiated and myxomatous types. PCR on the fixed tissue determined that exogenous ALV was present and sequencing of the complete gp85 was consistent with subgroup A.

Key Words: chickens, ALV-A, myxosarcomas, sequencing, gp85

T140 Testing of a new disinfectant process for poultry viruses. L. A. Gay*[SC] and E. S. Mundt, *University of Georgia*, *Athens*.

The objective of this study was to investigate the application of a new chemical compound as a disinfectant against an enveloped as well as a non-enveloped virus. As model viruses a low pathogenic avian influenza virus (lpAIV, H5N2) and a vaccine strain of infectious bursal disease virus (IBDV, D78) were used. Unlike many disinfectants currently in use, the biocide used in this process forms a gas, and therefore has a strong penetrating capability in sealed poultry houses. After treatment, once properly ventilated, the poultry houses are safe for subsequent use without the risk of further infection or contamination. Rendering the poultry litter free of virus and pathogens would allow the reuse of some or all of the litter safely. This would reduce the cost of poultry production, as litter has been increasing in cost rapidly. The results show that lpAIV in contaminated chicken litter is inactivated in less than one hour when the disinfectant is used at the recommended dose. This result was also obtained at one third the recommended dose. The impact of the disinfectant on chicken litter contaminated with IBDV was comparable. IBDV was inactivated in less than one hour with full and half doses. These results indicate that routine treatment of the facilities and litter at Broiler, Egg and Turkey production companies would greatly control or eliminate the risk of transmission of virus between flocks which are raised on used litter. Use of this disinfectant after a disease outbreak to disinfect litter seems feasible.

T141 Analysis of the host immune response during *Mycoplasma gallisepticum* vaccination or infection. S. A. Leigh*, S. D. Collier, and S. L. Branton, *USDA-ARS Poultry Research Unit*, *Mississippi State, MS*.

Infection of layer chickens with *Mycoplasma gallisepticum* (MG) can result in decreased egg production compared to uninfected controls. Although multiple live MG vaccines are available, little is known about their induced immune response and how they protect the host from subsequent infection. The immune response from chickens vaccinated with either FVAX-MG® or Mycovac-L®, chickens infected with MG strain R-low, and control (unvaccinated/uninfected) chickens was compared. Results were obtained from FACS analysis of peripheral blood lymphocytes stained with fluorescently labeled antibodies against chicken CD8, CD4, and Bu-1. Based on preliminary studies, no major differences between chickens treated with MG verses control birds were observed. The results do suggest an increase in CD4 and Bu-1 expressing lymphocytes over the course of the experiment and a decrease in CD8 expressing lymphocytes, suggesting the importance of humoral immunity in response to MG infection. These results are consistent with the results of other researchers who noted the infiltration of B-lymphocytes into the tracheal mucosa along with a decrease in the detection of MG DNA. These results suggest that an antibody mediated immune response is important in controlling MG infection. Current results are ambiguous regarding the response of CD8 lymphocytes to MG infection.

Key Words: *Mycoplasma gallisepticum*, immune response, CD4, Bu-1

T142 Effect of dosage and vaccination route on transmission of a live attenuated *Mycoplasma gallisepticum* vaccine: A broiler model. J. D. Evans*, S. L. Branton, and S. A. Leigh, *USDA-ARS Poultry Research Unit*, *Mississippi State, MS*.

Mycoplasma gallisepticum (MG) is an economically significant pathogen of poultry species and among the table egg sector of the poultry

industry, live attenuated strains of MG are commonly utilized to limit production losses associated with MG-induced disease. The vaccine, however, may be problematic to broiler- and turkey-related industries due to associated virulence and therefore, understanding the transmissibility of the live MG vaccines is of particular importance. In the current study, a broiler model addresses the effect of vaccine application route and dosage on the transmission of the MG vaccine, FVAX-MG® to commingled unvaccinated subjects for 7 wks post-vaccination. Vaccinations occurred via eye-drop or spray application at 1X, 10^{-3}X, or 10^{-6}X of the manufacturer's recommended dosage and transmission to unvaccinated subjects was measured. Serological response and presence of MG DNA indicate FVAX-MG® transmission only within the 1X FVAX-MG® eye-drop treatment and among no other treatment was transmission of FVAX-MG® detected. The results of the current study demonstrate that the dosage and vaccination route have direct implications on subsequent transmission of FVAX-MG®.

Key Words: *Mycoplasma gallisepticum*, mycoplasmosis, transmission, attenuated vaccine

T143 Discerning genetic differences in *Salmonella* Enteritidis isolates by RAPD, a powerful molecular tool for understanding *Salmonella* epidemiology in poultry integrators. D. L. Mathis*[SC], M. D. Lee, R. D. Berghaus, and J. J. Maurer, *University of Georgia*, *Athens*.

Salmonella continues to cause significant cases of foodborne illnesses in the United States. With poultry recognized as an important vehicle in past outbreaks, pressure has been placed on the industry to reduce poultry and egg contamination with *Salmonella*. To effectively reduce or eliminate *Salmonella* from poultry operations will require identifying its source(s), and while serotyping has been helpful, it has been sufficient in identifying *Salmonella* to strain level. Pulsed-field gel electrophoresis (PFGE) has been a useful tool in typing most *S. enterica* serovars. However, *S.* Enteritidis (SE) is refractory to typing by PFGE, due to its clonal nature. We have been able to identify genetic differences in SE isolates using random amplified polymorphic DNA (RAPD) PCR. While we were able to discern some differences in SE isolates by RAPD, no single typing, PCR primer was sufficient to type SE by this method. However, when we collate the different RAPD DNA patterns for each typing primer into a single phylogenic tree, we could identify sufficient genetic differences to discriminate SE isolates. From our analyses, we are able to identify geographical differences in the distribution of SE types, and discern source(s) of SE within a poultry integrator. This molecular approach to typing SE will prove to be a powerful epidemiological tool in determining the source of SE in poultry and layer operations.

Key Words: *Salmonella*, *Salmonella* Enteritidis, RAPD PCR, biosecurity, genetic differences