

Poster Session: Pathology

401P False negative results using serology to diagnose *Mycoplasma gallisepticum* in turkeys. J. P. Vaillancourt*¹, G. Hebert¹, D. Ojkić², and D. Ley³, ¹University of Montreal, St-Hyacinthe, PQ, Canada, ²University of Guelph, Guelph, ON, Canada, ³North Carolina State University, Raleigh.

A multi-age turkey breeder farm experienced elevated mortality in a flock in July 2008. Coughing and sinusitis were only observed in early August. The flock was slaughtered two weeks later. The diagnosis was airsacculitis caused by *Mycoplasma gallisepticum* (MG). A polymerase chain reaction (PCR) test was used for diagnosis.

In early September, a flock closest to the MG positive flock culled in August showed clinical signs and tested PCR positive for MG. Days later, two additional flocks were coughing. The three flocks were tested (15 sera) with RPA and PCR. All RPA tests were negative, but PCR results were positive. A second testing one month later with RPA remained negative. The same sera were then tested by hemagglutination inhibition (HI). Three were suspicious, but all were considered negative. Further testing with three different ELISA tests resulted in 3 positives out of 15 for one ELISA, and one positive out of 15 for each of the other two tests.

Several factors may explain the observed results. Antigenic variations: the field strain being much different from the ones used to develop the diagnostic tests. Some strains of MG are known to trigger a very limited immunogenic reaction, and turkeys have been reported to respond less than chickens. An investigation in North Carolina in 1999 showed that clinical signs and PCR positivity are detected before any seroconversion. The outbreak reported here shows that, although RPA is generally very sensitive, it is possible for this test to miss a case (false negative). The limited number of positive ELISAs also suggests that testing a low number of sera may lead to a false negative result. MG diagnosis in turkeys must emphasize close scrutiny of clinical signs and PCR in addition to serology. Low sampling (< 10 samples per barn) may substantially reduce the ability to detect an infected flock (i.e., sampling 8 birds in a flock using a sensitive test would likely fail to identify an infected flock if less than 25% of the birds have seroconverted). It is therefore highly advisable to not rely completely on only one test when trying to diagnose MG.

Key Words: mycoplasma, gallisepticum, serology, diagnosis, turkeys

402P Assessment of APEC virulence gene profiles and yeast diversity in turkey crops. F. Lago, D. Karunakaran, T. G. Rehberger, and G. R. Siragusa*, *Agtech/Danisco, Waukesha, WI.*

Turkey crop microbiota is highly diverse and normally composed of a stable and dynamic population. Avian pathogenic *E. coli* (APEC) and yeast are part the normal or healthy crop microbiota. Both organisms cause disease in the turkey that is transparent to the grower until clinical signs appear. APEC generated colibacillosis has been related to some specific virulence markers genes including *iss*, *iucC*, *tsh*, *cvaC* and *irp2*. *C. albicans* has been linked to crop mycosis. However, genotypic diversity of the two organisms has not been fully investigated in crop. Herein, crops from turkeys (n=37) were harvested and analyzed for level of total *E. coli* and yeast. *E. coli* were profiled for specific virulence gene content. *Candida yeast* species was determined using differential agar culture and 18S-rRNA analysis. APEC counts averaged 6.3×10^5 cfu/g. *E. coli* were tested (n=108) by multiplex PCR for the presence of *iss*, *iucC*, *tsh*, *cvaC* and *irp2*. From the total, 55 isolates (50.9%) had

two or more virulence genes. The most dominant gene was *tsh*, 87% followed by *cvaC* 43.5%, *irp2* 28.7%, *iss* 19.5%. The least dominant gene was *iucC* 9.2%. Yeast counts averaged 1.7×10^4 cfu/g. and were composed of 45% *C. catenulata*, 30% of *C. rhodotorula*, 20% *C. albicans* 2% *Saccharomyces cerevisiae*, and 3% of unknown. A significant correlation between APEC level and yeast level was observed ($r^2=0.06$ $p<0.05$) suggesting a coexistence between APEC and yeast in both the healthy and mycotic crop. This study demonstrated a significant level of APEC and potentially pathogenic yeast in the crop suggesting targeting the crop as a site for potential biocontrol.

Key Words: turkeys, crop mycosis, avian pathogenic *E. coli*, *Candida albicans*, yeast infection

403P A PCR-based survey of the prevalence of the *netB* gene in *Clostridium perfringens* isolated from broiler chickens diagnosed with necrotic enteritis. M. C. Hibberd, T. G. Rehberger, and G. R. Siragusa*, *Agtech/Danisco, Waukesha, WI.*

Necrotic Enteritis (NE) is a significant economic burden to the poultry industry, in terms of antibiotic costs and losses due to acute mortality. *Clostridium perfringens* Type A is the primary etiologic agent of NE; recently, research regarding the NetB toxin of this organism has challenged the implication that the *C. perfringens* α -toxin (Phospholipase C) is the main virulence factor in NE disease. A PCR assay for the *netB* gene was designed and integrated into a multiplex PCR procedure for detecting *C. perfringens* Type A and was used to assess the prevalence of *netB* in *C. perfringens* Type A isolated from broiler gastrointestinal tracts and the correlation of *netB* to the diagnosis of acute NE. The assay was validated by comparing known *netB* positive and *netB* negative *C. perfringens* and proper negative controls, and reliably and specifically detected the *netB* gene and the *C. perfringens* α -toxin (*plc*) simultaneously. To date, the survey includes 773 total isolates collected from the Upper Midwest, Upper East Coast, and Southern regions of the USA: 667 *C. perfringens* Type A collected from non-NE birds and 106 *C. perfringens* Type A collected from birds presenting symptoms of NE. Of the *C. perfringens* Type A isolated from birds with no NE symptoms, 389 of 667 (58.3% of total) were *netB* positive. *C. perfringens* Type A obtained by sampling birds from reported NE breaks and/or birds presenting characteristic symptoms possessed the *netB* toxin in 95 of 106 isolates (89.6% of total). Statistical correlation between the presence of the *netB* gene and the presence of observed NE disease was significant ($P<0.0001$, Fisher's Exact Test). Our results support the hypothesis of a primary role for *netB* in the etiology of NE in broiler chickens, and warrants increased attention to the NetB toxin as a specific target for studying, treating, or preventing the disease.

Key Words: *Clostridium perfringens*, necrotic enteritis, *netB*, broiler, NE

404P Nicarbazine anticoccidial dose response. G. F. Mathis*, *Southern Poultry Research, Inc., Athens, GA.*

A series of anticoccidial sensitivity tests were designed to determine an anticoccidial dose response to Nicarbazine. The treatments were Nonmedicated, un-infected (NMU) and infected (NMI), Nicarbazine 36 (N36), 72 (N72), 99 (N99) and 125 (N125) ppm. Five mixed species field isolates (all contained highly pathogenic levels of *E. acervulina* (EA), *E. maxima* (EM), and *E. tenella* (ET) were examined. Birds were

inoculated at 14 days of age and weighed and lesion scored 6 days post inoculation. Across all isolates there was a linear improvement in performance and decrease in coccidiosis lesions. Within all categories there were not significant differences between the NMI and N125 except for *E. acervulina* lesion scores (NMI 0.0 and N125 0.4). All other treatments and categories were significantly different. Feed conversions were NMU 1.447, NMI 2.594, N36 1.956, N72 1.886, N99 1.667, and N125 1.458. Average percent weight reductions compared to NMU were NMI 58.7, N36 40.8, N72 13.3, N99 6.4, and N125 1.7. Average lesion scores (Avg. of EA+ EM+ ET) were NMU 0.0, NMI 2.9, N36 1.8, N72 0.8, N99 0.3, and N125 0.1. Nicarbazin is often combined with other anticoccidial drugs typically at 36 ppm level. This information can be used to determine coccidiosis control at each level in combination or as a standalone anticoccidial drug.

Key Words: nicarbazin, anticoccidial, coccidiosis, eimeria, lesion

405P Comparison study of an impedance biosensor and rRT-PCR for detection of avian influenza H5N2 from infected chickens.

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The ongoing H5N1 highly pathogenic avian influenza (HPAI) outbreaks, originating from Southeast Asian countries, and spreading to Middle

Eastern, European and African countries, have driven global attentions and battles in fighting with deadly H5N1 virus to domestic poultry and also humans. Early detection or rapid diagnosis and effective control measures are needed to control the HPAI outbreaks and prevent its spread to other areas. This study's objective was to compare a newly developed impedance biosensor with real time RT-PCR (rRT-PCR) for detection of avian influenza virus (AIV) from tracheal and cloacal swab samples collected from experimentally H5N2 AIV infected specific-pathogen-free (SPF) chickens. After inoculation of 11-wk-old SPF chickens with H5N2 AIV, tracheal and cloacal swabs were collected daily for 7 days. rRT-PCR was performed on tracheal swab samples per NVSL protocol. The impedance biosensor was based on the use of a magnetic separator, magnetic nanobeads coated with AIV group and subtype specific monoclonal antibodies, and interdigitated microelectrodes in a polydimethylsiloxane (PDMS) microfluidic channel. After mixing the coated nanobeads with the swab sample, the target virus was captured, separated, and concentrated by the magnetic separator. Sample impedance was then measured using the interdigitated microelectrode. The presence of the virus caused a change in the impedance, which is linearly correlated to virus concentration. Of the 15 positive samples tested using the impedance biosensor and rRT-PCR, 14 were tested positive by both assays, for only 1 sample the biosensor was not in agreement with the rRT-PCR results (false negative). Detection time for the impedance biosensor is less than 1 hour compared to roughly 3-4 hours for rRT-PCR. This study indicates that the biosensor was comparable to rRT-PCR in detection of live AIV virus in poultry swab samples. Also, the impedance biosensor is portable and has great potential for in-field use, which would dramatically reduce the turnaround time for AIV detection.

Key Words: avian influenza, impedance biosensor, rapid screening, rRT-PCR, infected chickens

Poster Session: Physiology, Endocrinology, and Reproduction

406P Retinoic acid-induced meiosis of chicken primordial germ cells (PGCs). J. N. Petite*¹, C. Ge², J. Angerman-Stewart¹, and C. Zhang², ¹North Carolina State University, Raleigh, ²Zhejiang University, Hangzhou, China.

In mammals, germ cells enter into meiosis and arrest at prophase I in the embryonic ovary, whereas the drop of retinoic acid (RA) by Cyp26b1 in the embryonic testis protects male germ cells from initiating meiosis until sexual maturity. Organ culture of embryonic gonads and the long-term culture of chicken PGCs was used to investigate if RA plays a conserved role in regulating entry into meiosis in the chicken and if RA acts directly or indirectly on PGCs. Using organ culture, the addition of RA to stage 27 chicken gonads of both sexes significantly increased the mRNA expression of the premeiotic Stra8, as well as the meiotic markers Sycp3 and Dmc1. Using long-term cultured chicken PGCs, RA dramatically upregulated Stra8 expression in both male and female PGCs with similar increases in the expression of Dmc1 and Sycp3. Flow cytometry analysis for DNA content showed that after 4 days of RA treatment, 29.5% male PGCs and 58.37% female PGCs were at sub G1 phase, indicating that the cells had entered meiosis. Analysis of giemsa stained cells suggested a small difference between female and male PGCs in their capability to enter into meiosis where male PGCs appeared to have a lower capability to progress beyond zygotene/pachytene stages. This study shows that RA could induce chicken PGCs

of both sexes to enter meiosis and that RA acts directly on the PGCs of both sexes to enter meiosis.

Key Words: meiosis, germ cells, chicken, retinoic acid

407P Detection of SED1 and GalTase-I in rooster gonadal tissue: an investigation of two key molecules involved in sperm-egg fusion. A. P. Benson*¹, A. J. Davis², and B. D. Shur³, ¹Georgia Gwinnett College, Lawrenceville, ²University of Georgia, Athens, ³Emory University, Atlanta, GA.

Fertilization in eutherian mammals is dependent upon the binding of spermatazoa to the egg coat, or zona pellucida, prior to penetration of the ovum. Two key molecules, Beta 1,4-Galactosyltransferase-I (GalTase-I) and SED1 (MFG-E8 or lactadherin), have been determined to play significant roles in facilitating this binding and are thus critical in mediating sperm-egg interactions. Specifically, in mice, SED1 is involved in the initial binding of spermatazoa to the zona pellucida while the receptor protein GalTase-I promotes the acrosome reaction upon binding of the oocyte's zona pellucida protein, ZP3. Due to the paucity of information that exists regarding the function of SED1 and GalTase-I in avian sperm-egg interactions, we investigated the presence of these molecules in the testes of the domestic rooster, *Gallus gallus*.