

159 Analysis of mucosa-associated and luminal bacteria in chicken intestine following feeding dietary additives. Z. Uni*, A. Smirnov, and R. Perez, *Hebrew University, Rehovot, Israel.*

A diverse bacterial community exist throughout the chicken small intestine and have an important metabolic and protective function in the gastrointestinal tract. Most of the knowledge about bacterial diversity in the chicken intestine has been obtained by analysis of the luminal content, either by culture methods or by ribosomal DNA and PCR based molecular techniques. However, the mucosa-associated bacteria (MAB) was not considered although its presence and composition might be critical in determining epithelial health through their ability to exclude pathogens.

In this study, a PCR-based method was used for quantifying the selective bacterial species in MAB fraction and in the luminal content. For the experiment Cobb hatchlings were fed commercial diet containing either probiotic (Pro) supplement (PrimaLac 2 g/kg) or antibiotic growth promoter (AGP) (Virginiamycin, 2 mg/kg). Control group was fed diet with no additives. Jejunum was taken at 7 and 28 days and the intestinal content was collected for analysis of luminal bacteria. MAB was removed from the everted intestine by incubation in citrate buffer containing 0.5 mM DTT. Bacterial DNA was isolated and relative amounts of the bacteria species was analyzed in luminal and MAB samples by PCR amplification of the 16S ribosomal DNA targets. Universal primers were used to determine total bacterial DNA and specific primers were directed to the *Lactobacillus* and *Bifidobacterium* species. Results showed that feeding dietary probiotic led to 32% increase in relative amount of mucosa-associated *Lactobacillus* at day 7 in comparison to the control group ($P < 0.05$). Mucosa-associated *Bifidobacterium* was undetectable at day 7, whereas at day 28 only Pro and control groups showed *Bifidobacterium* presence. The relative amount of the *Lactobacillus* species in luminal content was unaffected by the treatments while *Bifidobacterium* was detected only in luminal content of Pro and Control groups.

The later demonstrated the importance of analysis both luminal and mucosa-associated bacteria.

Key Words: Intestine, Bacteria, Feed additives

160 Maternal dietary fatty acids alter the retention of long chain n-3 fatty acids in the cardiac and hepatic tissue of growing chickens. G. Cherian* and M. P. Goeger, *Oregon State University, Corvallis.*

The effect that egg yolk or maternal n-6 and n-3 fatty acids have on cardiac and hepatic tissue long chain (>20C) n-6 and n-3 polyunsaturated fatty acids (PUFA) of chicks during growth was investigated. Long chain PUFA of n-6 and n-3 series are the precursors of eicosanoids that are lipid mediators of inflammation. Eicosanoids derived from n-3 PUFA are less proinflammatory than those derived from n-6 PUFA. Fertile eggs with diverse n-6 and n-3 PUFA were obtained by feeding New Hampshire hens a corn-soybean meal-based diet containing 3.0% sunflower oil (Low n-3), 1.5% sunflower oil and 1.5% fish oil (Medium n-3), or 3.0% fish oil (High n-3). The hatched chicks were fed a commercial diet containing 3.4% alpha-linolenic acid (LNA 18:3 n-3), but was devoid of long chain n-6 or n-3 PUFA. The fatty acid composition of cardiac and hepatic tissues at days 7, 14 and 21 of chick growth were determined. At days 7, 14 and 21 the cardiac and hepatic tissue of chicks hatched from hens fed High n-3 diet retained higher levels of docosahexaenoic acid (DHA, 22:6 n-3) and eicosapentaenoic acid (EPA, 20:5 n-3) than Low n-3 diets ($P < 0.05$). With time, the levels of EPA and DHA declined significantly in all treatments and, after day 14, dietary LNA did not sustain the content of long chain n-3 PUFA in chick heart and liver ($P < 0.05$). No difference due to maternal diet on cardiac and hepatic arachidonic acid (20:4n-6) was observed after day 7 of growth ($P > 0.05$). No effect of maternal diet on chick cardiac tissue saturated or monounsaturated fatty acids was observed ($P > 0.05$). However, monounsaturated fatty acids were higher at day 7 in chicks from hens fed High n-3 diets. The alteration in n-3 PUFA in cardiac and hepatic tissue may affect the synthesis of eicosanoids derived from n-3 PUFA and may lead to increased health to chickens.

Key Words: n-3 Polyunsaturated fatty acid, Chicken, Heart

Pathology: Pathology I

161 Ionophore toxicosis in White Leghorn pullets. R. Porter*^{1,2}, D. Zoromski¹, and J. Brown³, ¹*Wisconsin Veterinary Diagnostic Laboratory, Madison, Wisconsin*, ²*University of Wisconsin School of Veterinary Medicine, Department of Pathobiological Sciences, Madison*, ³*Centurion Poultry, Inc., Lexington, Georgia.*

A flock of 213,000 white leghorn pullets were raised in three-tier, drop-through cages with nipple drinkers. Livability was normal until the birds were diagnosed with coccidiosis at four weeks of age. At five weeks, shortly after delivery of feed containing an ionophore, many of the birds were depressed, laterally recumbent and unable to walk when manually positioned upright. Many of the affected pullets were thought to be dead, lying motionless in the cage and flattened laterally by the weight of other cagemates, but when examined were actually alive. Recumbent pullets, when removed from cages and separated from other birds, were often alert and walking in several hours or less. At this time a preliminary diagnosis of ionophore toxicosis was made and 20 tons of replacement feed were delivered to the farm, but mortality continued for four days. Necropsy of affected birds revealed bruising on the ventral aspect of the wings and mild subcutaneous edema over the thigh and tibiotarsus. There was multifocal intramuscular hemorrhage of varying degrees in the caudal aspect of the thigh, digital flexor muscles of the tibiotarsus, and lumbosacral muscle (gluteal muscles). Histologic examination of affected muscles revealed attenuation, hyalinization and myocytolysis of muscle fibers with loss of cross-striations, varying infiltrates of mononuclear cells, mostly macrophages, and proliferation of satellite and fibroblast nuclei. Total mortality directly resulting from ionophore toxicosis was 3.9% and an additional 2.2% of the flock was culled over the next thirteen weeks for lack of uniformity and poor growth. A pre-mix error resulted

in pullets consuming ionophore at five times the recommended concentration of 110 grams/ton. When replacement feed was delivered to the farm, the new feed was not mixed with the remaining 10,000 pounds of toxic feed, and pullets continued to consume toxic levels of ionophore for four days.

Key Words: Ionophore, Toxicosis, Pullets

162 Myopathy in turkeys: Are the mechanisms similar to those in broilers and pigs? Effects of halothane. M. Mitchell*¹, L. Gatcliffe², and D. Sandercock¹, ¹*Roslin Institute, Roslin, Midlothian, United Kingdom*, ²*BUT, Broughton, Chester, United Kingdom.*

Previous studies in this laboratory described the induction of myopathy in rapidly growing modern broiler chickens by exposure to halothane anaesthesia. It was proposed that the mechanism of this pathology might involve elevation of intracellular calcium through activation of the intracellular sarcoplasmic reticulum (SR) calcium channel or ryanodine receptor (Ryr). Further studies demonstrated that acute heat stress induced myopathy in broiler chickens was ameliorated by treatment with the specific Ryr antagonist dantrolene sodium. These findings further implicated the SR calcium channel in the etiology in spontaneous and stress-induced myopathy in broiler birds. A defective halothane sensitive Ryr in pigs and humans is responsible for malignant hyperthermia syndrome and porcine stress syndrome. Other workers have attempted to establish a link between halothane sensitivity, myopathy and meat quality problems (e. g. a PSE like condition) in both broilers and turkeys but the findings remain equivocal. The present study therefore examined the effects of halothane upon muscle

membrane integrity (plasma creatine kinase or CK activity) in a commercial turkey line and related these measures to other physiological responses to the anaesthesia. Groups of 8 turkeys (17kg body weight) were allocated to control, halothane anaesthesia or anaesthesia plus dantrolene treatments. Anaesthesia was of 15 minutes duration. Blood samples and body temperatures were obtained immediately before and after anaesthesia and after 4 hours recovery. Samples were analysed for pH, pCO₂, sodium, potassium and CK. There was no evidence of malignant hyperthermia or acid base disturbance in these birds or of a halothane-induced myopathy. Plasma potassium was elevated by anaesthesia an effect apparently reversed by dantrolene. The findings differ markedly from those in broilers and do not support the hypothesis that the similarities in muscle pathology and meat quality problems in pigs, broilers and turkeys are attributable to the same mechanisms.

Key Words: Turkeys, Myopathy, Halothane

163 Bacteriology of ascites in broilers. Y. Vizzier Thaxton*, M. Putsakum, and S. Anderson, *Mississippi State University, Mississippi State.*

Previous work in our laboratory demonstrated the ability of *E. faecalis* to produce pulmonary hypertension syndrome in broilers. No work was done to determine whether or not the organism was present in ascitic fluid subsequent to the disease. Other researchers have produced ascitic fluid in birds with a variety of methods of infectivity as well as bacterial toxin. However, none of these examined the fluid itself. As a result, we decided to determine whether or not ascitic fluid contained bacterial cells. Using broilers with naturally occurring ascites, samples of the fluid were aseptically removed from the pericardial sac. Smears were made and stained with Gram stain and Wright's stain. Additionally, the fluid was plated on tryptic soy agar plates and incubated for 48 hours at 37C. Representative colonies were picked from the plates for identification. Bacteria were present in the fluid as were blood cells. The majority of the birds had fluid containing members of the facultatively anaerobic genus *Staphylococcus*.

Key Words: Ascites, Bacteria, Ascitic fluid

164 Dynamics of duodenal and ileal microbial ecology in chickens vaccinated and challenged with mixed *Eimeria* spp. E. O. Oviedo-Rondón^{1,2}, M. E. Hume^{*3}, and S. Clemente-Hernández^{1,4}, ¹Stephen F. Austin State University, Nacogdoches, Texas, ²North Carolina State University, Raleigh, ³USDA, ARS, Southern Plains Agricultural Research Center, College Station, Texas, ⁴Universidad Autónoma de Chihuahua, Chihuahua, Chihuahua, México.

Intestinal microflora ecology plays an important role in responses against coccidia infection. This research determined the dynamics of duodenal (D) and ileal (I) microbial communities (MC) of broilers vaccinated with viable attenuated oocysts (Advent[®]), challenged with field mixed *Eimeria* spp., and fed diets supplemented with specific essential oil (EO) blends (Crina POULTRY[®] (CP) or Crina ALTERNATE[®](CA)). Five treatments were compared: Two controls, Uninfected-Unmedicated (UU), Unmedicated-Infected (UI), and three vaccinated treatments. Coccidi-vaccinated treatments included one group without feed additives (no-FA), and two with either of the EO blends at 100 ppm. Cobb-500 male chickens were raised up to 13 d of age on used litter top-dressed and latter moved to battery cages. Challenge with *E. acervulina*, *E. maxima*, and *E. tenella* was done when broilers were 19 d old. Digesta samples were taken from 12 birds per treatment just before and 7 d after the challenge, pooled in 6 samples, and frozen. DNA was isolated, and denaturing gradient gel electrophoresis was used to examine PCR-amplified fragments of a 16S ribosomal DNA variable region from bacteria. Data is presented as % similarity coefficients (SC). Dendrograms of amplicon patterns indicated that vaccination with attenuated oocysts of *Eimeria* spp. had a small effect on MC in the D and I compared with UU (86.5% - 94.5% SC, respectively). EO blends CP and CA do affect (65.3 to 86.9% SC) MC of vaccinated birds in both gut sections. Both EO had similar effects in the I (93.3% SC), but were different in the D (65.3%). Coccidia challenge changed MC in D (36.7%), and I (55.4%). After the challenge, coccidi-vaccinated broilers fed CP had similar D- MC (89.8%) to chickens fed no-FA diets, and all vaccinated treatments had similar MC to UU (80.9%), but different from UI (48.4%). Treatments fed EO had I- MC similar (89.8-83.2%) to UU, but different (66.5%) from the vaccinated no-FA and UI groups. We concluded that coccidi-vaccination causes small changes in D and I MC. EO supple-

mentation to cocci-vaccinated birds modulates MC avoiding drastic shifts after a mixed challenge.

Key Words: Coccidia vaccine, Microbial ecology, DGGE

165 Prophylactic effects of orally administered CpG-ODNs during clinical challenge with *Eimeria acervulina* and *E. tenella*. K. Ameiss^{*1}, J. El-Attrache¹, H. Danforth², A. McElroy³, and D. Caldwell¹, ¹Texas A&M University, College Station, ²USDA/ARS, Beltsville, Maryland, ³Virginia Polytechnic Institute and State University, Blacksburg.

Recent studies in chickens using synthetic oligodeoxynucleotides containing CpG-motifs(CpG-ODNs) have demonstrated that CpG-ODNs can improve the immune response to protein antigens and reduce pathogenicity of bacterial pathogens and protozoan parasites. The goal of this study was to determine whether a single oral administration of CpG-ODNs could provide protection from a clinical challenge dose of either *Eimeria acervulina* or *E. tenella*. In the first model, co-administration, chicks were given 50 µg of CpG-ODN or a control non-CpG ODN simultaneously with a clinical challenge dose of 1.25 X 10⁵ oocysts of *E. acervulina* or 1.0 X 10⁵ oocysts of *E. tenella*. In the second model, pre-administration, chicks were administered 50 µg of CpG-ODN or the control non-CpG-ODN one day before clinical challenge with 1.25 X 10⁵ oocysts of *E. acervulina* or 1.0 X 10⁵ oocysts of *E. tenella*. Body weights were taken for each bird on days 15 and 21 post-hatch. Individual weight gains were calculated and lesions were scored on day 25. Mean weight gain and lesion scores among the challenged groups in the co-administration model for both *E. acervulina* and *E. tenella* revealed no differences among the challenged groups. Mean weight gain in the pre-administration model for both species also revealed no differences among challenged groups. No differences in mean lesion score were observed among the *E. acervulina* challenged birds either. However, over two replicates, significantly lower (P<0.001) mean lesion scores were observed in *E. tenella* challenged birds pre-administered CpG-ODNs (1.5 ± 0.2 and 1.1 ± 0.2) as compared to those pre-administered non-CpG-ODNs (2.3 ± 0.2 and 1.9 ± 0.2) or those given the challenge only (2.1 ± 0.2 and 2.3 ± 0.2). These findings indicate that a single oral administration of CpG-ODNs given one day before clinical challenge can decrease lesions associated with *E. tenella*.

Key Words: Eimeria, CpG-ODNs, Oral

166 Effects of F-strain *Mycoplasma gallisepticum* inoculation at twelve or twenty two weeks of age and diet supplementation on blood characteristics of commercial layers. S. Park¹, E. Peebles^{*1}, S. Branton², M. Kidd¹, S. Whitmarsh¹, and P. Gerard¹, ¹Mississippi State University, Mississippi State, ²USDA-ARS, Poultry Research Unit, Mississippi State, Mississippi.

The effects of 12 or 22 wk F-strain *Mycoplasma gallisepticum* (FMG) inoculations and various dietary supplementation regimens on blood characteristics in Hy-Line W36 hens between 24 and 58 wk were investigated. At 12 and 22 wk of age, 120 sham- (control) and 120 FMG- (treated) inoculated birds were randomly assigned to individual cages in one of two enclosed and isolated ends of a caged layer facility according to inoculation treatment. One of four isocaloric diets was randomly provided to birds in each end at 20 wk of age. Diets included: 1) normal basal diet (NB); 2) NB with 1.25 % additional poultry fat (PF); 3) NB with 2.00 % additional PF; and 4) NB with 2.00 % additional PF and supplemental phytase (600 FTU/kg) and 25-hydroxyvitamin D₃ (0.025 %). Inoculation type, inoculation time, and diet treatment combinations were thrice replicated, with ten hens in each replicate group. Parameters investigated were plasma total protein, serum cholesterol, serum triglycerides (TRIG), serum calcium (CA), and hematocrit. Only age effects were noted for serum cholesterol and hematocrit. Plasma total protein was higher in FMG-inoculated birds compared to sham-inoculated controls. In FMG-treated birds, TRIG was lower at 44 wk of age in birds fed the 2.00 % PF, phytase, and vitamin D₃ combination diet compared to birds fed one of the other 3 diets. Furthermore, the 1.25 % PF diet had variable influences on CA in sham-inoculated control birds, whereas diet had no influence on CA in FMG-inoculated birds. Added dietary phytase and vitamin D₃ may suppress TRIG levels in layers inoculated with FMG at 12 or 22 wk of age.

Key Words: Blood, Layers, *Mycoplasma gallisepticum*

167 Effects of F-strain *Mycoplasma gallisepticum* inoculation at twelve or twenty two weeks of age and diet supplementation on egg yolk characteristics of commercial layers. S. Park¹, E. Peebles^{*1}, S. Branton², M. Kidd¹, S. Whitmarsh¹, and P. Gerard¹, ¹Mississippi State University, Mississippi State, ²USDA, ARS, Poultry Research Unit, Mississippi State, Mississippi.

The effects of 12 or 22 wk F-strain *Mycoplasma gallisepticum* (FMG) inoculations and various dietary supplementation regimens on egg yolk characteristics in Hy-Line W36 hens between 24 and 58 wk were investigated. At 12 and 22 wk of age, 120 sham (control) and 120 FMG- (treated) inoculated birds were randomly assigned to individual cages in one of two enclosed and isolated ends of a caged layer facility according to inoculation treatment. One of four isocaloric diets was randomly provided to birds in each end at 20 wk of age. Diets included: 1) normal basal diet (NB); 2) NB with 1.25 % additional poultry fat (PF); 3) NB with 2.00 % additional PF; and 4) NB with 2.00 % additional PF and supplemental phytase (600 FTU/kg) and 25-hydroxyvitamin D₃ (0.025 %). Inoculation type, inoculation time, and diet treatment combinations were thrice replicated, with ten hens in each replicate group. Determinations were made on wk 24, 34, 44, 50, and 58 for yolk lipid (YL) and yolk moisture (YM), on wk 24 and 50 for yolk cholesterol, and on wk 58 for yolk fatty acids. Only an age effect was noted for yolk cholesterol. YL was higher from birds fed the phytase, vitamin D₃, and 2.00 % PF combination diet compared to those fed the control diet at 24, 34, and 50 wk. Also, in birds sham-inoculated at 12 wk, YL was higher from birds fed the combination diet compared to the control diet. Compared to sham-inoculated controls, YM was higher at 44 wk but was lower at 58 wk in FMG-inoculated birds. Yolk palmitic acid was decreased and yolk linoleic acid was increased from birds fed the 2.00 % added PF diet compared to the control diet. Yolk characteristics were altered by diet but not by FMG-inoculation and its timing. Furthermore, supplemental phytase and vitamin D₃ may elevate yolk lipid content.

Key Words: Egg yolk, Layers, *Mycoplasma gallisepticum*

168 Molecular dating of the epidemiological events in relation to the emergence of very virulent infectious Bursal Disease Virus. C. -C. Hon^{*1}, Y. -F. Lee², W. -K. Ching², K. -P. Ng², T. -W. Ng², and F. C. Leung¹, ¹The University of Hong Kong, Hong Kong, HK-SAR, China, ²The University of Hong Kong, Hong Kong, HK-SAR, China.

The Very Virulent (vv) strains of Infectious Bursal Disease Virus (IBDV) were isolated first in Europe during the late 80s and soon after causing worldwide pandemic outbreaks in the early 90s. The enhanced virulence of vvIBDV was later proposed to be related to the unique mutations on both genome segments A and B. Using sequence data of both genome segments collected locally during 2001-04 and available database sequences with known sampling dates, here we estimate the time of divergence of the most recent common ancestor (TMRCA) of all vvIBDV pandemic strains and its demographic history in a Bayesian framework using Markov Chain Monte Carlo sampling (BMCMC) under both strict and relaxed molecular clock methods. Based on the VP2 sequences, the TMRCA of all vvIBDV strains was estimated to be at around 1965 (1956-1975), more than 20 years before the onset of its worldwide pandemic outbreaks. The VP1 sequence of all vvIBDVs formed a mono-phylogenetic group that excludes all the other serotype I and II IBDVs, indicating that VP1 of vvIBDV may be reassorted from an unidentified reservoir and this reassortment event was estimated to be at around 1977 (1966-1979) coincided with the TMRCA of most of the vvIBDV pandemic strains at 1979 (1975-1983). Moreover, our estimates on the demographic behavior of vvIBDVs showed a transition from a constant population size to rapid exponential population growth at around 1982 (1978-1985) coincided with the early reported outbreaks and its explosive transmission manner in the late 80s. Based on these findings, we conclude that vvVP2 may have been emerged more than 20 years before the observed vvIBDV outbreaks in Europe. The rapidly worldwide spread of vvIBDV in the late 80s is likely to be related to a single epidemiological event, which is proposed to be the reassortment of the vvVP1

Key Words: IBDV, Very virulent, TMRCA

169 Effect of dietary protein level on broiler performance and lesion development during vaccination and following field strain *Eimeria* challenge. J. T. Lee^{*1}, N. Eckert¹, K. Ameiss¹, S. Stevens¹, P. Anderson¹, A. P. McElroy², H. D. Danforth³, and D. J. Caldwell¹, ¹Texas A&M University, College Station, ²Virginia Tech, Blacksburg, ³USDA-ARS, Beltsville, Maryland.

Two floor pen studies were conducted to evaluate dietary protein level on broiler performance and intestinal lesion development following vaccination with Coccivac[®]-B and subsequent clinical *Eimeria* challenge. Experiment 1 measured intestinal lesion scores, body weight gain, and feed conversion of non-vaccinated and vaccinated broilers fed three corn-soy rations varying by dietary protein level (20, 22, and 24%). Three weeks post-placement, broilers were challenged with a three species inoculum of *Eimeria*. Intestinal lesions were significantly higher (P<0.05) in non-vaccinated/challenged broilers compared to vaccinated/challenged broilers. Body weight gains of broilers were significantly higher (P<0.05) and feed conversion ratios lower (P<0.05) during the challenge period in vaccinated/challenged broilers compared to non-vaccinated/challenged broilers. Feed conversion ratios of vaccination/challenged broilers were similar to all non-challenged broilers. Intestinal lesions associated with *E. maxima* in vaccinated/challenged broilers were significantly greater at the 20% protein level compared to the 24% level. Lesions associated with *E. tenella* challenge were significantly higher in the 24% protein level as compared to the 20 and 22% levels. Similar results in growth characteristics were observed in Experiment 2. Intestinal lesions were higher (P<0.05) in non-vaccinated/challenged broilers compared to vaccinated/challenged broilers, with the exception of the mid-intestinal lesions in the 20% vaccinated/challenged broilers. Lesions from *E. maxima* challenge in vaccinated/challenged broilers were again greater (P<0.05) in broilers fed 20% protein as compared to the 22 and 24% protein diets. These data suggest live oocyst vaccination is influenced by dietary protein level and is effective in reducing lesion development, improving weight gain, and reducing feed conversion following *Eimeria* challenge.

Key Words: *Eimeria*, Vaccination, Protein

170 Novel staining method to enhance visualization of the chicken Peyer's patch *in situ*. L. Vaughn^{*}, P. Holt, and R. Moore, USDA, ARS, ESQ, Athens, Georgia.

The chicken ileal Peyer's patches (Pp) are classified as secondary gut-associated lymphoid tissue of the mucosal immune system. Young birds may possess multiple Peyer's patches, yet as the bird ages these Pp sites tend to regress in number. Also, Peyer's patches of aging chickens are often difficult to observe grossly. There is one Pp located anterior to the ileocecal junction which appears to be consistently present in various age groups. This single Pp may serve as an important tissue site for monitoring inflammatory & immunological responses of the host against enteric pathogens. However, a simple technique to enhance visualization of the Pp is lacking. Therefore, we designed a novel staining method that is quick, easy & accurate to aid in gross identification & recovery of the chicken Pp.

Segments of lower GI tract, jejunioleal junction extending distally to colon, were excised from SPF white leghorn spent hens & commercial broilers 4-6 wks of age. GI segments were flushed with phosphate buffered saline (PBS) to remove ingesta & cellular debris. Eosin-Y & a modified-Crystal Violet were then infused into the GI tract segments in a sequential fashion. First, Eosin-Y diluted in PBS was infused into the GI segment via 30cc syringe with 16 gauge, 1 inch needle until the GI segment was fully distended. The Eosin-Y stain was allowed to permeate for 1 minute then gently extruded. Second, modified-Crystal Violet diluted in PBS was infused into the GI segment. Upon addition of Crystal Violet, the lymphoid tissue area became apparent at the GI serosal surface. The distal ileal Peyer's patch appeared as a pale whitish-pink ovoid area with surrounding gut tissue stained light purple. The exact site of the Pp lymphoid tissue could be delineated with the unaided eye from both serosal & mucosal surfaces and then properly excised. Tissue was verified by histology.

The novel Eosin-Y + Crystal Violet staining technique promotes rapid identification of & accurate recovery of chicken Pp lymphoid tissue from fresh tissue specimens. The method renders specific Pp lymphoid tissue suitable for use in various research applications.

Key Words: Peyer's patch, Eosin-Y, Crystal Violet