The US produced 8550 MM chickens and 242 MM turkeys in 2010. This impressive production of poultry was almost entirely done under confinement. This level of production could not be achieved without coccidiosis control. The first anticoccidial added to poultry feed that proved to be effective and was approved by FDA in 1948 was sulfadimidine. This was the start of the chemophylactic era and paved the way for the initial expansion of the industry and the development of a multitude of new anticoccidials. Some are still successfully used today, among them; nicarbazin approved in 1955 and arsanilic acid, approved in 1949 (now replaced by roxarsone). Up to the early 1970s the chemophylactic control of coccidiosis was based on the use of synthetic anticoccidials. Pharmaceutical companies employed scientists responsible for screening a variety of chemical compounds synthesized in their laboratories for their effectiveness as anticoccidials. The ones that were effective and well tolerated by poultry were further developed until an approval for marketing was secured. With a few exceptions, such as nicarbazin, the problem with these “chemicals” was that most of them had very strong anticoccidial activity and this lead to selective pressure upon the Eimeria spp. that in most cases resulted in complete resistance to the drug within a few years. Pharmaceutical companies had to be discovering, testing and approving new anticoccidials every few years. This changed in 1971 with the approval of monensin, the first ionophore. The main difference was that due to its different mode of action a fast development of resistance was not occur. This lead to selective pressure upon the Eimeria spp. that in most cases resulted in complete resistance to the drug within a few years. Pharmaceutical companies had to be discovering, testing and approving new anticoccidials every few years. This changed in 1971 with the approval of monensin, the first ionophore. The main difference was that due to its different mode of action a fast development of resistance did not occur. This lead to the development and introduction of other ionophores; lasalocid (1976), salinomycin (1986), narasin (1988), maduramicin (1989) and semduramicin (1996). Since then, prevention of coccidiosis by in-feed anticoccidials has remained the backbone of control programs. It is remarkable that in spite of over 55 years of use, “chemical” anticoccidials like nicarbazin continue to be effective, and after more than 40 years of the introduction of the first ionophore, these drugs continue to be effective in chickens, and some (monensin and lasalocid) also in turkeys.

Key Words: coccidiosis, anticoccidials

Coccidiosis in poultry is still considered as one of the main diseases affecting performance of poultry reared under intensive production systems. With currently practically available diagnostic methods such as oocyst counts and lesion scoring, an interpretation of the impact of (subclinical) coccidiosis is not easy. Another problem difficult to address to date, is the interpretation of the efficacy of an anticoccidial program. Anticoccidial sensitivity testing of the different anticoccidial drugs available is the only reproducible method available today, but interpretation is far from easy. The result of all this is that coccidiosis is, all over the world, underestimated as a health hazard and thus also remains an important economic threat. Continuous optimization of anticoccidial programmes is therefore thought to be advantageous to the broiler industry. In addition to this, a link between subclinical coccidiosis and bacterial enteritis complicates choosing the right tools and strategy for poultry producers mainly in countries producing poultry meat without growth promoters and/or with vegetable diets only. Implementing sound shuttle and rotation programs, making use of all available and validated preventive tools (anticoccidials and live vaccines) is essential to not only control clinical, but mainly also subclinical coccidiosis. While basic rules for preventive programs are applicable all over the globe, regional differences (poultry meat organoleptic requirements, legislation in and availability of anticoccidial drugs and vaccines, management and impact of climatic conditions on coccidiosis challenge) make that designing anticoccidial prevention programs are per definition tailor made and solutions should continuously be evaluated.

Key Words: Eimeria, poultry, prevention, anticoccidial, vaccine

Coccidiosis mediated effects on energy cost at 5 age intervals throughout the broiler growth curve to 48 days. R. G. Teeter*,1, A. Beker1, C. Brown1, C. Broussard2, S. Fitz-Coy2, J. Radu2, and L. Newman2, 1Oklahoma State University, Stillwater; 2Intervet Schering Plough Animal Health, Union, NJ.

A metabolic chamber experiment was conducted utilizing Cobb x Cobb males to evaluate coccidiosis impact and calorific costs. Two groups of birds were reared in coxi free environments with one vaccinated at hatch (Coccivac-B) and the other maintained as naive to cocci. Birds were selected from the 2 backgrounds at 5 weekly intervals for chamber placement. The 5 challenge periods consisted of an oral dose of sterile saline or a mixture of 3 Eimeria species administered as oocysts to naïve birds at 14, 21, 28, 35, and 42 d. Variables examined 6 d post challenge included live weight, FE, gross lesion scores (upper small intestine: USI; mid small intestine: MSI; ceca: C), heat production (HP) and body composition. Metabolic costs of cocci challenge included appetite suppression, maintenance energy elevation, excreta calorie elevation and reduced live weight gain, FE and ration net energy (P < 0.05). Though coccidiosis challenge occurring early in the production cycle had energy cost, birds exposed late (35, 42 d) exhibited higher costs (P < 0.05). Effective calorific value (ECV) places calorific density equivalents upon nutritional and nonnutritional factors. In this study coccidiosis mediated lesion scores 6 d post oocysts challenge exhibited marked (P < 0.01) deleterious impact upon ECV. Lesion score 1 and 2 reduced the dietary energy value from an initial 3,200 Kcal/Kg ration by 125 and 596 Kcal for 800 g broilers and by 625 and 2,277 Kcal/Kg for 3000 g birds, respectively. Lesion score cost far exceeded consequences for inadequate lighting program and poor pellet quality. Calorimetry data substantiated the lesion consequence with increased maintenance energy need, heat production and malabsorption. Results demonstrate the importance of time dependency on coccidiosis control and calorific cost associated with lesions score.

Key Words: coccidiosis, broiler, energy, malabsorption
A quarter of a century ago, broiler coccidiosis control using in-feed anticoccidial medication was highly effective. Rotation and shuttle programs maximized efficacy, and control could be maintained with minimum flock-to-flock variation regardless of environmental conditions. But as sensitivity of the field coccidia decline, we must accept that farms will be subjected to some degree of Eimeria challenge or “coccidiasis” during the life of the flock. The genetics of the modern high-yield broiler allow slaughter by 30 to 36 d in many markets, and slaughter age will often coincide with the peak of Eimeria challenge. Coccidiosis or coccidiasis is no longer just a disease of a single bird or a single flock. It is a dynamic population that produces different clinical or economic outcomes depending upon many field factors such as stocking density, environmental humidity, slaughter age and the influence of the coccidiosis control program of the previous flock. Studies utilizing gross lesion scores, microscopic lesion scores, and sequential oocyst counts can be used to examine the influence of each of these factors on the behavior of the Eimeria challenge population on the farm. Eimeria reproduction is slowed by in-feed anticoccidial medication, low stocking density and low environmental humidity. This results in populations that peak at 28 d of age or later. When this peak corresponds to the final 2 weeks before slaughter, negative economic impact is maximized (Teeter et. al.). A peak in Eimeria shedding immediately before slaughter appears to result in the carryover of a higher challenge to the subsequent flock, even when cleaning and disinfection are employed. This early challenge may alter the clinical and economic outcome of the Eimeria population dynamics in that flock, which may, in turn, influence the next flock in sequence. Understanding coccidiosis population dynamics will help producers to develop coccidiosis and environmental management strategies to maximize broiler economic return as the broiler growth rate continues to increase and sensitivity to in-feed anticoccidials continues to decline.

Key Words: Eimeria, economics, coccidiosis, population, environment

The move toward drug-free poultry feeds has increased use of coccidial vaccines, however, the curtailing of feed and therapeutic antibiotic use worldwide has increased enteric diseases, including necrotic enteritis (NE). NE, caused by Clostridium perfringens (Cp) is one of the most economically important enteric diseases of broilers. Coccidial vaccination may predispose birds to NE. The purpose of this presentation is to place necrotic enteritis within the context of digestive health and microbial ecology by testing the hypothesis that coccidial vaccination alone does not lead to Cp overgrowth. The studies were designed to test whether diet and dietary additives also play a role in Cp overgrowth. To study the role of diet in the development of dysbacteriosis, a model has been developed that does not include a Cp challenge. Rather, the model uses a high viscosity diet that has been associated with Cp dysbacteriosis. Using this model, a series of experiments was conducted to study factors that can contribute to or mitigate the effects of subclinical enteritis. In a proof of principle experiment broilers were fed a 22% CP, 1.21%/1.07% total/digestible lysine mash diet was fed that contained 33% rye, 25% wheat, and 31% soybean meal. A 3x overdose of a live oocyst vaccine and an antibiotic or an NSP enzyme mixture were compared with unchallenged and/or untreated controls. Results indicated that diet played a significant role in Cp growth while coccidiosis challenge had no significant effect. This model is being used to test novel feed additives. Determining the dietary and enteric conditions that precede clinical NE are essential in the development of dietary and feed solutions for sustainable drug free agriculture. The purpose of the research described here is to examine the relationship between coccidial cycling and dysbacteriosis involving C. perfringens in broiler chickens with the goal of identifying nutrition guidelines and feed additives that reduce the incidence of Cp overgrowth in the distal ileum of broiler chicks.

Key Words: coccidiosis, necrotic enteritis, vaccination, dysbacteriosis

Considering the complex life cycle of Eimeria parasites, the interactions of this pathogen with the host have been historically difficult to track at the molecular level. Particularly, immune responses are diverse with the belief that cell-mediated immunity plays the predominant role against this intracellular pathogen. Over the past decade however, especially since the description of the highly conserved pattern recognition receptors, scientists have shown a much greater role of the innate arm of the host immune system. Traditionally regarded as an ancient scavenger system that immediately responds to slow down infection until adaptive immunity kicks in, components of the innate system have proven to perform more duties in orchestrating subsequent immune responses. In recent years, a plethora of publications has characterized both innate and acquired immune responses during Eimeria infections. These include the involvement of numerous immune elements like Toll-like receptors, antimicrobial peptides (e.g., LEAP-2), cytokines, and others engaged in inducing inflammation, activation of immune cells, and production of effector molecules. It has become evident that initial encounter of pathogens with the innate system leads not only to impeding the infectious process, but also to recruitment of various immune components, as well as induction and modulation of the adaptive immune system. As part of innate defenses, cells of the epithelial linings also contribute to such processes by secreting their own defense molecules. Overall, significant progress has been accomplished for better interpretation of the complex immune responses to coccidiosis, yet much remains to be explored. Current advanced tools of genomics, proteomics, and next generation sequencing technologies have become readily available and more economical for scientists to delve deeper into the intricate world of coccidiosis and the parasite interactions with its host.

Key Words: innate immunity, adaptive immunity, coccidiosis, Eimeria, poultry

The 2 predominant means by which producers control avian coccidiosis are medication of feed with anticoccidial compounds and administration of live Eimeria oocysts vaccines. While these approaches are sufficient to prevent major coccidiosis outbreaks, there is need for improvement in application of both control strategies. One improvement would be a rapid, inexpensive method for identifying species and strains of Eimeria present in a poultry house. This information would be useful for identifying drug-resistant or immunovariant strains, and
may guide producers on adopting alternative control strategies (e.g., switching between different drugs or vaccines). At present, there are several molecular methods based on PCR amplification of specific gene sequences, followed by analysis using gel- or capillary-electrophoresis or by real-time PCR to detect a particular *Eimeria* species. Efforts are underway to develop assays based on microsatellite markers for differentiating *Eimeria* strains, but no methods are available at present to discriminate *Eimeria* oocysts beyond the species level. The second area that needs improvement is live oocysts vaccine delivery. It is well established that chicks given *Eimeria* oocysts by oral gavage at hatch acquire immunity sufficient to resist a challenge infection by 2 weeks of age. Several commercial vaccines are available based on virulent or precocious *Eimeria* strains, and the 2 most often used methods of vaccine delivery are spray vaccination at hatch or inoculation into 18d embryos. Anecdotal evidence suggests that vaccination is neither efficient nor uniform, and that “cycling” of oocysts by chickens once inside the poultry house is required to achieve complete immunity. Our research is directed at improving the delivery of live vaccines by incorporating *Eimeria* oocysts inside gelatin-beads, and subsequent application onto feed for consumption by day-old chicks. Our preliminary data indicates that this method has promise for achieving uniform immunity against coccidiosis challenge infection. Comparison of gel-bead delivery to other immunization methods and obstacles for practical application will be a topic of discussion.

**Key Words:** coccidiosis, *Eimeria*, detection, vaccination, delivery

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**250 Precocious lines and attenuated coccidia.** R. H. Fetterer*, Animal Parasitic Diseases Lab, USDA/ARS, Beltsville, MD.

Poultry coccidiosis caused by several species of *Eimeria* remains an important disease of chickens raised in intensive production systems. The biology of *Eimeria* is characterized by a direct life cycle with 3 or more meront stages which allow for asexual expansion of the parasite resulting in sexual stages leading to production of oocysts. The duration of the life cycle stages and the prepatent period were originally considered to be fixed characteristics. However, seminal studies by T. K. Jeffers and others demonstrated that the prepatent period could be shortened by selecting strains of parasites derived from the earliest produced oocysts. This selection for precocious development is a result of one or more meront stages being eliminated with the resultant attenuation of the life cycle. The precocious strains are desirable for use in live vaccines since they lack the virulence and fecundity of natural strains but retain the ability to illicit protective immunity. Considerable research effort has led to the development of commercially successful vaccines containing precocious strains. In addition to precocious strains there has been some interest in attenuation of *Eimeria* development by gamma irradiation of sporulated oocysts. Using the appropriate radiation dose it may be possible to limit parasite development to the trophozoite or early meront stages thus preventing pathology, eliminating oocysts production and inducing acquired immunity. A vaccine consisting of irradiated oocysts would not require genetic selection and production would not be limited by the reduced fecundity observed in precocious strains. Development of new methods to attenuate the life cycle of *Eimeria* will require an increased knowledge of the regulation of parasite development. The selective knock out of genes controlling developmental stages (genetic attenuated development, GAD) has been achieved in other Apicomplexan parasites but the ability to genetically manipulate genes of *Eimeria* is lacking. In the future, GAD may be the key to understanding the role of developmental stages in the acquisition of immunity to avian coccidiosis.

**Key Words:** coccidiosis, *Eimeria*, poultry, disease

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**251 Using immunology and genomics as tools to investigate innate immunity to *Eimeria* with the goal of developing antibiotic-free, disease control strategies against avian coccidiosis.** H. Lillehoj*, Animal and Natural Resources Institute, US Department of Agriculture, Agricultural Research Service, Beltsville, MD.

Host-pathogen interactions in avian coccidiosis are complex. At the level of the host, a multitude of defense effector mechanisms are mounted against *Eimeria* parasties, including innate and acquired components of intestinal immunity. At the pathogen level, virulence is manifested through diverse and multifaceted genes and gene products. Although the underlying host immune response against *Eimeria*, and the counteracting response by parasites, are governed by genetically-controlled traits, recent evidence also highlights an important role for environmental factors affecting the outcome of this host-pathogen interaction. This complexity is further magnified by the microbiota of commensal bacteria which naturally inhabit the gut and influence the nutritional and metabolic states of both host and parasite. Therefore, a large array of management strategies to enhance gut health and increase disease resistance need to be considered if avian coccidiosis is to be effectively controlled. This talk will underscore recent basic research findings on poultry immunity and genetics that may prove beneficial for future development of novel alternative disease control strategies, thereby mitigating the use of anti-coccidial drugs.

**Key Words:** avian, coccidiosis, immunology, genomics, *Eimeria*

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**252 Coccidiosis control programs: Effects on gastrointestinal microbiota and incidence of clostridial infections in broiler chickens.** G. D. Ritter*¹ and A. P. Neuman³, ¹Mountaire Farms Inc., Millsboro, DE, ²Danisco USA Inc., Waukesha, WI.

Coccidiosis is a ubiquitous disease of economic importance in commercial broiler production. Prophylaxis treatment to prevent or minimize economic losses due to reduced absorption of feed nutrients is typically achieved using ionophoric compounds, other classes of anticoccidial chemicals, and live oocyst vaccines. While interventions have been successful at reducing the impact of coccidia, they can have other unintended consequences on the natural communities of beneficial microbes in the GI tract. Disruptions in these communities can lead to reduced performance and disease. Commercial broiler production data over a 6-year period from a large broiler integrator located on the Delmarva Peninsula will be presented relating incidence of Clostridial gangrenous dermatitis (GD) to various coccidial control programs. Based on this field data a pen research study was completed to investigate the effects of anticoccidial feed additives on the composition of the gastrointestinal microbiota. Interventions evaluated included in-ovo vaccination using a live oocyst coccidial vaccine, the ionophores; monensin and salinomycin, the chemical products; Clinacox, Deccox, and Maxiban, a chemical ionophore shuttle program (nicarbazin/salinomycin), and a non-medicated control. Intestinal mucosa samples and cecal contents were collected periodically during the rearing period for microbial community profiling using molecular analysis techniques DGGE, T-RFLP and cloning and sequencing of select samples. Research results and associations/correlations with field data will be discussed.

**Key Words:** microbiota, ionophore, coccidiosis, gangrenous, dermatitis

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**253 Prevalence of *Eimeria* spp. in European broiler farms.** M. Pages*¹, M. Dardi¹, J. Rubio-Perez¹, D. Blake², and E. Del Cacho³.

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Avian coccidiosis is one of the most important diseases affecting the intensive poultry industry worldwide. Despite its outstanding importance and well-known relation with other factors that may influence health status of the birds and commercial performance, few studies are available on the distribution of species in the field. In Europe few field surveys of *Eimeria* species are available. Commonly, the published studies have been focused on identifying the 7 species of *Eimeria* which affect *Gallus gallus* in a European country. The studies available in Europe until the present date are, Czech Republic (KuÄ era, 1990), France (Williams, 1996), UK (Chapman, 1982, Shirley 1995, Shirley 1997, Williams 2006, Eckert 1995), Norway (Haug, 2008) and Sweden (Thebo, 1998). The number of studies that have been done using samples from broiler farms is scarce and only the study by Haug 2008 has been done exclusively with samples from broilers. In the present study litter samples obtained in broiler farms from Spain, Belgium, Italy and France were evaluated for the presence of *Eimeria* species. The evaluation was done using a polymerase chain reaction (PCR) developed at IAH to specifically detect *E. acervulina*, *E. maxima*, *E. mitis*, *E. praecox* and *E. tenella*. Together with this molecular tool for detection of *Eimeria* species in litter samples, oocyst counts and evaluation of percentage of species by using a morphometry test was also done to further evaluate the samples. Results obtained provide interesting data on the prevalence of species which affect the broiler birds in Europe as well as the percentage of farms with each species. Summary of data collected from litter samples in broiler farms using the PCR.

**Key Words:** *Eimeria*, PCR, broiler, coccidiosis

### 254 Pathogenicity and prevalence of the lesser species of chicken *Eimeria*. S. H. Fitz-Coy*, Intervet/Schering-Plough, Millsboro, DE.

Five of the chickens *Eimeria* are regarded as lesser species (*E. mitis, E. praecox, E. mivati, E. hagani and E. acervulina*). These are considered “lesser species” due to the perceived less pathogenic impact on the host. There is scant documentation on research with the *E. hagani*, *E. praecox* and *E. mivati*; however, *E. acervulina* and *E. mivati* have had a reasonable share of scientific investigations. *E. acervulina* may cause severe growth depression, impairment in feed efficiency and cessation in egg production. Clinical signs are watery, viscid and mucoid droppings. Gross lesions are white transverse bands which may coalesce in severe infections in the upper third of the small intestines. *E. acervulina* is one of the most prevalent species of chicken. The description of *E. hagani* was brief, but was recently re-described. Infection confined to the upper half of the small intestine. *E. hagani* produces hemorrhagic spots and watery intestinal contents. Recent samples have had organisms identified as *E. hagani*. *E. mitis* produces no lesions but cause growth suppression and cessation in egg production. The prevalence of *E. mitis* is less than 15%. *E. mivati* is the most pathogenic, infections cause growth depression in broiler chickens, cessation in egg production and mortality in susceptible chickens. Signs are watery and mucoid droppings with a tinge of blood and gross lesions are white spots “a star burst appearance” throughout the small intestines with higher intensity in the upper half. Mortality can occur and may vary from 10% to 40%. The prevalence of *E. mivati* in the US is estimated to be 20–30%. *E. praecox*, has a shortened pre-patent period, the pathogenicity is often overlooked due to the lack of gross lesions. The prevalence is less than 15%. Although these 5 species of chicken *Eimeria* are referred to as the lesser species, some members of this group may be moderately pathogenic even causing mortality.

**Key Words:** *Eimeria*, lesser species, pathogenicity, chickens

### 255 *Eimeria* in gamebirds: Development of PCR-based diagnosis and tests of immunity and drug efficacy. L. R. McDougald*, R. Gerhold, and R. B. Beckstead, University of Georgia, Athens.

Outbreaks of coccidiosis are common and severe in gamebirds raised commercially. In cooperation with the North American Gamebird Association we conducted a limited survey of chukars, pheasants and bobwhite quail. Samples received from the field in 2% potassium dichromate were examined microscopically, then passaged in young cage-reared birds. Oocysts were collected from the droppings and the lesions examined by microscopy at necropsy. Single-oocyst isolates of these cultures were used to prepare species-specific PCR primers. Genus-wide *Eimeria* PCR primers were used to amplify the internal transcribed spacer region 1 (ITS-1) of the ribosomal RNA of each species. PCR products were cloned, sequenced, and aligned for phylogenetic analyses. In the Chinese ringnecked pheasant, the most predominant and pathogenic species were *E. phasiani, E. colchici, E. duodenalis, E. pacifica* and *E. tetartooomia* were also identified. In the Chukar partridge, the predominant species was *E. kofoidi* (18 isolates). *E. legonensis* was identified in 3 isolates. Another species, identified only by PCR in one isolate, was unidentified. Bobwhite quail had 3 species common in most isolates. The most pathogenic and prominent were *E. lettyae* and *E. dispersa*. The 3rd species was probably *E. colini*, although the description of this species is incomplete. Tests were also conducted to evaluate the potential vaccination against these coccidia in each bird species. Several anticoccidial drugs were tested for efficacy against selected isolates.

**Key Words:** coccidiosis, *Eimeria*, PCR, gamebirds

### 256 Tom Jeffers: Pioneer of coccidiosis research. H. D. Chapman*, University of Arkansas.

For much of his career Dr T. K. (Tom) Jeffers was involved in animal health research and administration for one of the largest pharmaceutical Companies in the world. In 1963, as a student at Cornell University, he co-authored a paper on the genetics of allelism in the fowl which was published in Nature. During the 1970s, at Hess and Clark and Elanco, he made several unique contributions to our understanding of the disease coccidiosis in poultry. These included the most comprehensive set of data concerned with resistance to anticoccidial drugs ever published, the phenomenon of synergism and collateral sensitivity to certain drugs, and genetic recombination between them. Jeffers was the first to show that it was possible to develop resistance to an ionophore, the most widely used drugs for the control of coccidiosis. He co-invented a novel anticoccidial drug combination now widely used to control coccidiosis. His most significant contribution, however, was the demonstration that it was possible to attenuate a coccidian by selection for precociously in the chicken. This eventually led to the development of live, attenuated vaccines for the control of coccidiosis. He proposed that alternating cycles of immunization using drug sensitive strains and chemotherapy could provide effective long-term control of coccidia in commercial poultry production. Tom Jeffers was a true pioneer of coccidiosis research.

**Key Words:** Jeffers, coccidiosis, drugs, vaccines