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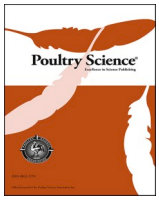

Symposium

Coccidiosis in poultry: Disease mechanisms, control strategies, and future directions

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ABSTRACT

According to a 2023 survey by the American Veterinarians in Broiler Production, Coccidiosis is the number one disease in the broiler poultry industry. Coccidiosis results in the reduction of growth rate, decrease in feed efficiency, and poor body weight uniformity. The more we understand this disease the more we can move forward towards control. The purpose of this symposium was to increase our understanding of coccidiosis. The topics discussed were the diagnosis, immune response, control/prevention, medications (natural and chemical), and interactions with other diseases. The Coccidiosis Symposium provided up to date information from both research and field experiences. This information will be useful for production managers, nutritionists, and veterinarians, as well as providing opportunities for future research.

Introduction

Over the past several years, the members of the Association of Veterinarians in Broiler Production (AVBP) have been polled about the disease and non-disease issues they faced. The survey showed that the number one disease each year was coccidiosis. Coccidiosis is caused by *E*imeria, intercellular intestinal protozoan parasites, and is an enteric disease which causes malabsorption, enteritis, depressed weight gain, uniformity issues, increased FCR, and mortality. This disease leads to economic losses and animal welfare issues. Blake et al. (2020), estimated the world-wide cost of coccidiosis in chickens was ~13 billion US dollars. This included production losses, and the cost associated with prophylaxis and treatment. This symposium examined the disease, its prevalence, impact on the host, interaction with other diseases, and control/management programs.

Describing the disease, its diagnosis, and methods to judge pathogenicity are the basics for all coccidiosis research and field observations. Nine *Eimeria* species infect broiler chickens. *Eimeria acervulina, E. maxima, E. brunetti, E. necatrix,* and *E. tenella* are thought to be the most pathogenic chicken specific species. However, the remaining species, referred to as lesser species, are very prevalent and often impact production. Their importance is reviewed in the Prevalence and Pathogenicity of the lesser species section of the symposium.

Coccidiosis triggers a myriad of host reactions, both innate and adaptive immune responses. The immunity section of this symposium brought together the impact on intestinal immunity, integrity, and physiology. The disease process damages the intestinal epithelium, which allows opportunistic pathogenic bacteria to invade the host. The damage associated with a coccidiosis infection and the proliferation of *Clostridium perfringens* often leads to necrotic enteritis. “Holes” in the epithelium allow bacteria such as *Escherichia coli, Staphylococcus*, and *Clostridium* species to translocate to different internal tissues. These secondary infections may be more costly than coccidiosis by itself.

Poultry coccidiosis is controlled by the use of prophylactic feeding of anticoccidial drugs or vaccinating with live coccidia oocysts vaccines. Generally, the drugs have a board species spectrum of activity and are very effective. However, the number of drugs are limited, and resistance is a major issue. Only 12 FDA approved anticoccidial drugs are available

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for the USA poultry market. Based on many factors, several feed additive alternatives to anticoccidial drugs have been researched and used in poultry production. Some of the phytogenic feed additives have shown some anticoccidial activity. Understanding their mode of action and how to correctly use them is paramount to their continued use. Vaccination utilizes live coccidia to produce immunological protection. Strong and lasting immunity develops to all the vaccinated *Eimeria* species. In order for adequate protective immunity to develop, coccidia must multiply repeatedly. This process can lead to intestinal damage and affect broiler performance. The pros and cons of both programs were discussed. Also discussed in the symposium were ways to improve the vaccination process.

This coccidiosis symposium was designed to share knowledge from leading coccidologists. Attendees can use this information to add to their coccidiosis foundation when assessing the disease in the field or using the parasite in research studies. Hopefully ideas will grow from this symposium and someday coccidiosis will no longer be the number one disease challenge.

Coccidiosis – The Disease, Diagnosis, Lesion Scoring

Hector M. Cervantes

Introduction

Coccidiosis remains an important disease of chickens and turkeys, especially of young poultry. The disease is caused by protozoan parasites of the genus *Eimeria* that infect different segments of the intestinal tract causing destruction of epithelial cells leading to inflammation, increased permeability, malabsorption of nutrients, impaired growth, poor feed utilization and increased susceptibility to secondary bacterial infections like necrotic enteritis.

Important species and life cycle

The "big three" species of coccidia in broiler chickens are *E. acervulina, E. maxima* and *E. tenella* (Cervantes et, al.,2020), while in meat turkeys the "big three" are *E. adenoides, E. gallopavonis* and *E. meleagrimitis* (Durairaj et al., 2023). These species are the most important for their economic impact and the most studied since 1929.

As in other avian species, the coccidia of chickens and turkeys have direct life cycles with one exogenous phase of the cycle (sporogony), happening in the litter of the poultry house, and another couple of endogenous phases (schizogony and gametogony) occurring inside the host (McDougald, 1998). Birds first become infected by ingesting sporulated/infective coccidian oocysts, these are usually picked up by a bird by pecking at the litter, although sometimes they can be found contaminating feed or water. Only sporulated oocysts are infective. This is important because when the oocysts are first passed with the droppings onto the litter, they are not sporulated, and therefore, unable to cause disease. Under the proper conditions of temperature, oxygen and moisture, oocysts sporulate within 24 – 48 h and become infectious. Management factors have a significant influence on the rates of sporulation of the oocysts present in the litter of a poultry house. By maintaining good ventilation, proper pressure, height and maintenance of nipple drinkers the litter can be kept drier slowing down the sporulation of oocysts. By minimizing the level of sporulated oocysts, producers can in turn, minimize coccidian challenge and favor good control of coccidiosis.

Once ingested by the bird, the sporulated oocysts are crushed by the mechanical action of the gizzard releasing up to 4 smaller cysts known as sporocysts. Each sporocyst contains two infective parasites known as sporozoites, the digestive action of the bile and pancreatic secretions (trypsin and chymotrypsin) present in the duodenum break down the outer wall of the sporocysts releasing them into the lumen of the intestine where, if they do not come into contact with an anticoccidial drug, they can travel and infect epithelial cells in their preferred areas of localization.

Once inside an intestinal epithelial cell, the parasites multiply very quickly by an asexual mechanism of multiplication known as fission, these process results in a large number of "daughter parasites" contained in a large body known as a schizont, when the schizont fills up with parasites it will rupture out releasing them into the lumen of the intestine or ceca, the parasites will then invade more epithelial cells of the intestinal or cecal mucosa causing more destruction and damage to the absorptive capacity of the intestines. At this stage, when viewed under a microscope, the parasites (merozoites) are shaped like a banana. This process of multiplication by fission will be repeated 2, 3 or more times, the first generation of "daughter cells" or merozoites is known as merozoites I, the second generation as merozoites II, and so on. The entire stage of multiplication by fission is known as the "schizogony" or "merogony" and constitutes the asexual multiplication phase of the life cycle. As the epithelial cells lining the intestinal mucosa are infected, and then ruptured, it causes additional damage to the intestinal tract, impairing its ability to absorb nutrients. As the damage also changes the permeability of the intestinal wall, proteins and fluids may leak into the intestine resulting in wetter droppings and favoring the growth of pathogenic bacteria like *Clostridium perfringens*, the causative agent of necrotic enteritis.

One final stage of multiplication with more destruction of the epithelial cells of the mucosa still occurs, this final stage is known as the "gametogony" or sexual multiplication phase of the life cycle. During this stage, the "daughter cells" sexually differentiate producing two types of cells, the smallest ones are motile and are known as microgametes, and are the equivalent of the spermatozoa, the larger ones are known as the macrogametes and are the equivalent of the ova. The microgametes fertilize the macrogametes resulting in multiplication and more destruction of the intestinal or cecal mucosa and the production of zygotes or immature oocysts which will be passed through the droppings onto the litter to complete the cycle.

Diagnosis

In chickens, from a practical point of view, the characteristic appearance and location of the gross lesions in conjunction with microscopic detection of significant number of parasites in mucosal scrapings from the affected areas is sufficient for diagnosis. In turkeys, coccidiosis can be suspected when turkey poults present signs such as diarrhea, ruffled feathers, huddling or increased mortality. However, confirmation should always be done by postmortem examination of representative birds and microscopic examination for the detection of parasites in mucosal scrapings from the duodenum, jejunum, ileum and ceca. Typically, turkeys do not display gross lesions of coccidiosis, therefore, microscopic examination of mucosal scrapings is required to confirm the diagnosis by the detection of large numbers of oocysts.

Lesion scoring

Although lesion scoring has been practiced since the 1950s, a standardized system was not widely adopted until 1970 when Johnson and Reid published a detailed description of gross lesions for each of the 5 species that produce gross lesions in chickens. In 2019, a similar system for scoring gross lesions of the 3 most pathogenic species of turkeys was published by Gadde et al. (2019), although its application in the field has been hampered by the common lack of gross lesions.

Immune responses to coccidiosis in poultry: Where do we stand?

Rami A. Dalloul

As the leading parasitic disease in commercial poultry production systems, coccidiosis continues to inflict major economic costs to the industry stemming from prevention, treatment, and mitigation of secondary stressors and infections. The complexity of the apicomplexan life cycle, including coccidiosis-causing *Eimeria* spp. that undergo several structural changes through asexual and sexual phases, triggers a myriad

of host reactions to the various antigenic molecules expressed at various developmental stages. Additionally, as per any enteric encounter, the host immune system responds via both innate and adaptive responses along with an array of non-specific intestinal defenses. As such, combinations of response variables are expressed by the host at any given time point during infection, from the early acquisition of oocysts through the late stages and parasite shedding. Not to ignore the parasite's own machinery that is capable of producing and expressing its own array of cytokines and receptors, respectively, thus influencing those specific host reactions and counterreactions (Dalloul and Lillehoj, 2006; Kim et al., 2014). One such cytokine is the macrophage migration inhibitory factor (MIF) that the parasite could produce at the infection site to slow down the host's innate and subsequent adaptive immune responses (Miska et al., 2013). In a series of studies, *Eimeria* MIF not only mimicked the functions of the host MIF (Kim et al., 2014; Park et al., 2016), but also did so via interactions with the same receptors on host cells (Dalloul, unpublished data).

Delineating the specific immune responses to coccidiosis has historically focused on indirect measurements of intestinal markers, chiefly by profiling cell populations and comparing relative mRNA abundance of 'relevant' immune response genes in intestinal tissues. The challenge with these approaches, particularly in field situations, is the assumption that such responses are solely due to a single infection. Simply attributing profile differentials to coccidiosis alone is certainly imprecise as the parasite is a major predisposing factor to other infections and stressors, which differ during the progression of parasite development (Emami and Dalloul, 2021). Also, correlating such parameters with concurrent physiological responses has been challenging at times and often misleading, especially when relying on indirect measurements of host response variables. In this context, another major issue lies in the eventuality that immune responses are inconsistent among birds and treatments as they often tend to be more circumstantial to each research setting and field condition (Soutter et al., 2020). While timing is key in terms of when samples are collected and assessed, which pertinent parameters and how to measure them are more critical. Further, implicit bias comes into play when interpretation of the results leans towards situational objectives sometimes resulting in ambiguous reporting and potentially poor conclusions. Comprehensive understanding of complex host responses continues to present challenges until more adequate and reliable immunological tools for poultry become available and feasible. Until then, our collective efforts as researchers will continue to sort out individual and group responses within the context of the designed laboratory studies as well as in field trials.

Coccidiosis Control: FDA approved Drugs and USDA approved Vaccines

Greg F. Mathis

Poultry coccidiosis is controlled by the use of prophylactic feeding of anticoccidial drugs or vaccinating with live coccidia vaccines. Anticoccidial drugs have been successfully used for over 50 years (McDougald, 2003). Currently, there are only 12 FDA approved anticoccidial drugs. FDA approved drugs are researched and approved for efficacy and safety, regulated for production quality, and with feed manufacturing oversite. Anticoccidials are broadly divided into synthetic (or chemical) and polyether Ionophorous antibiotics. Synthetic/chemical anticoccidials generally have a broad spectrum of activity, high anticoccidial efficacy, a potential for rapid resistance development, and generally allow limited immunity development. This type of drug does not have any antibiotic activity, thus can be used in production of birds raised without antibiotics. The ionophores also have a broad spectrum of anticoccidial activity. The ionophores do not eliminate coccidia (direct control). Thus, their mode of control relies on both direct and immunological control. The ionophores have some antibiotic activity and are compounds that have no presence in human medicine. Birds that are fed the ionophores cannot be labelled antibiotic free but can be labeled: raised without antibiotics that are important to human medicine. Limited options of available anticoccidial drugs and many years of use has reduced anticoccidial sensitivity/ resistance has resulted in increased use for all in-feed anticoccidial drugs by the poultry industry (Chapman, 1982). The use of chemical and ionophore anticoccidials in shuttle programs (changing drug program from one cycle to the next cycle) and rotating drugs (changing drugs within a grow out cycle) have extended the longevity of many of these drugs to be used in poultry feeds.

The increasing demand for antibiotic-free and drug-free birds, has led to the ever growing use of coccidiosis vaccination programs. Commercial live coccidia vaccines have been available since the 1950s. The use of a coccidia vaccine does not involve drugs, antibiotics or residues, in-order to produce immunity of all species contained in the vaccine, and generally contain less pathogenic strains than what is found in the field. USDA approved coccidia vaccines require potency determination for each lot of vaccine. Vaccination programs use live coccidia oocysts, which are administered using a hatchery (day of hatch) spray or gel, a gel puck placed into hatchery box, or in-ovo dosing. Field reapplication is also used to increase vaccine coverage. These methods provide a prescribed number of oocysts at an early age that enable immunity development to progress rapidly at a prescribed rate. A significant amount of immunological protection develops by 14 days of age, allowing birds to withstand a substantial challenge by 21 to 28 days of age. Coccidia vaccines are of two types; non-attenuated (not altered) and attenuated. All USDA approved coccidia vaccines contain at least *E. acervulina, E. maxima*, and *E. tenella*. Some vaccines contain *E. mivati, E. necatrix, E. brunetti*, and/or *E. mitis*, and possibly more than one strain of *E. maxima*. These *Eimeria* are all live, infective, and reproduce in the birds. Thus, coccidiosis develops within the bird which causes some degree of intestinal disruption. This disruption can lead to malabsorption, enteritis, depressed weight gain, uniformity issues, increased FCR, and mortality. Non-attenuated vaccines contain strains that are generally less pathogenic than field strains while maintaining their reproductive and immune stimulating characteristics. Attenuated vaccine strains have been selected for reduced pathogenicity by collecting the earliest coccidia oocysts shed post challenge. This selection for shorter life cycle, eliminates one of the pathogenic asexual stages of development, producing a strain that does not affect performance as much as the non-selected (non-attenuated) stains. Attenuation does decrease pathogenicity but also reduces fecundity and immunogenicity. Key factors for successful vaccination are application, vaccine storage, and farm management. Vaccination programs can provide equal effectiveness and performance to a drug program (Williams, 2002).

A combination of vaccination plus an anticoccidial drug is called a Bio-shuttle. The drug is used at the lowest approved level. This low-level drug is given after the bird has developed some coccidia vaccine related immunity and near the peak of coccidia cycling. The coccidia vaccine peak is generally 2-3 weeks, thus the change from starter feed to grower feed is the best time to start the drug in the Bio-shuttle program. Bio-shuttle programs are used to reduce and/or modulate coccidiosis at the peak of coccidia cycling. Consequently, this reduction/modulation decreases the damage caused by coccidia and potentially reduces necrotic enteritis development.

Even though coccidiosis is always present it can be controlled. The type of anticoccidial program one uses depends on many factors. Both FDA approved drugs and USDA approved vaccines have pros and cons. Cost, availability, equipment and expertise to apply and use, toxicity to certain drugs, marketing (size and antibiotic usage), attenuated versus non-attenuated vaccine, and housing and management are just a few of the factors to consider. Thinking long term while using the most effective program will provide successful coccidiosis control.

Prevalence and Pathogenicity of the Lesser Species of Chicken Eimeria

Steve H. Fitz-Coy

There are nine species of *Eimeria* named for the domestic chicken in the United States. These include *E. tenella* (Rillette and Leucet, 1891), *E. maxima, E. mitis*, and *E. acervulina* (Tyzzer, 1929), *E. praecox* and

E. necatrix, (Johnson, 1930), *E. brunetti* and *E. hagani* (Levine, 1938), and *E. mivati* (Edgar and Seibold, 1964). Some researchers tend to lump *E. acervulina, E. mitis, E. mivati, E. hagani*, and *E. praecox* into one group and often referred to the group as "lesser species" or "*E. acervulina* type". Proper species identification is important due to differences in pathogenicity and response to anticoccidial drugs. *Eimeria praecox* had the shortest prepatent period, 84 h post-infection (pi). *Eimeria mivati, E. acervulina, E. hagani*, and *E. mitis* were 93, 97, 99, and 99 h, respectively. The mucosal epithelium thickened with *E. acervulina* or *E. mivati* infections in contrast to fairly normal epithelium with severe infestations by *E. mitis, E. praecox*, and *E. hagani.* The mucosal thickening is caused by multiple parasites within the host cells. Historically, it all started in 1929 when Dr. Ernest E. Tyzzer identified and named several coccidia species that affect chicken, turkey, and quail. Between 1929 and 1960, five other coccidia species were described and named: *E. necatrix* and *E. praecox* by Johnson, *E. brunetti* and *E. hagani* by Levine, and *E. mivati* by Edgar. Following the naming of *E. mivati*, controversy commenced around *E. mitis*. This controversy was resolved in the early 1980′s; however, the taxonomy and validity of the "lesser species" *E. hagani*, and *E. mivati* remained.

Five of the chicken *Eimeria* are regarded as "lesser species" (*E. mitis, E. praecox, E. mivati, E. hagani*, and *E. acervulina)* due to their perceived lesser pathogenic impact on the host. Research on *E. hagani, E. praecox*. and *E. mitis* is limited. However, *E. acervulina* is one of the most prevalent species of chicken *Eimeria. E. acervulina* may cause severe growth depression, impaired feed efficiency and cessation of egg production. Gross lesions occur in the upper third of the small intestines. The description of *E. hagani* was brief, but a recent re-description has emerged. These parasites are confined to the upper half of the small intestine where they cause watery intestinal contents. Recent samples have organisms identified as *E. hagani. Eimeria mitis* produces no lesions but causes growth suppression and cessation in egg production. *E. mitis* prevalence is less than 15 %. *E. mivati* is the most pathogenic species, causing growth depression in broiler chickens, cessation in egg production, and mortality in susceptible birds. Symptoms include watery and mucoid droppings tinged with blood, gross lesions characterized by white spots with a "starburst appearance" throughout the small intestines, especially in the upper half. Mortality can occur and may be as high as 40 %. The prevalence of *E. mivati* in the US is estimated to be 30 %. *E. praecox* has a shortened prepatent period and the pathogenicity is often overlooked due to the lack of gross lesions. The prevalence of *E. praecox* is less than 15 %. Although these five species of chicken *Eimeria* are referred to as the "lesser species," some members of this group may be moderately pathogenic, even causing mortality. Cross immunization studies further characterized the differential specificity of these species (Table 1). In non-immunized birds challenged with single species, parasites developed in the areas described for these species. Invasion by *E. praecox, E. acervulina*, or *E. hagani* was anterior to the Meckel's diverticulum, whereas *E. mitis* and *E. mivati* infected the entire lower digestive tract. Birds immunized with *E. acervulina* and then challenged with *E. acervulina* were protected against the challenge. However, birds immunized with *E. acervulina* and challenged with *E. mitis* were susceptible. Birds immunized with *E. mitis* were resistant to the *E. mitis* challenge. Birds immunized with *E. mivati* resisted challenge with *E. mivati* but were not immune to *E. mitis, E. acervulina*, or *E. hagani*.

As for prevalence and pathogenicity, from the 1980′s to 2000′s while ionophores were heavily used, the prevalence of the lesser species was low. After the identifying and naming of some of the lesser species, some researchers considered them rare or non-existent. These included *E. hagani, E. mitis* and *E. mivati*. Since the 2010′s, when more chemical anticoccidials and biologics were gaining more usage due to the shift in customer preferences; the prevalences of some members of the lesser species had increased. During periods of moderate chemical anticoccidials usage in the fall and winter periods in the U.S. broiler industry, the prevalence of the lesser species has increased. However, during periods where biologics are heavily used, the prevalence of *E. hagani* and *E. praecox* was drastically reduced — 70 % during periods of chemicals vs 7 % during periods of biologicals. There appeared to be a reduction in the efficacy of selected chemical anticoccidials to some of the lesser species. The anticoccidial index (ACI) is a measurement used to determine the effectiveness of an anticoccidial treatment. The drug efficacy score averaged 44 % as compared to the negative controls at 100 % and the infected control at 31 %, respectively. The prevalence and pathogenicity for the *E. acervulina, E. mitis* and *E. mivati* vary enormously; *E. acervulina* is the most prevalent followed by *E. mivati* then *E. mitis* at 90 %, 40 % and less than 10 % in the U.S.A., respectively. However, the species have varying pathogenicity; *E. mivati* is most pathogenic and may cause mortality, followed by *E. mitis* and *E. acervulina* (Table 2 and Table 3).

To summarize, *E. acervulina, E. hagani* and *E. praecox* infect the small intestine anterior to the Meckel's diverticulum. *E. mitis* and *E. mivati* invade the small intestine, ceca, rectum including Meckel's diverticulum. *E. acervulina, E. mivati, E. praecox* and *E. hagani* are relatively prevalent in U.S. broiler industry. *E. mitis* is less prevalent in the USA broiler industry. Yet, precautionary measures must be taken to avoid contamination during propagation. The purity of isolates must be determined before critical studies are done.

Coccidiosis: Insights from Molecular Biology and Vaccination
Mark C. Jenkins

Table 2
Growth patterns for immunized and challenged groups of birds.

	Control*	*E. mitis*	*E. mivati*	*E. acervulina*
	% Growth			
Control*	100	60	58	65
E. mivati	100	65	95	60
E. acervulina	100	58	60	98

*Control = non-immunized, non-challenged.
Immunized = *Eimeria* sp. in rows while Challenged = *Eimeria* sp. in columns.

Table 1
Cross immunization studies of some lesser species of chicken *Eimeria*.

Eimeria species		Regions of intestines parasitized				
Immunized	Challenged	Duodenum	Jejunum	Meckel's	Ileum	Rectum
None	*acervulina*	G, O**	G, O**	none	none	None
None	*mitis*	G	G	G, O**	G, O**	G, O**
None	*mivati*	G, O	G, O	G**, O	G**, O	G*, O
None	*hagani*	G**	G**	none	none	None
acervulina	*mitis*	G, O	G, O	G**, O	G**, O	G*, O
acervulina	*acervulina*	none	none	None	none	none
mivati	*mivati*	none	none	None	none	none
mivati	*mitis*	G, O	G, O	G, O	G*, O	G*, O
mivati	*acervulina*	G, O**	G, O**	none	none	None
none	none	none	none	none	none	None

Key = *Many, **Moderate, G and *O* = gametocytes and oocysts.

Table 3
Microscopic scores for immunized and challenged groups of birds.

	Control	*E. acervulina*	*E. mivati*	% Mortality
E. acervulina	7.0	0	5.0	0
E. mivati	11.0	11.0	0.4	20

Control = immunized not-challenged.
Immunized = *Eimeria* sp. in rows while Challenged = *Eimeria* sp. in columns.

Until the advent of molecular tools, unequivocal species identification of *Eimeria* in fecal droppings and in litter was nearly impossible because of the similarity in morphology among *Eimeria* oocysts. Although average size of each *Eimeria* species oocyst is known, there is considerable overlap in the size range (l x w) of the *Eimeria* oocysts that infect chickens which makes discerning one species from another difficult (Table 4). Ascribing performance issues and necrotic enteritis (NE), the latter often associated with an *Eimeria* infection, to coccidiosis frequently requires necropsy and visualizing lesions in specific regions of the gut. For instance, overt lesions in the duodenum may indicate an *E. acervulina* infection whereas lesions in the jejunum may reflect infection with *E. maxima*. A factor complicating diagnosis based on gross or microscopic lesions is that two *Eimeria* species (e.g. *E. maxima* and *E. necatrix*) infect the same intestinal region and in severe cases migrate beyond the primary site of infection. Knowing the *Eimeria* species composition in necropsied intestinal tissue sample is important because the predominance of one *Eimeria* species may reflect an underlying problem with drug-resistance or immunovariablity. It is well known that certain anticoccidial drugs are more effective against one *Eimeria* species and thus switching to an ionophore or synthetic compound that may control an emerging drug-resistant isolate may be an option to consider.

Techniques based on PCR amplification of internal transcribed spacer 1 (ITS1) or internal transcribed spacer 2 (ITS2) ribosomal DNA or Sequence-Characterized Amplified Region (SCAR) DNA are useful for analyzing mixtures of *Eimeria* species and for determining the *Eimeria* species composition in fecal droppings and litter (Fernandez et al., 2003; Gasser et al., 2005; Jenkins et al., 2006; Haug et al., 2007). This information has been helpful in understanding the dynamics of drug-resistance and immunovariability (Morris et al., 2007; Jenkins et al., 2017). Moreover, PCR directed to rDNA coupled with DNA sequencing has identified 3 new *Eimeria* species of chickens- *E. lata, E. nagambie*, and *E. zaria* previously known as OTU-x, y, and z that appear only in the Southern hemisphere, but pose a threat to the worldwide poultry industry (Cantacessi et al., 2008; Hinsu et al., 2018; Blake et al., 2021; Soares Júnior et al., 2023). It is improbable that these 3 new *Eimeria* species would have been discovered without the availability of molecular techniques.

While *Eimeria*-specific PCR can provide a list of *Eimeria* species present in a sample, they cannot provide insight on the relative abundance of each *Eimeria* in that sample. This is because the target DNA sequence, typically ITS1 or ITS2, being amplified exists in multiple copies and may vary among the *Eimeria* infecting chickens. Thus, there is no reliable way to estimate the relative number of any single *Eimeria* species in a sample using nuclear genes that vary in copy number among *Eimeria* spp. genomes. My laboratory is developing a quantitative metagenomics assay that is based on amplification of highly conserved mitochondrial sequences. After amplification and sequencing, the sequence reads are mapped to mitochondrial sequences present in GenBank. Using equal mixtures of *E. acervulina, E. maxima*, and *E. tenella* oocysts as controls, the number of reads mapping to each species appears consistent to the relative number of input *Eimeria* species oocysts. This advance was possible only after exhaustive searching and testing of primers whose sequences were conserved and thus amplified with nearly equal efficiency homologous mitochondrial sequences among different *Eimeria* species. This metagenomic approach should allow for a single tube analysis of *Eimeria* oocysts in litter and fecal droppings from chickens during anticoccidial drug or coccidiosis vaccine programs.

Table 4
Average size (l x w) and range in length (l) and width (w) of *Eimeria* species oocysts infectious for chickens. Sizes are given in microns (Long and Reid, 1982).

Eimeria sp.	Average Size (l x w)	Range in Length (l)	Range in Width (w)
E. acervulina	18×15	18 – 20	14 – 16
E. brunetti	25×19	21 – 30	18 – 24
E. maxima	31×21	22 – 42	17 – 30
E. mitis	16×14	12 – 19	11 – 18
E. necatrix	20×17	13 – 23	11 – 18
E. praecox	21×17	20 – 25	16 – 20
E. tenella	22×19	20 – 26	17 – 23

Vaccination against *Eimeria* by immunizing newly-hatched chicks with a low dose mixture of virulent or attenuated (precocious) *E. acervulina, E. maxima*, and *E. tenella* oocysts is a widely used approach to preventing coccidiosis in the poultry industry. Vaccination has many benefits, not least of which is using drug-sensitive *Eimeria* in the vaccine to replace drug-resistant *Eimeria* in litter that eventually arise during anticoccidial drug programs. Vaccination is often employed in warmer months of the year when certain synthetic chemical anticoccidials have deleterious side-effects on chick health. The basis for vaccination is the well-documented dose-dependent protective immunity that develops in chickens after a primary *Eimeria* infection [for review see Fatoba and Adeleke, 2018]. Immunity is extremely species-specific with little cross-immunity among different *Eimeria* species. The vaccines are typically applied at the hatchery by spray vaccination of chicks in hatching trays with *Eimeria* oocysts in an aqueous or gel suspension. Due to the well-documented inefficiency and non-uniformity of spray vaccination (Jenkins et al., 2012; Price et al., 2014), my laboratory and others have developed alternative delivery methods. These include *in ovo* injection of *Eimeria* oocysts (Sokale et al., 2017; Weber and Evans, 2003; Weber et al., 2004) and application of *Eimeria* oocyst-impregnated alginate or gelatin beads directly to poultry feed (Jenkins et al., 2012; Norton and Joyner, 1986). Vaccine uptake as estimated by measuring oocyst excretion on days 5-8 after infection was greater and more uniform when *Eimeria* oocysts were applied as gelatin beads compared to spray vaccination (Fig. 1). This increased uptake may explain the greater protection against *Eimeria* challenge as measured by greater weight gain and lower feed conversion ratios in gelatin bead delivery compared to spray vaccination (Fig. 2). Although effective, there are practical problems with preparing and applying alginate or gelatin beads, such as the need to manufacture the beads in one location and then transporting and distributing the beads to each house. *In ovo* injection requires a sterile vaccine or the use of antibiotics such as gentamycin which obviates use in chicks grown without antibiotics (e.g. ABF, NAE). Another approach that overcomes these limitations is delivering *Eimeria* oocyst vaccines into the drinking water system. *Eimeria* oocysts are introduced into the drinking water when chicks are 3 days of age. Comparison of vaccine uptake as measured by oocyst output between a commercial vaccine given in the water system compared to this same vaccine sprayed onto chicks at the hatchery revealed startling differences in vaccine take (Jenkins et al., 2023). Water vaccination led to 88-94 % uptake compared to 0 % uptake in chicks immunized by spray vaccination at the hatchery (Table 5). At least one poultry company is using this water vaccine delivery technology under select grow-out conditions.

In conclusion, molecular techniques are crucial to understanding the epidemiology of avian coccidiosis by giving insight into *Eimeria* population dynamics. This technology when used in conjunction with standard parasitological evaluation (e.g. *Eimeria* oocyst counts, microlesion scores) can aid in describing the cause of increased NE or poor performance in chickens. Molecular methods can also assist in assessing the effectiveness of anticoccidial drug treatments and *Eimeria* vaccination, such as pinpointing drug-resistance or immuno-variation. As vaccination methods improve through water delivery or other improved, then outbreaks of coccidiosis should diminish.

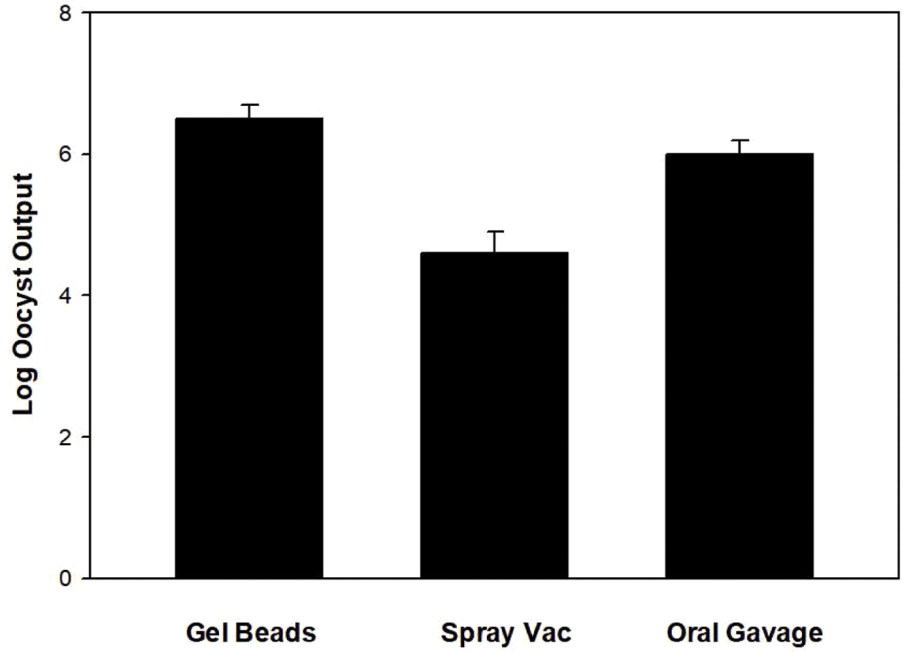

Fig. 1. Average log total *Eimeria* oocysts output between days 5-8 post-immunization in broiler chicks vaccinated at one day of age by 3 different delivery methods (gel-beads, spray-vaccination, or oral gavage) with a mixture of *E. acervulina* (4.5×10^3), *E. maxima* (10^3), and *E. tenella* (4.5×10^3) oocysts. Data is the mean and standard error of the mean (S.E.) of 3 individual trials.

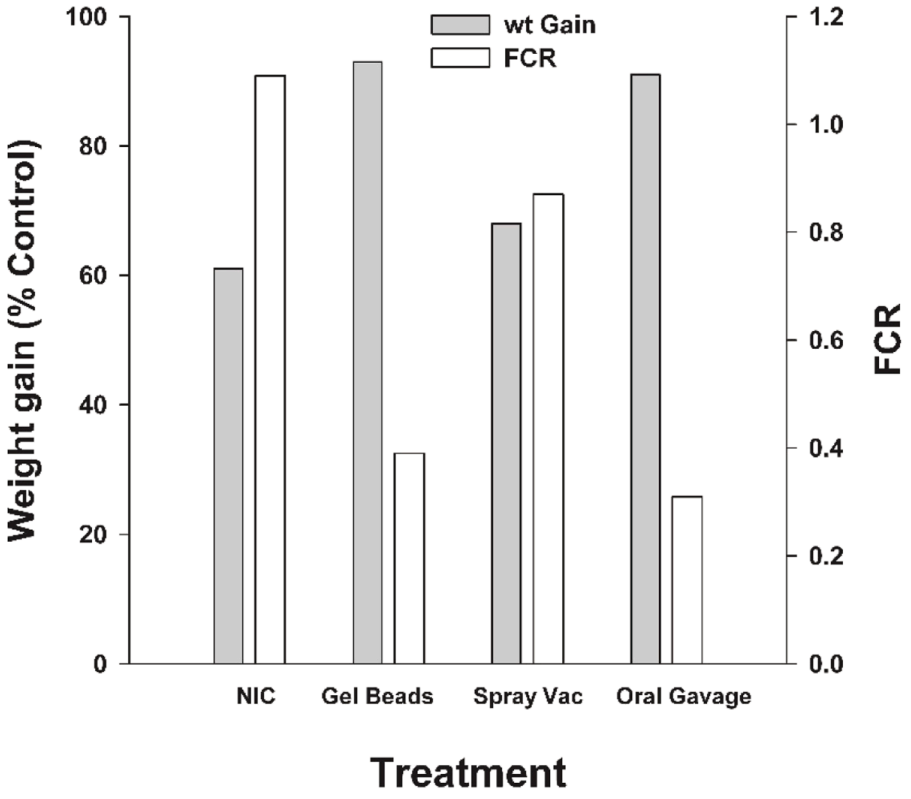

Fig. 2. Average percent body weight gain and increase in feed conversion ratio relative to non-immunized, non-challenge controls over a 7 day infection period in broiler chickens that were immunized at one-day of age with a mixture of *Eimeria* acervulina, *E. maxima*, and *E. tenella* oocysts by different delivery methods (gel-beads, spray-vaccination, or oral gavage), and then challenged at 4 weeks of age with a high dose of *E. acervulina, E. maxima*, and *E. tenella* oocysts. Data is the mean 3 individual trials.

Coccidiosis and Disease Interactions

Matthew K. Jones

Eimeria in poultry is important due to the primary infection, but the resulting disease processes that stem from this initial insult are equally critical. During the cycling of *Eimeria,* the later asexual phases of reproduction cause damage to the intestinal epithelium. This disruption

Table 5
Uptake of commercial *Eimeria* oocyst vaccine administered to chicks either at the hatchery by spray vaccination (day 0) or in a broiler house through the drinking water system (day 3). Uptake is defined as the number of chicks excreting detectable numbers of *Eimeria maxima* oocysts between days 5-8 post-vaccination.

Trial No.	Delivery Method	Percent Positive (# of positive chicks/total # of chicks)
A	Hatchery Spray (day 0)	0.0 (0/16)
	Drinking Water (day 3)	87.5 (14/16)
B	Hatchery Spray (day 0)	0.0 (0/16)
	Drinking Water (day 3)	87.5 (14/16)
C	Hatchery Spray (day 0)	0.0 (0/16)
	Drinking Water (day 3)	93.8 (15/16)
Mean ± *S*. D.	Hatchery Spray (day 0)	0.0 ± 0.0
	Drinking Water (day 3)	89.6 ± 3.6

can also be measured through changes in intestinal morphology, expression of tight junction proteins, absorption of nutrients, and increased permeability (FITC-d analysis) (Liu, et al., 2021). The epithelial layer of the intestine is an essential physical and immunological barrier protecting the birds from pathogens in the lumen of the intestine. *Eimeria* species erode this barrier and allow opportunity for pathogenic bacteria, and potentially even commensals, to come through and locally or systemically invade the host. Reproduction of the protozoal parasites also damages the principal site of nutrient absorption in the host, epithelial cells in the small intestine. This damage alters luminal nutrients and microbial populations which can also result in secondary health issues.

The most documented secondary infection in the US broiler industry associated with a primary *Eimeria* infection is necrotic enteritis; however, mycotoxins, small grain diets (higher in non-starch polysaccharides), high protein diets, management changes, and other stressors can also increase the risk of necrotic enteritis in broilers. Most coccidia species can induce necrotic enteritis, but some species, such as *Eimeria maxima,* pose greater risk than others (Nicholds et al., 2021). The disruption caused by *Eimeria* allows pathogenic *Clostridium perfringens* to infect the host and release toxins which cause both a systemic toxemia and necrosis of the intestinal epithelium. While the clinical impact of these two pathogens can be striking, the depression in performance in birds that do not succumb to the disease may be of greater economic and welfare impact due to the number of individuals affected. In experimental conditions which lack *Eimeria* challenge, there is minimal consequence to the birds (Liu et al., 2021). The broader implication here being that by limiting the coccidia influence, the disease can be more easily controlled.

The physical and physiological breach of the intestine by *Eimeria* has the potential to allow other organisms to infiltrate the host. *Escherichia coli, Staphylococcus aureus,* and *Enterococcus* species are intestinal commensals which translocate to different internal tissues. The presence of these bacteria has been well documented in disease conditions such as bacterial chondronecrosis with osteomyelitis (BCO) and spondylitis. Researchers studying interactions between *Eimeria* and bacterial infection have not observed a positive correlation (Baba et al., 1990; Borst, et al., 2019; Tellez, et al., 1994). In internal trials, there appears to be an increase in secondary bacterial causes of mortality, including polyserositis, airsacculitis, femoral head necrosis, and BCO, after early necrotic enteritis infections (*Eimeria* and *Clostridium perfringens* challenge) than in unchallenged groups. Excluding necrotic enteritis, mortality from secondary infection in necrotic enteritis studies was approximately double (0.086 %) the rate of the unchallenged groups (0.035 %; (Table 6). This may suggest an association between early intestinal damage and secondary bacterial disease later in the grow out of the broilers.

Not all interactions between *Eimeria* and bacterial disease are correlated with intestinal breach. *Eimeria tenella* infects the distal intestine in the ceca and has been associated with increased enumeration of *Salmonella* in intestinal samples and internal organs. Both *Salmonella* Enteritidis and *Salmonella* Typhimurium colonize at greater levels when there is coinfection with *E. tenella* (Arakawa, et al., 1981; Qin, et al., 1995). Conversely, low levels of *Eimeria tenella* have been associated with lower *Salmonella* colonization. This same group of researchers has reported *Salmonella* colonization can also respond to other *Eimeria* species (Takimoto, et al., 1984). So, in addition to impacts on bird health, there are also implications between *Eimeria* and food safety.

Necrotic enteritis, secondary infections, and *Salmonella* are each major issues for the poultry industry. These have direct impact on human health, animal welfare, and economic outcomes for the poultry industry. Coccidia by itself is a substantial concern to the poultry industry, but when secondary infections are considered the true depth of consequences resulting from *Eimeria* infections are much greater.

The Alternative Arsenal for Coccidiosis Management

Kayla R. Price

Coccidiosis, caused by *Eimeria* spp., continues to be a high ranked disease across the globe in commercial poultry production with much time and money being allocated to management. The global cost of coccidiosis has grown from $0.8 billion (2002) to $13.2 billion (2020), partly due to increased poultry production and higher percentage meat production being transitioned to programs that limit or eliminate antibiotic use, which often include ionophores (Blake et al., 2020). Additionally, in USA caged and caged-free pullets and layers, coccidiosis remains within the top three and ten challenges, respectively (USAHA, 2024). The impact of coccidiosis on the flock is further exacerbated by concomitant stressors.

Animal production has made use of feed additives (also known as alternatives) in nutrition for many years (Ilias et al., 2023). However, heightened voluntary and mandated regulation over antibiotics and, in some cases, ionophores as well as managing on-farm resistance has influenced the growth of this market (Ahmad et al., 2024; Kim and Lillehoj, 2019). The global feed additives market – including vitamins, minerals, and gut health products – has expanded over time with the market being valued around USD $36 billion in 2023 and expected to increase (Fortune Business Insights, 2024). In 2023, the USA broiler industry spent just over USD $ 1 to just under $ 4 per short ton of feed on gut health products to manage mostly parasitic and bacterial challenges (Agristats, March 2024, unpublished). In 2023 USA turkey production, alternatives and coccidiosis vaccine use represented ~18 % to 14 % of the surveyed market (~195 million turkeys), respectively, and were used to supplement anticoccidial drug programs (USAHA, 2023). Thus, the use of alternatives as a part of the holistic program to manage coccidiosis and its impacts on the host are more commonplace than in the past, albeit the products used and when they are applied can be unique to each location and flock.

A variety of alternatives and their mixtures have been used that include probiotics, pre-biotics, post-biotics, phytogenics, and antioxidants (Aguiar-Martins et al., 2023; Ahmad et al., 2024; Broom, 2021; El-Shall et al., 2022; Lee et al., 2022). Some of these additives have been

Table 6
Average% mortality per day.*.

Experiment Type	Daily Percent Mortality
Non-challenged	0.035 %
Necrotic Enteritis Challenge	0.086 %

* Average daily mortality from secondary bacterial disease from 14 to 42 days of age.

demonstrated to act diversly such as reducing parasite numbers, influencing the microbiota, supporting intestinal function, modulating oxidative stress, and supporting protective immune responses (Aguiar-Martins et al., 2023; Ahmad et al., 2024; Broom, 2021; El-Shall et al., 2022; Lee et al., 2022). Due to the dynamic interaction between *Eimeria* spp, intestinal microbiota, intestinal physiology, and host immunity, a program that supports the modulation of each component, or some combination, has been demonstrated in commercial production to be beneficial to strengthen resilience of poultry. As a result, synergistic blends have been developed and demonstrated to enhance the flock's defenses (Duffy et al., 2005; Mathis et al., 2016).

Between and within each category of feed additive there are many differences including selection criteria, growing conditions, processing, bioactive components, and mixtures. This variation has resulted in differing mode of actions, efficacy, and success in the field (Broom, 2021). Additionally, many studies on these additives have been conducted *in vitro* and may not be reflective of *in vivo* results (Aguiar-Martins et al., 2023; Broom, 2021; Sandu, 2019; Sundar et al., 2017). Regardless of the differences, numerous additives have shown promising results in research settings (Duffy et al., 2005; El-Shall et al., 2022; Lee et al., 2022) and have been implemented in the field.

The feed additives that are used to support coccidiosis management can be used in many ways, such as: 1) on their own; 2) with an anticoccidial drug program; 3) with a coccidiosis vaccine program; or 4) a combination of programs. Sometimes multiple strategies may be used with different flocks throughout the year, such as one strategy through the summer versus winter months, and the program can be unique to a particular company or even flock (Sandu, 2019).

Commonly, the challenge of coccidiosis and enteritis is "death by a thousand cuts" where multiple, simultaneous stressors that influence the dynamic Eimeria-microbiota-host connection escalate the impact. Consequently, understanding how alternatives act and perform in relation to challenges, controllable changes, and goals of production is essential to select a suitable combination and flexible program to complement management for location specific needs.

Declaration of competing interest

The authors declare no conflicts of interest.

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