Medicon Agriculture & Environmental Sciences
Volume 2 Issue 1 January 2022
Editorial

Current Approaches for Diagnosis and Prevention of Poultry Coccidiosis

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Received: December 15, 2021; **Published:** December 20, 2021

The global chicken industry is a thriving enterprise containing many sectors, including meat and egg production, from which approximately 3.5 billion chickens are produced annually. *Eimeria* spp is one of major hurdle in raising chickens profitably. Highly host-specific Eimeria are ever present in galliform birds and causes immense economic losses approximately US$ 2 to 3 billion annually. Coccidiosis has remained the focus of anxiety in the commercial poultry producers not only owing to the mortality losses in acute infections or lowered production, but also as a consequences of cost input required for effective chemoprophylaxis and immunoprophylaxis. The coccidiosis usually manifests itself in modern, intensive rearing environments where large numbers of immunologically naïve hosts are housed in close confinement. All species of *Eimeria* are responsible for causing coccidiosis with adverse impacts on growth rate and feed conversion ratio in naïve chickens exposed with large numbers of oocysts; however, some species viz. *E. acervulina, E. maxima, E. necatrix* and *E. tenella* are associated more frequently with clinical coccidiosis (i.e. diarrheal disease and obvious impacts on bird rearing profitability) with the eruption of macroscopic mucosal lesions typically seen during necropsy of affected birds. Infections with *E. brunetti*, *E. mitis*, and *E. praecox* may impact growth and performance but rarely demonstrate such macroscopic lesions and infrequently cause mortalities. In contrast, birds severely infected with *E. necatrix* or *E. tenella* will have bloody diarrhea, dramatic mucosal lesions (likely with obvious hemorrhage) and some mortalities are expected to result. Other species, such as *E. maxima* and *E.* acervulina (and occasionally *E. necatrix*), more often may predispose chickens to other diseases, specifically necrotic enteritis. Due to the ubiquitous and fecund nature of the parasite, finding a flock that is not shedding oocysts is rare. If house management and biosecurity measures are not proper, clinical disease is bound to occur. So, the diagnosis of the infection and identification of different species of Eimeria present in a farm are central to the prevention, surveillance and control of coccidiosis.

Diagnostic approaches for poultry coccidiosis

The classical parasitological methods of diagnosis include morphological identification and enumeration of oocyst, lesion scoring and demonstration of schizonts, gamonts or other pathogenic stages of *Eimeria* spp in the intestinal mucosal scrappings. However, The number of oocysts per gramme (OPG) in faeces or litter has a weak relationship with the parasite's impact on a flock's performance. Furthermore, identifying various species based on oocyst morphology is difficult, time-consuming and expertise is required. Lesion scoring is a method of interpreting macroscopic apparent lesions. Eimeria spp.-caused lesions are usually graded on a scale of zero to four. For a Total Mean Lesion Score of (TMLS) individual scores for all species are routinely compiled (six per flock). The procedure is incredibly time-consuming, intensive, subjective at times, and only reliable when performed by trained professionals The link between lesion scores and performance is thought to be stronger than with other variables. OPG, however it's still difficult to gauge the severity of lesions in terms of their impact on the environment.

Lesion scoring, on the other hand, is still the most often used diagnostic approach today. Lesion scores of more than 1.5 per species are deemed clinical, whereas those of less than 1.5 are considered subclinical and do not require treatment. It has been proven that macroscopic and microscopic lesions might have a poor relationship, stressing that macroscopic lesion scoring alone is insufficient to detect all economically important coccidiosis infections.

COCCIMORPH, a computational approach for identifying Eimeria pp. from chicken and rabbit, is a very innovative technique for oocyst identification. Curvature characterisation, size and symmetry, and internal structure characterization are all examined on images from sporulated oocysts from a proven species. Users can submit digital photographs of unidentified oocysts, and the computer will identify the species. This is quite accessible, and the low cost is a significant benefit. One downside is that only sporulated oocysts can be recognised, making this technique only useful for litter sample identification. While traditional methods for diagnosing coccidiosis had limitations, significant advances in biochemical and molecular diagnostics such as multilocus enzyme electrophoresis (MEE), southern analysis, pulsed field electrophoresis (PFGE), inversion electrophoresis (FIGE), amplified fragment length polymorphism random amplification polymorphic DNA or arbitrarily-primed (AP-PCR), sequence characterised amplified regions (SCARS), and sequence characterised ampl Several polymerase chain reaction (PCR)-based tests targeting various sections of the Eimeria genome have been developed, including ITS-1 (first internal transcribed spacer), ITS-2 (second internal transcribed ribosomal DNA), EASZ240/160 (subunit sporozoite antigen gene), and others.

Prevention and control poultry coccidiosis

Biosecurity and disease preventive strategies must be implemented to combat coccidiosis in commercial poultry. Many aspects of the chicken industry may be contaminated by biosecurity. In a single flock or across flocks, appropriate house management may limit the danger of contamination among birds or from pests (vermin or flying insects) due to carryover of used litter or an inadequately cleaned chicken house. Anticoccidial live vaccination is used to prevent coccidiosis. A range of measures are employed to lessen the negative impact of coccidiosis, including anticoccidial medication prophylaxis, selection of disease resistant chicken breeds, and augmentation of immunity. Prophylactic in-feed anticoccidial medication has been employed by the chicken industry as the prevention control method shortly after their discovery in the 1940s. Coccidiostatic activity (endogenous development halted but can resume after drug withdrawal) and, depending on the type of drug used, coccidiocidial activity (Eimeria species parasites are killed during endogenous development) were both observed at preventative doses of prophylactic in feed medication. However, as these preventative medications became more effective, the parasite's reliance on them created an ideal environment for drug resistance to evolve. Increased customer concerns about drug residues in chicken products, in addition to drug resistance, created a niche (and rising) market for antibiotic-free poultry management.

Vaccination is a long-term approach for coccidiosis control that may be utilised on a large commercial scale and is not limited by drug resistance. In terms of vaccinations, there has been some resistance in the broiler (meat) sector to using vaccines based on live, attenuated oocysts for a variety of reasons, including cost, potential harmful effects on chicken growth, and the rapid induction of protection in short-lived birds. Live virulent organisms, live attenuated parasite strains, non-infective parasite derivatives, and genetically designed subunit vaccines are among the Eimeria spp. vaccines available.

First commercial vaccine to fight poultry coccidiosis was registered under the name Coccivac and launched in the USA in 1951. The vaccine was a cocktail of a counted number of live oocysts of *E. acervulina*, *E. maxima* and *E. mitis* developed for the broilers. All the vaccines present in the world market are based upon varied formulations of live virulent or live attenuated parasites. The process of attenuation has been achieved by several passages in chicken embryos or using the praecocoius lines (early shed oocysts). Because of the smaller schizont size and fewer schizogonic phases, early oocysts have been proven to be less harmful. CoccivacB and CoccivacD, VACM, ADVENT, and Inovocox are live virulent (non-attenuated) vaccines available in the United States; ImmucoxCI and ImmucoxC2 are registered in Canada; and NobilisCox ATM is registered in the Netherlands. Eimeria Vac (China), Eimeriavax 4m (Australia), Hipracox Broilers (Spain), Inmuner Gel Coc (Argentina), LivacoxQ and LivacoxT (Czech Republic), Paracox5 and Paracox8 (UK), and Supercox (China) are the live attenuated vaccines.

Use live vaccine increasing poultry production with chickens reared on the floor with litter in cages. Live vaccines require handling that must incorporate basic understanding the parasite life cycle, specifically the exogenous and transmission portion the life cycle. Since live Eimeria vaccines administer small dose live, infective parasites to the host, this control method partly dependent what the

oocyst needs to become infective and how the vaccine progeny oocysts are transmitted in the field. Thus, live Eimeria vaccines are constrained by the atmospheric and physical barn environment ensure the vaccine reaches its full protective potential.

The production of recombinant vaccines for coccidiosis being attempted of late as both the Eimeria genome and genes encoding the chicken immune system have been investigated. While the mouse-prokaryotic pathogen system has been studied extensively, identification the genes and specific antigens the numerous Eimeria species and life cycle stages that are responsible for inducing protective immunity in chickens have acted the rate limiting step in recombinant coccidiosis vaccine discovery. Recombinant coccidiosis vaccines hold the promise of delivering antigens effective in inducing protective (possibly complete) immunity in a safe, sustainable, cost-effective form suitable for mass application without resorting to live oocyst use. Main targets of recombinant vaccine development are being pursued are: 1) sporozoites and merozoites; and 2) gametocytes (gamonts). The former may be the natural choice for vaccine development because sporozoites initiate infection, therefore blocking this stage should halt an infection prior to any damage to the host. The latter has been targeted in the commercial vaccine CoxAbic® a means to block oocyst wall formation during gametogony thus reducing oocyst formation and shedding. Native gametocyte (sexual stage of the parasite) antigens (APGA) from parasites are used to make CoxAbic®. Because there are no in vitro culture techniques for the growth of Eimeria's sexual phases, parasites must first be separated from their intracellular location within chicken intestines.

A large number of putative coccidial antigens have been identified and cloned. AMA1, MICI, MIC2, MIC3, MIC5, IMP1, SAG, HSP70, Lactate dehydrogenase, Rhomboid-like proteins, TA4, GAM56, GAM82, GAM22, profiling, S07, and other potential antigens are being investigated and tested in laboratories throughout the world. for their immune and vaccine potential. However, genetic polymorphism of the candidate genes in different isolate/strains of the parasite from diverse geographical locations need to be studied in order to come out with an effective vaccine for wide acceptability.

A delivery mechanism for coccidial vaccines that produces optimum resistance to challenge infection has yet to be determined. Immunogenic Eimeria antigens have been given as isolated proteins with adjuvants, as recombinant antigens in live vectors such as nonpathogenic E. coli strains, Salmonella enterica poxviruses, fowlpox virus, and turkey herpesvirus, and by direct plasmid DNA injection with varying degrees of effectiveness. Immune stimulating antigens with established potential functions throughout various stages of parasite establishment, invasion, and propagation provide important information for creating cocktail vaccines that target the parasite at various stages of development.

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