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The diagnostic aspects of chicken coccidiosis

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Abstract

Coccidial diagnosis in poultry is a fundamental and critical issue due to the disease's significant economic impact, the losses it causes that affect production and material returns, and the fact that the disease can occasionally take an invisible form. Clinical signs, necropsy Findings, Laboratory may be assist to diagnosis the disease. In recent years, molecular methods have been used. With proper management, the use of anticoccidial and sulfa medications, vaccinations, and some natural food additives, this disease, as well as monetary losses, can be avoided.

Keywords: Coccidiosis, necropsy, infection site, laboratory diagnosis

1. Introduction

Intra Chickens are the world's most intensively raised poultry and offer a nutritious, affordable, and high-quality protein source. *Eimeria* is the most economically significant poultry parasite [1]. Blake and colleagues (2020) Chlamydia causes coccidiosis, a common avian disease, most notably in chickens, which can have serious consequences for the development of poultry production, Numerous tropical and subtropical areas exhibiting advantageous ecological and managerial circumstances have endemic oocyst coccidians sporulation and development [2]. The most intensively raised fowl in the world, chickens are a good, affordable, and healthful source of protein. *Eimeria* is the poultry parasite that has the biggest economic impact [1]. Blake and colleagues (2020) Chlamydia causes coccidiosis, a common avian disease, most notably in chickens, which can have serious consequences for the development of poultry production [2]. The majority of tropical and subtropical areas with ideal ecological and management circumstances have endemic occidian oocyst development and sporulation [3]. Cellular parasites spreading to new hosts *Eimeria* belongs to the Apicomplexa phylum, Coccidia class, Eucoccidiorida order, and Eimeriidae family of protozoa. With an apical complex that facilitates host cell penetration, invasive extracellular sporozoites carry these obligatory intracellular parasites to new host cells [4]. The nine species of *Eimeria* that can infect hens are gumboro, *Eimeria acervulina*, *Eimeria mivati*, *Eimeria maxima*, *Eimeria necatrix*, *Eimeria brunetti*, *Eimeria pracox*, *Eimeria tenella*, and *Eimeria hagani* [5]. *E. tenella* is a species with a high rate of pathogenicity of oocyst production that causes coccidiosis in young chickens, whereas *E. nectarix* is equally virulent but produces fewer oocysts that cause the disease in older birds. The most important factor influencing the prevalence of coccidiosis infection in chickens is their age [6]. Between the ages of 32 and 46 be associated with a higher prevalence of coccidiosis, Young birds are more susceptible to disease show symptoms sooner, whereas older chickens have a higher level of resistance due to prior infection [7].

Aim of study

Coccidial infections are a major economic concern, and this disease has been linked to the occurrence of other diseases such as necrotic enteritis, requiring knowledge of the methods for diagnosing this disease and thus lowering the risk of this disease.

Etiology

Coccidiosis in chickens is caused by the intracellular protozoan parasite *Eimeria* species. This organism is a member of the class Coccidia, phylum Apicomplexa, order Eucoccidiorida, family Eimeridae, genus *Eimeria*, and kingdom Protozoa.

Of the nine coccidians, only seven (*Eimeria*) species previously identified as pathogenic for poultry coccidiosis have been identified. *E. necatrix* and *E. tenella* are two pathogenic *Eimeria* spp. *E. acervulina*, *E. maxima*, and *E. mivati* are among the *Eimeria* spp. with low to moderate pathogenicity (*E. mivati*). While *E. brunetti* is uncommon, it can be dangerous when it does appear. *E. mitis*, *E. praecox*, and *E. hagani* are non-pathogenic *Eimeria* species [8].

Many species of *Eimeria* share the same coccidia oocyst shape. They have sporocysts, an ellipsoidal or circular form, and a strong cell wall. *Eimeria* oocysts are typically ovoid in shape.

The largest *Eimeria* species is *E. maxima* (30.5 x 20.7 m), which is followed by *Eimeria mitis* (15.6 x 13.4 m) (15.6 x 14.2 m) and *Eimeria mivati* (15.6 x 13.4 m). *E. necatrix* is shaped differently than *E. tenella*, *E. maxima*, *E. acervulina*, and *E. hagani*. *Eimeria* species are host-specific and develop in the gastrointestinal tract (intestinal villi epithelial cells or crypt cells), and they are all ovoid in shape. The site of development in afflicted chicken guts and the species of *Eimeria* that infect chickens are shown [9].

Diagnoses

Clinical symptoms, coprology, pathomorphological, and pathohistological examinations are used to diagnose coccidiosis [10]. Recently, various biochemical and molecular techniques have been used.



Bloody feces, ruffled feathers, anaemia, reduced head size, and somnolence are all observed clinically [11].

Symptoms include pallor, cozziness, agitation, and depression. After four to five days, chickens infected, coccidia become weak and fatigued, and young birds frequently have blood on their faces. The birds' eyes eventually close, their wings fall off, and many die [12]. The development of clinical coccidiosis and the management method are related to the number of sporulated oocysts a bird consumes at one time, the virulence of the *Eimeria* species, and the age of the diseased chicken. Infected chickens rapidly degrade their performance and productivity. Egg production in laying hens will fall [13]. Most of the total economic loss is caused by subclinical coccidiosis, which shows up as low weight gain and reduced feed conversion efficiency.

Findings from a Necropsy

Coccid In chickens, postmortem examination of a

representative number of birds is most effective method of identifying coccidiosis is best diagnosed in chickens through postmortem examination of a representative number of birds [14]. The nature of the intestinal contents, the location of the lesions on the digestive system, their appearance and severity, and any other noticeable changes related to them, can aid in the determination of a diagnosis. Under a microscope, a lesion is examined. Smears taken from suspected lesions can reveal coccidial development stages. It is possible to see oocysts, schizonts, and gametocysts, which are pathogenic to some coccidioid species. The severity of coccidiosis lesions is determined by the degree of digestive tract inflammation and damage. Intestinal wall thickness, mucoid to blood-tinged exudates, hemorrhagic enteritis, petechial hemorrhages, necrosis, and mucous profuse bleeding in the ceca are a few of these signs.

Coccidiosis causes the intestines to thicken, giving them the appearance of sausages. Light-colored spots on the gut's surface are possible, as are hemorrhages and streaks within the gut. *Eimeria species* are revealed by the type and location of lesions in the intestine [15].



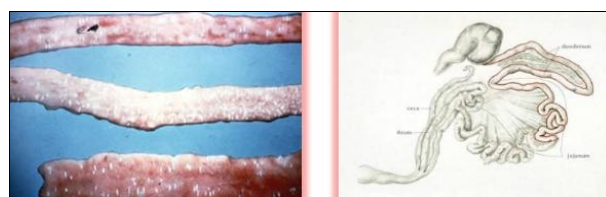
Significant congestion and dilatation of the caecum with a significant amount of blood exudate (A), a friable and mucosa-free caecal wall, especially in figures B and C [23].



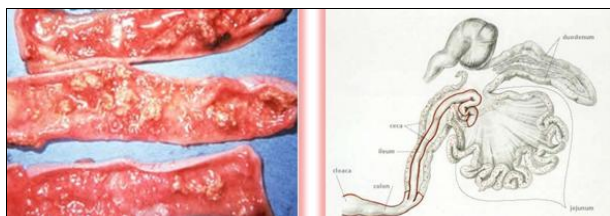
In a broiler chicken, gross lesions include *E. tenella* lesions with obvious bleeding into the cecal pouches [12].



Gross lesions: frank bleeding in the middle of the stomach *E necatrix* [16].



E acervulina gross lesions (white longitudinal plaques in the duodenum of a broiler chicken) [16].



E. brunette gross lesions in a broiler chicken's small intestine ^[16]

Parasite morphology

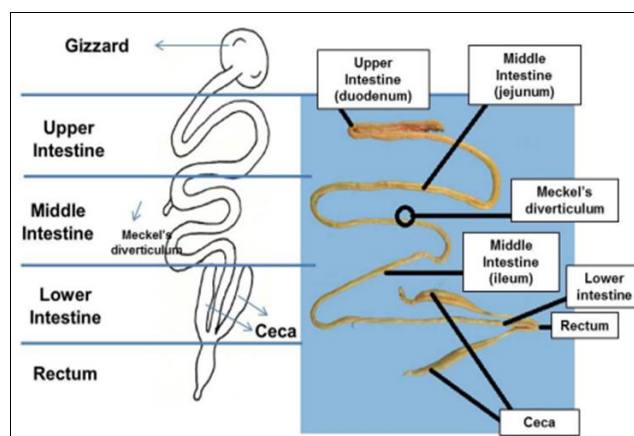
Coccidian parasites have three developmental stages: schizonts, gonadotrophs, and oocysts.

The size of schizonts varies according to parasite species, host location, and stage of maturity. Mother meronts are small, spherical, basophilic cells that dwell intercellularly within host cells.

Generate a large number of daughter merozoites through endogenous nucleotide division and cytokinesis. Membrane-bound clusters of small basophilic bodies represent mature schizonts. Individual schizonts are typically larger than megalo-schizonts (as in a bunch of grapes) (up to 1 mm in diameter) (17). demonstrates that female macrogamonts are uninucleate eosinophilic cells with a single ovoid nucleus, while male microgamonts are multinucleate basophilic stages that finally shed microscopic biflagellated microgametes. The robust outer oocyst walls are formed by the many eosinophilic wall-forming bodies found in growing oocysts. The growing sporoblast in unsporulated oocysts eventually sporulates, creating sporocysts that contain infectious sporozoites. The characteristic 1:4:2 shape of *Eimeria* oocysts indicates that each oocyst has four sporocysts, each of which has two sporozoites. Oocysts can have specific structures including polar caps, micropyles, residuals, and crystalline bodies. They are 10–40 mm long by 10–13 mm wide ^[18].

Infection site

Male microgamonts are multinucleate basophilic stages that eventually shed microscopic biflagellated microgametes, while female macrogamonts are uninucleate eosinophilic cells with a single ovoid nucleus. The stiff outer oocyst walls are the result of the presence of many eosinophilic wall-forming bodies in developing oocysts. In unpopulated oocysts, a growing sporoblast sporulates and generates sporocysts that contain infectious sporozoites. Because of their distinctive 1:4:2 configuration, *Eimeria* oocysts contain four sporocysts, each of which has two sporozoites. With specific features including polar caps, micropyles, residuals, and crystalline bodies, oocysts are 10–40 mm long and 10–13 mm wide ^[18].

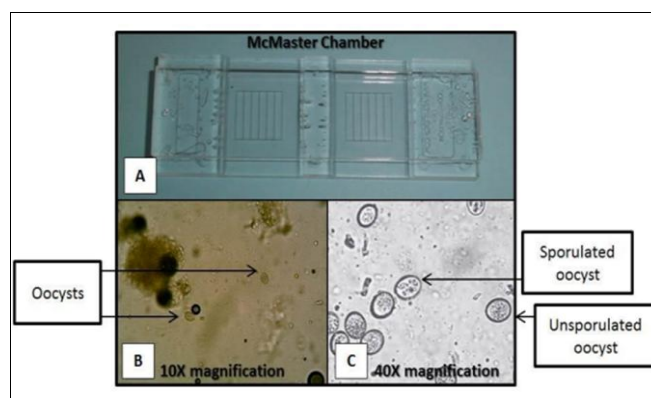


Normal poultry intestine ^[19]

<i>Eimeria</i> spps.	Oocyst size	Host species	Site infection	Pathogen-necity
<i>Eimeria acervulina</i>	18x14Mm	Chickens	Anterior small intestine	High
<i>E. brunetti</i>	26x22Mm	Chickens	Small and large intestine	High
<i>E. maxima</i>	30x20Mm	Chickens	Mid small intestine	Moderate
<i>E. necatrix</i>	20x17Mm	Chickens	Small intestine, caecum	High
<i>E. tenella</i>	23x19Mm	Chickens	Caecum	High

Laboratory Diagnosis

Clinical chemistry values, bone marrow counts, and blood smears are only occasionally checked. To find oocysts in the excrement of sick birds, the flotation approach using a saturated salt or sugar solution is essential. McMaster developed the counting chamber technique. The number of oocysts found per gram of feces is recorded. The McMaster counting chamber technique is described in detail here.



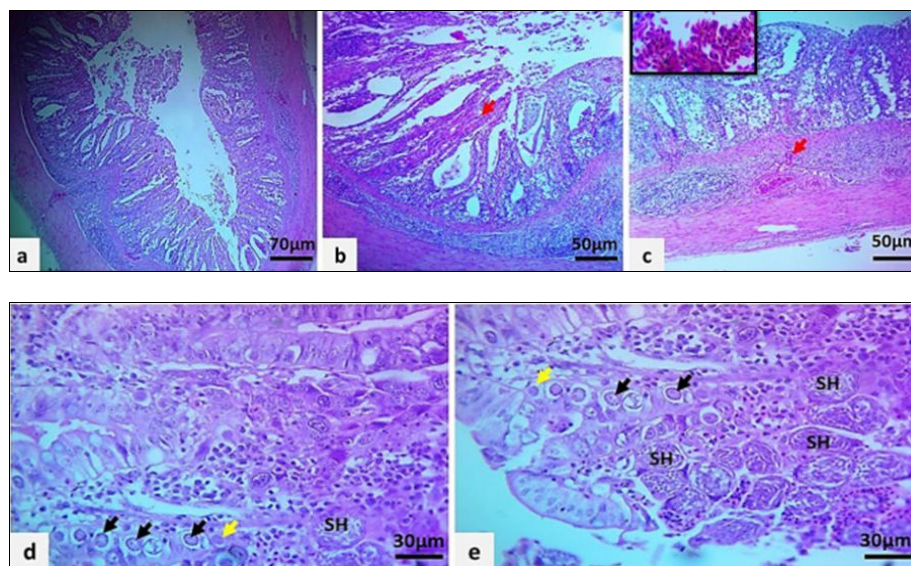
The McMaster chamber (A) can be used to count oocysts. Oocysts in a fecal sample can be seen at low (B) and high (C) magnifications against a background of plant material and air bubbles. At a higher magnification (C), it is easier to distinguish between sporulated and unsporulated oocysts. Photographers: A, B ^[20].

It can find subclinical infections, *Eimeria* oocysts from the digestive contents of chickens are also collected using the concentration flotation technique. To isolate *Eimeria* oocysts, measurements using a calibrated ocular micrometer at 400x magnification are required.

Eimeria species identification is aided by the size, shape, and minimum sporulation time of oocysts ^[21].

Histopathological analysis

The microscopic features revealed that the mucosal lining epithelium was widely shed, that necrotic material was shed in the lumen, that the mucosal fold was noticeably atrophying, which led to blunting of the mucosa with atrophy of the caecal crypts, and that these cells replaced chronic inflammatory cells, which spread throughout the caecal layers (a-c). *Eimeria* develops in stages in the submucosal layer: gametocytes, such as macrogametes, which have a central nucleus and eosinophilic bodies lining their border; immature and mature oocysts, which are identified by their oocyst wall and central nucleus; and microgametes, which have many crescent-shaped merozoites in addition to growing schizonts grouped in a cluster (d. e) ^[22, 23].



Microscopic analyses of the caecum in naturally infected chickens with *Eimeria* species revealed: a–c: severe sloughing of the lining mucosa with necrotic debris in the lumen, atrophy of the mucosal folds, and transmural inflammatory cell infiltration with significant bleeding (red arrows and inset). d and e: The several stages of *Eimeria* include oocytes, macrogametes (black arrows), microgametes (yellow arrows), and schizonts (SH) with many eosinophilic coccidial merozoites in the submucosal layer (H&E stain) [23].

Bloody faeces, dysentery, and diarrhea are all symptoms of coccidiosis. ELISAs are capable of identifying antibodies in blood samples (sera) and can be configured to identify antibodies that are specific to particular epitopes, despite the fact that serology is the most widely used disease surveillance technique in commercial poultry. Because ELISAs detect antibodies rather than parasites, they can identify which species. Even when the parasites are gone, hens still have an immunological reaction. Additionally, they enable the assessment of vaccination-induced immunological responses. In diagnostic labs, ELISAs are high-throughput assays that are used to rapidly screen a large number of samples [24].

Molecular analysis

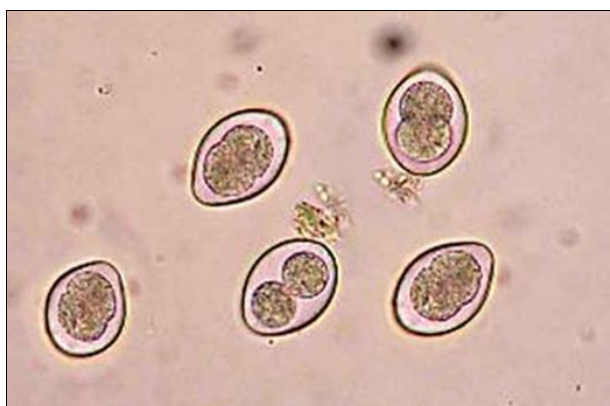
The development of molecular diagnostic technology has advanced significantly in recent years. Because they simply employ the DNA sequence, they have a few advantages over traditional methods. Of the species *Eimeria*, primers that target distinct sections of the *Eimeria* genome have been used in a number of PCR-based experiments that have been published [25]. Even though PCR-based techniques are crucial for identifying *Eimeria* species, one drawback of this assay is the challenge of obtaining DNA from *Eimeria* oocysts. Because *Eimeria* oocysts isolated from feces are resistant to chemicals, disrupting them is essential for obtaining high-quality DNA. Numerous chemical compounds have been shown to disrupt oocysts [26]. To identify *Eimeria* species, several investigations have employed quantitative techniques such as real-time PCR (qPCR) amplification of ITS-1, ITS-2, and SCAR markers in addition to PCR [27, 28]. Melting curve analysis and primers conserved in different protozoa were utilized in a qPCR experiment to identify *E. acervulina* in an oocyst combination [29]. used a high-resolution melting curve analysis in conjunction with a qPCR targeted at ITS-1 sequences to validate the identification of all seven *Eimeria* species from pure oocyst cultures. The expensive cost of this assay has prevented its widespread adoption. For the identification of

Eimeria species, loop-mediated isothermal amplification (LAMP) has been devised as an inexpensive substitute for molecular approaches [30]. Reactions can be carried out without the use of thermocyclers or electrophoresis since this method makes use of isothermal enzymes and intercalating dyes. Consequently, the technology currently performs better than previous molecular techniques. The LAMP test has been used to detect apicomplexan parasites in a number of published publications [31]. But before this method can be regarded as a standard assay, a lot more work needs to be done. The existence of intraspecific and intragenomic changes in these genes, which contribute to their poor species delineation, is the only disadvantage to their extensive usage in *Eimeria* identification. Given this [25], reported that cytochrome c oxidase subunit 1 (COI) is very successful in identifying the species of a variety of organisms, including *Eimeria*. Given this, cytochrome c oxidase subunit 1 (COI) has been shown to be quite successful in identifying the species of a variety of organisms, including *Eimeria*. There are numerous published research on the application of COI to *Eimeria* species identification [15, 32]. One of this gene's limitations in species identification is the absence of reference sequences. Therefore, a new method for identifying *Eimeria* species that is presently being studied is combining various loci, such as ITS and COI. Preventing Coccidiosis Because of the severe effects of coccidiosis on poultry, a number of management measures have been put in place. Strict biosecurity and preventative measures were the initial methods of disease control. Synthetic medications including amprolium, nicarbazine, diclazuril, and toltrazuril have been effectively used for many years to treat coccidiosis in poultry [33]. The medications target the merozoite and sporozoite phases of the parasite's development. However, this approach to controlling coccidiosis disease is limited by the advent of drug-resistant *Eimeria* parasite isolates [34]. A significant percentage of field isolates of *E. acervulina*, *E. maxima*, and *E. tenella* from 28 farms across 12 US states showed partial or whole resistance to popular synthetic medications such as narasin and nicarbazin (Bafundo *et al.* 2008). Public pressure to lower medication levels in the food chain has led to the search for alternate coccidiosis control methods [34].

Species Recognition

Because of their distinct gross lesions, five of the species, *E. acervulina*, *E. brunette*, *E. maxima*, *E. necatrix*, & *E. tenella*, are well known and easily identified. The pathogenicity of

these organisms ranges from mild to severe. *E. praecox* and *E. mitis*, on the other hand, do not kill chickens or cause pathognomic lesions and are frequently misunderstood. Experimental infections, on the other hand, cause enteritis, diarrhea, and decreased feed efficiencies, indicating that two species are almost certainly capable of causing commercial losses and must therefore be controlled ^[24]. Clinical signs, characteristic lesions at specific infection locations in the chicken intestine, and assessment of the prepatent time, oocyst size, and intracellular morphology can all be used to identify the five most pathogenic species listed above. Less dangerous species of *E. mitis* and *E. praecox* might go undiscovered. Regular *Eimeria* testing and diagnosis techniques use colored images of big lesions caused by the most common species of *Eimeria*. The application of a molecular biological method to differentiate species according to isozyme composition was first introduced by Aleen and Fetterer (2002). Using rRNA and rDNA probes with unique restriction fragment patterns, individual species were identified. A randomly amplified polymorphic DNA assay was used to identify within-strain variations between *E. acervulina* and *E. tenella*. Different strains of *E. tenella* were identified using recombinant DNA techniques, along with markers for drug-resistant and precocious strains. Six *Eimeria* species were identified and differentiated using PCR amplification of internal transcribed spacer region I from genomic DNA ^[35]. Eight species, including *E. hagani*, were reportedly identified using a two-step PCR process, while six were identified using a PCR-linked restriction fragment length polymorphism technique.



Coccidia oocyst with multiple sporocysts ^[36]

D. Diagnosis

Sometimes, hemorrhagic enteritis, necrotic enteritis, and other intestinal disorders are mistaken for intestinal coccidiosis. Salmonellosis and histomoniasis can infect caecal coccidiosis due to their comparable lesions ^[37].

Conclusion

This disease is clearly one of the most important in poultry because of its economic impact. As a result, we advocate for the possibility of conducting research and identification studies in early diagnosis, particularly since the subclinical form of infection occurs occasionally with some species of infection, and thus the possibility of controlling and avoiding infection.

Conflict of Interest

Not available

Financial Support

Not available

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