CEVA HANDBOOK
OF POULTRY DISEASES

5 COCCIDIOSIS

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DEFINITION

Coccidiosis is a common protozoan disease found in domestic birds and other fowl, characterized by enteritis and bloody diarrhoea. The intestinal tract is affected, with the exception of the renal coccidiosis in geese.

HISTORY AND SYNONYMS

In 1910, H. B. Fantham, a parasitologist at the University of Cambridge, UK, described the life cycle of the coccidiosis parasite in birds. Further progress in coccidiosis research was made about 30 years later at Harvard University – USA, by E. Tyzzer. His studies laid the foundations of our contemporary understanding of coccidiosis and the *Eimeria* species involved in this illness. The results from the studies conducted by W. T. Johnson at Western Washington, Oregon Agricultural Experiment Station can also be added to these achievements, as a major contribution in the field (Chapman, 2003). The first live anti-coccidial vaccine appeared in the middle of the 20th century (Shirley and Long 1990).

CHARACTERISTICS AND CLASSIFICATION OF THE PATHOGEN

Nine species of *Eimeria* are capable of infecting chickens. Depending on the localisation of lesions in the intestines, the coccidioses are divided into caecal, induced by *E. tenella*, and small intestinal, induced by *E. acervulina, E. brunetti, E. maxima, E. mitis, E. mivati, E. necatrix, E. praecox and E. nagani*. All are intracellular parasites belonging to the two genera of the Eimeridae family: *Eimeria* and *Isospora*. The differences between both genera of parasites is related to their exogenous development, with the *Eimeria* forming in their oocysts 4 sporocysts with 2 sporozoites, and the *Isospora* forming 2 sporocysts with 4 sporozoites each. Under suitable external conditions, with warmth (the optimal temperature is 29°C), moisture and oxygen, sporulation occurs within 24–48 hours. At temperatures below 8°C, most of the oocysts perish, but in those which survive, sporulation could go on for more than 8 weeks (Obreshkov et al., 1978). Transmission of *Eimeria* from chickens to other bird species is impossible, with the exception of cases in which severelyimmunosuppressed birds were used (McDougald & Fitz-Coy, 2008). After entering the digestive tract, the oocyst wall is broken, which marks the beginning of the endogenous development. The

released sporozoites enter the cells of the intestinal mucosa and asexual division – schizogony – is initiated. After 2 or 4 generations of asexual development, of micro - and macrogamettes are formed, which is the beginning of the second (sexual) stage of the coccidia's development - gametogony. There follows the release of a zygote-containing oocyst from the intestinal mucosa into the intestinal lumen and its excretion with the faeces. This endogenous development (schizogony and gametogony) continues for 4–6 days, while exogenous development in completed within 2 days, so that the entire reproduction process takes 7–8 days (McDougald & Fitz-Coy, 2008).

Seven species infect turkeys – the big three of concern are *Eimeria meleagrimitis, E. adenoeides*, and *E. gallapovonis* (Helm, 1999).

Oocysts exhibit a considerable resistance in the environment, capable of retaining their viability for years. The environment most conducive to their survival is a depth of 5 cm down in the soil. At lower temperatures (–6°C to –10°C) fewer than half of the oocysts remain vital. After 2–3 consecutive frosting/ Defrostingcycles, all perish. At temperatures of 60°C they can survive for 15–20 min, at 70°C – for 2 min, while at 80°C they die instantly (Obreshkov et al., 1978).

EPIDEMIOLOGY AND PATHOGENESIS

These bacteria primarily affect younger birds as immunity develops relatively quickly after exposure, protecting against repeated infection at a later age. It should be taken into consideration, however, that there is no cross-immunity between the *Eimeria* species in birds, and consequent outbreaks could be caused by different species. In birds at a later age, most commonly around the beginning of laying, heavy outbreaks with high mortality rates may occur, provoked by stress as well as by the short direct cycle and the high reproductive potential of the coccidia. Depending on the amount of ingested oocysts, the illness could be mild or acute

(McDougald & Fitz-Coy, 2008).

Chickens are infected through feed that has been contaminated with oocysts from the environment. In field conditions, it is nearly impossible for chickens remain uninfected. Sources of oocysts are adult birds, as well as ill or recovered birds. The infected chickens shed oocysts via their faeces for several weeks.

Predisposing conditions are poor hygiene, breeding in close proximity to mixed-age flocks, overcrowding, unbalanced feeding, etc. The wet litter and the heat in premises favour sporulation and therefore, the outbreak of coccidiosis. Most oocysts can be found in the litter of broilers aged between 3 and 5 weeks. The main route of oocyst spread is mechanical –via staff, equipment, etc. The ubiquitous spread of coccidia makes their elimination or prevention through sanitation, disinfection or quarantine impossible (McDougald & Fitz-Coy, 2008).

Damage to the mucosa and the changes in the functions of the intestines that occur as a result of the life cycle of the oocysts create an opportunity for the multiplication of various pathogens, most of all clostridia, as well as *Salmonella spp., Histomonas*, etc. (Arakawa et al., 1981; Mcdougald & Hu, 2001).

CLINICAL SIGNS AND PATHOLOGY

Caecal coccidiosis is among the most commonly observed conditions. It affects commercial broilers, growing stock layers and their breeders. They are caused by *E. tenella,* which damages the caecal mucosa, as a result of which severe haemorrhages occur. It is characterised by high morbidity, dehydration, weight loss and mortality.

Fig.1

Clinically, bloody faeces, ruffled feathers, anaemia, reduced head size and somnolence are observed.

Fig.2

The area around the vent is stained with blood.

Fig.3

Pathoanatomically, dehydration and a high degree of anaemia of the body and viscera are discovered.

Fig.4
Anaemic appearance of internal organs.

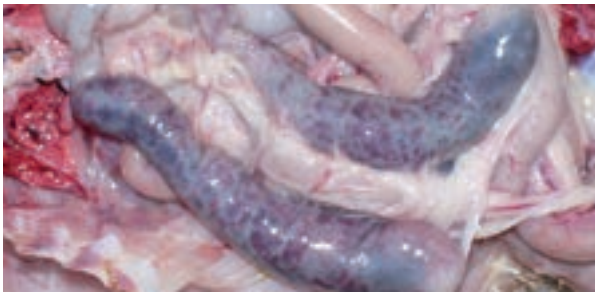

Fig.5
In caecalcoccidiosis, a marked typhlitis is present.

Fig.6
Caecalcoccidiosis. Haemorrhages are seen through the intestinal wall.

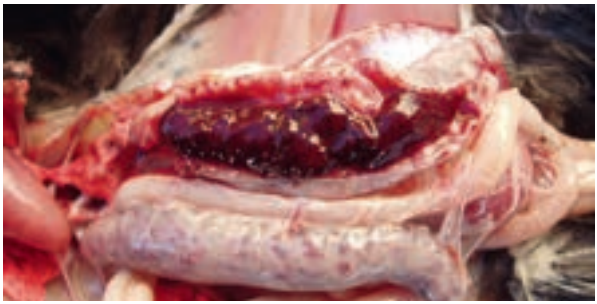

Fig.7

The caeca are filled with fresh or clotted blood.

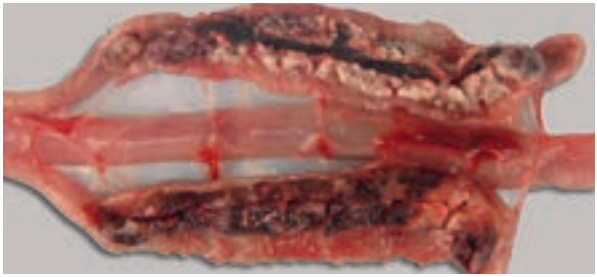

Fig.8

At a later stage, the caecal content becomes thicker, mixed with fibrinous exudate and acquires a cheese-like appearance.

In small intestinal coccidioses, depending on the Eimeria species, haemorrhages of various intensities are observed in different parts of the intestine.

Fig.9

In many instances, the haemorrhages are petechial and visible through the intestinal wall.

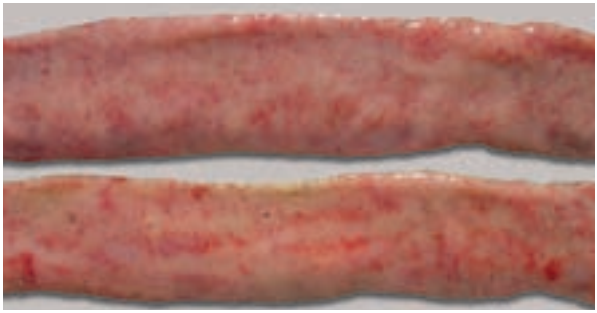

Fig.10

Haemorrhages are usual found on the mucosal surface after opening of the intestinal lumen.

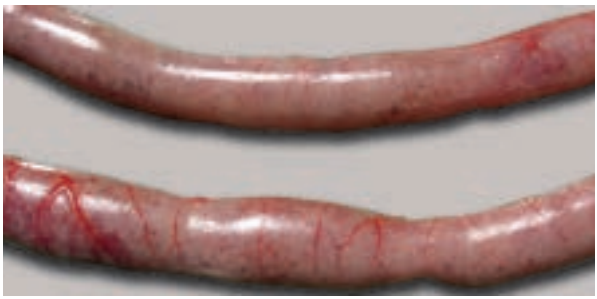

Fig.11

Sometimes, a reaction of the intestinal lymphoid tissue is present.

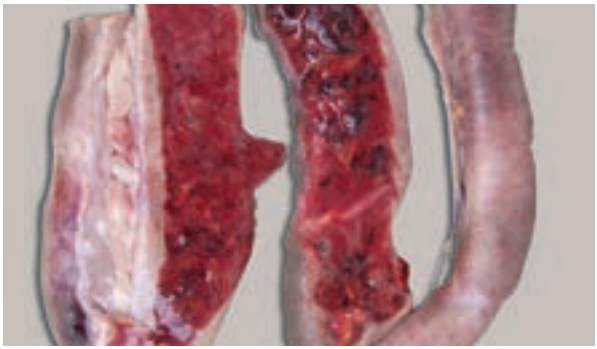

Fig.12

The content is mixed with fresh or clotted blood, and the mucous coat is mottled with multiple petechial or larger haemorrhages.

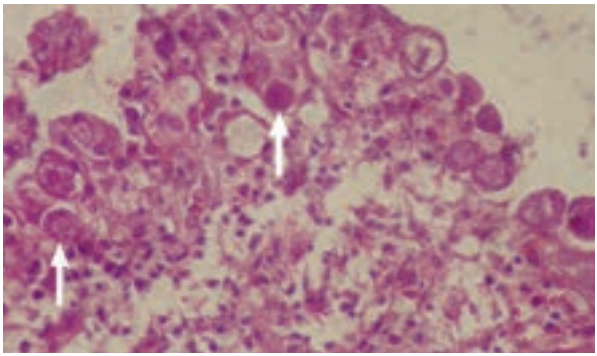

Fig.13

Histologically, developmental forms (arrows) in a different stage of *Eimeria* life cycle are detected in epithelial cells of intestines. H/E, Bar = 25 µm.

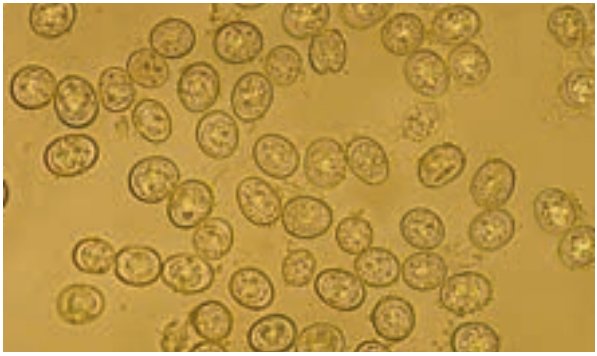

Fig.14

The microscopic examination of a native preparation of intestinal content or of superficial mucosal layer reveals numerous oocysts in one observation field, native preparation, Bar = 10 µm.

DIAGNOSIS

Diagnosis is made on the basis of a thorough analysis of the complex clinical circumstances, macroscopic lesions, imprint preparations, histological study and flotation.

To differentiate between the species, the following features have to be taken into account: area of intestinal lesions, oocyst morphology, minimum sporulation time, minimum prepatent time, size and location of schizont development, etc. (Conway & McKenzie, 2007).

Macroscopically, diagnosis is

possible if major lesions are present. In subclinical or chronic cases, microscopic examination is necessary in order to detect oocysts. Samples for detection of the oocysts can be taken from faecal matter, intestinal content or the litter. Litter samples should be collected from several different areas of the surface layer, avoiding the moist litter under the watering troughs. The separate samples should be mixed well, with 5 g of this mixture retained. The sample is then suspended in 2.5% potassium bichromate for a few hours, after which it is filtered and resuspended in a saturated NaCl solution for analysis (Conway & McKenzie, 2007).

Retrieving oocysts from faecal matter or infected intestinal tissue is possible after homogenization, mixing with water and filtration through a sieve into a glass container. The supernatant is thrown away and the oocysts are resuspended in potassium bichromate. The oocysts are then separated from the surface layer with a pipette.

To detect coccidia in the intestinal tissue, routine histological techniques can be used. Various stages of development of the *Eimeria* organism can be detected by staining cross sections with hematoxylin-eosin.

DIFFERENTIAL DIAGNOSIS

Coccidioses should be differentiated from NE, UE and histomonosis (typhlohepatitis).

TREATMENT

Sulfonamides are widely used: sulfadimethoxine, sulfaquinoxaline, sulfamethazine. They should not, however, be used in layer hens. Vitamin A and K supplements can aid recovery.

MANAGEMENT, PREVENTION AND CONTROL

Coccidiosis prevention depends on a number of factors, including dosage of coccidiostatics and vaccines, building and equipment management, as well as feed quality control (Conway, 1996).

The use of coccidiostatics with forages on a rotation basis is the most widely-used method. Coccidiostatics can be included in the feed of broiler chickens from one day of age up to a few days before slaughter. Errors in the coccidiostatic programme, restrictive feeding in growing breeder flocks, or withdrawing the coccidiostatic a few days before slaughtering of the birds can increase the risk of a coccidiosis outbreak. In commercial broiler production, the polyether ionophores play a major role in anticoccidial protection in rotational or shuttle programmes. In shuttle programmes, chemical coccidiostatics are used in starter feeds on a rotational basis, with ionophore drugs used in grower feeds (Eckman 1993). An inversed system is also possible: ionophores in the starter feed and chemical coccidiostatics in the grower (Conway et al. 2001). This latter arrangement does raise certain concerns regarding seasonal effects, as well as past experience. Based on empirical observations, and in light of medication resistance issues, the use of chemical coccidiostatics with polyether ionophores in shuttle programmes for 3–4 fattening cycles, or in rotational programmes for 1–2 cycles in rotation with polyether ionophores or anticoccidial vaccines, is recommended (Conway & McKenzie, 2007).

The duration of coccidiostatic programmes depends on the quality of the feeds, as well as on the proper mixing of the coccidiostatic in each batch of feed.

Environment management

Environmental factors that are of significance for the control of coccidioses and poultry health include: stocking density, litter condition, temperature, feeding and water provision, ventilation and illumination equipment. At temperatures causing discomfort, feed consumption decreases and thus so does consumption of the coccidiostatic. Insufficient ventilation could lead to moist litter, which facilitates the proliferation of *Eimeria*. An increase in litter humidity by 15% – 25% is conducive to the sporulation of oocysts.

Moist litter after the application of anticoccidial vaccines could foster the recurrence of the infection (Conway & McKenzie, 2007).

High population density also poses

an increased risk of coccidiosis due to the competition for food and water among the chickens, as well as a higher concentration of oocysts (Hamet et al. 1982). An insufficient feeding and drinking width due to overcrowding has an influence on feed consumption, and thus over the intake of coccidiostatics.
The quality of the feeds, i.e. adequate levels of protein, minerals, nutritional supplements and coccidiostatics, is of considerable importance for the prevention of coccidioses.

Anticoccidial vaccines

Live non-attenuated vaccines are able to induce long-term protective immunity. The host's immunity is species-specific and the live vaccines must contain a mixture of *Eimeria* species, each with a varying degree of pathogenicity. A balance must be struck between the infective dose and pathogenicity, and care taken to avoid including new pathogenic strains in vaccines for flocks that have not been previously exposed.
Another problem is the antigenic variability between the species of *Eimeria* included in the vaccine and those encountered in the field (Martin et al., 1997). Because only a small number of the more virulent *Eimeria* species can be used for live vaccines, protective immunity depends on live parasites infecting the host as a result of several cycles of faecal proliferation and repeated ingestion, which increases the oocyst dose. The parasite load thus increases over time, gradually developing immunity.

Live attenuated vaccines

Vaccines incorporating attenuated live parasites allow us to avoid some of the problems associated with pathogenic field strains. Successful attenuation through serial passages in embryonated eggs has been accomplished for *E. mitis*, *E. necatrix* and *E. tenella*, but not *E. acervulina*, *E. maxima* and *E. praecox* (Shirley & Long, 1990). This method, however, was soon abandoned due to the loss of virulence during the parasites' passages through the eggs. The attenuation of oocysts has also been achieved through irradiation (Gilbert et al., 1998). Irradiation affects the parasites' reproductive potential.

Recombinant protein vaccines

When developing recombinant protein vaccines, a critical consideration is the identification of the

stage of the life cycle at which the parasite induces protective immunity. Sporozoites are the preferred parasitic form for the preparation of recombinant vaccines, as they are procured relatively easily. Brothers et al. (1988) characterised the surface antigen of the *E. tenella* protozoites and cloned the corresponding gene into *E. coli*. It has been reported that the recombinant antigen of *E. acervulina* sporozoites stimulates the host's natural immunity, while the recombinant antigen associated with the later stages of development (merozoites) induces cellular and humoral response, which are effective in natural and passive immunity (Jenkins et al., (1988). The recombinant antigen (GX3262) of the *E. acervulina* sporozoite induces partially protective immunity after a single inoculation of chickens at the age of 2 days, which is visible from the caecal lesions, compared to non-inoculated chickens (Miller et al., 1999). Cellular surface antigens may also be suitable components of vaccines, due to their direct role in the relationship between the parasite and the host (Lillehoj & Lillehoj, 2000).

Recombinant DNA vaccines

DNA vaccines use genes coding for immunogenic proteins of the pathogens. DNA vaccination requires gene transfer and antigen expression in a tissue accessible to the immune system, such as the skin or mucosal surface. Two methods of DNA administration are used: independent DNA introduction, and insertion into the tissues through particle bombardment (gene gun). The observed result is immune protection with considerable reduction of faecal oocyst proliferation in chickens vaccinated subcutaneously with DNA-coded protein of *E. acervulina* inducing production of IFN-γ (Lillehoj et al., 2000).

In order to enhance and prolong the specific immunity to coccidiosis, various adjuvants and immunomodulators have been tested as potential stimulators of humoral and cellular immunity after vaccination against coccidiosis. Prolonged and stronger effects in such cases are attributed to the increased production of IFN-γ, or its combination with a specific cellular immune response. These results are typical for IFN-γ in immune prevention control against coccidiosis in commercial broiler flocks (Lillehoj & Dame).

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