Pathology caused by three species of *Eimeria* that infect the turkey with a description of a scoring system for intestinal lesions

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**ABSTRACT**

Three-week-old turkey poults were infected with pure lines of three species of *Eimeria* (*E. adenoeides*, *E. gallopavonis*, and *E. meleagrimitis*) recently isolated from commercial turkey farms. The lines had been propagated from a single oocyst and identified by species-specific PCR amplification of the mitochondrial cytochrome c oxidase subunit I (COI) gene. Five to six days after infection their intestines were removed and examined for the presence of intestinal lesions. A description and review of the pathology caused by these parasites is provided, and a scoring system developed by which the severity of the lesions can be evaluated. The system is similar to that described by Johnson, J. and Reid, W. M. [1970. Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. *Experimental Parasitology* 28, 30–36] for chickens in which a score of zero to four is assigned to lesions of increasing severity. The intestinal lesions observed here, and their assigned scores, are supported by representative illustrations. It is hoped that they may prove a useful tool for evaluating the pathology caused by *E. adenoeides*, *E. gallopavonis*, and *E. meleagrimitis* in the turkey.

**RESEARCH HIGHLIGHTS**

- A scoring system has been developed for intestinal lesions caused by three species of *Eimeria* that infect the turkey.
- The lesions attributable to these species are illustrated.

**Materials and methods**

**Birds and husbandry**

Newly hatched (day-old) turkey poults (Nicholas breed) that had not been vaccinated against coccidiosis were utilized in the experiments. They were reared in...
brooder cages at a stocking density of 257 cm²/poult until they were 14 days of age. Poults were moved to grower cages at 2 weeks of age, where they were kept until the end of the experimental period. Husbandry and management followed Guidelines for the Care and Use of Animals in Agricultural Research (FASS, 2010) and all experiments were approved by the Institutional Animal Care Committee. All poults were fed an unmedicated, nutritionally adequate corn-soybean diet (NRC, 1994) and had free access to water.

Parasites

All three parasites used in this study were initially obtained from faecal samples collected from farms in the USA where turkey poults were raised commercially. Oocysts were harvested using standard methods (Shirley, 1995) and propagated in poults that had been reared in the absence of infection. A pure line of each species was obtained by isolating a single oocyst according to the procedure described by Shirley & Harvey (1996) and subsequent propagation in naïve poults. Identity of each species was confirmed by species-specific PCR amplification of the mitochondrial cytochrome c oxidase subunit I (COI) gene according to procedures described by Rathinam et al. (2015). In addition, the PCR product for each species was sequenced and subjected to BLAST analysis to confirm identity. For all three species, BLAST analysis of the sequence resulted in high identity (100% coverage and 100% identity) to respective GenBank sequences (KJ608415.1 for E. adenoeides; KJ608413.1 for E. gallopavonis, and KJ608414 for E. meleagrimitis).

Characterization of gross lesions

In order to characterize lesions, groups of 21-day-old poults (four replicates/group; eight poults/replicate) were inoculated with 0.05 − 4.0 × 10⁵ sporulated oocysts of each species per bird. Control birds were sham inoculated with water. On days 5 and 6 after inoculation they were euthanized, and their intestines examined for the presence of lesions.

Results

Based upon examination of 256 poults infected with each species of Eimeria, a scoring system and a description for lesions of increasing severity were established (Tables 1–3). The lesions evident in the intestines of selected poults are illustrated in Figures 1–3. The infection doses and the average lesion scores observed for each dose are provided in Table 4. An increase in lesions was apparent with an increase in the dose for all three species. Of the two days when lesion scoring was performed, lesions were pronounced on day 5 for E. adenoeides and E. meleagrimitis and on day 6 for E. gallopavonis.

### Table 1. Lesion scoring system for E. adenoeides.

| Score | Description |
|-------|-------------|
| 0     | No gross lesions are present. |
| 1     | Caecal contents are watery, clear or creamy white in colour, frothy and may or may not contain grains of caseous material. Petechial haemorrhages may be seen on the caecal mucosal folds. |
| 2     | Caecal contents contain frothy exudate. Caseous material is organized into small clumps/clots and is seen mixed with the exudate. Petechial haemorrhages may be seen on the caecal mucosal folds. |
| 3     | Caecal contents are replaced by caseous material organized into cores as multiple fragments (and not into one plug). Petechial haemorrhages may be seen on the caecal mucosal folds. |
| 4     | Caeca appear pale and are filled with caseous material. Caseous material is organized into one long plug, taking the shape of the caecal pouch. Petechial haemorrhages may be seen on the caecal mucosal folds. Birds dying due to coccidiosis will be assigned a score of 4. |

### Table 2. Lesion scoring system for E. gallopavonis.

| Score | Description |
|-------|-------------|
| 0     | No gross lesions are present. |
| 1     | Contents of the ileum appear normal. Petechial haemorrhages may be seen in the lower ileal mucosa, the ileo-caecal junction, and rectum along with a few small, white, round spots. Caecal contents are normal but grains or clots of caseous material may be present in the caecal neck. |
| 2     | Contents of the ileum appear normal. Numerous petechiae and white spots (wider spots) are visible in the lower ileal and rectal mucosa. A white caseous exudate may accumulate in the caecal neck forming into a plug, sometimes extending into the proximal third to the middle of the caecal pouch. |
| 3     | Ileal contents contain a small amount of soft cheesy material that accumulates in the lumen and is sometimes mixed with orange clots, fibrin strands, and green mucoid material. Numerous petechiae and white spots are seen in the ileal mucosa that may coalesce to form a white layer that completely covers the lower ileal mucosa and extends into the upper ileum. Petechial haemorrhages are seen in the rectum and the rectal contents may be watery or mixed with cheesy exudate. Caseous plugs in the caecal neck (sometimes extending into proximal parts of caecal pouch) may be present. |
| 4     | The serosal surface of the ileum and rectum appears white with prominent blood vessels. Large amounts of white cheesy exudate fill the entire length of the gut from Meckel’s diverticulum to rectum, sometimes forming clots or plugs. Caseous exudate may be mixed with orange clots/fibrin strands/green mucoid content. Caseous plugs in the caecal neck (sometimes extending into proximal parts of caecal pouch) may be present. Birds dying due to coccidiosis will be assigned a score of 4. |

### Table 3. Lesion scoring system for E. meleagrimitis.

| Score | Description |
|-------|-------------|
| 0     | No gross lesions are present. |
| 1     | Duodenum has watery contents with fibrin strands, clots and mucus. Duodenal mucosa may or may not have petechiae. |
| 2     | Fibrin in the duodenum present in a thin layer covering the duodenal mucosa and extending into the proximal part of the jejunum. Petechiae may be present on the duodenal mucosa. Watery contents present in the distal portion of the jejunum. |
| 3     | Fibrin layer is mixed with small blood clots and extends from the duodenum into the jejunum. Duodenal wall is thickened and petechiae may be found on the mucosa. |
| 4     | Duodenal wall is greatly thickened. Extensive necrosis and haemorrhage visible on the mucosal surface of the duodenum and jejunum. Birds dying due to coccidiosis will be assigned a score of 4. |
caseous exudate and formation of plugs. Petechial haemorrhages were observed sporadically in the caecal mucosa but were not consistently correlated with any score. While the parasite develops in the lower ileum, in all the birds that were sampled, no discernible lesions were observed in the lower ileum and rectum (Table 1; Figure 1(a)–(e)).

Pathology and lesions of *E. gallopavonis*

Lesions were primarily seen in the ileo-caecal junction, caecal neck, and rectum, and included petechiae and the presence of few to numerous white round spots on the ileal and rectal mucosa (Figure 2(b,c)). In severe infections, lesions extended throughout the ileum all the way to the Meckel’s diverticulum with an accumulation of soft, white, cheesy caseous exudate that was occasionally mixed with orange or green mucoid content. The accumulation of caseous exudate in the caeca was confined to the caecal neck or proximal one-third of the caeca with the remainder of the caecal pouch unaffected (Table 2; Figure 2(a)–(e)).

Pathology and lesions of *E. meleagrimitis*

Lesions were primarily seen in the duodenum and proximal parts of jejum and included oedematous intestines with accumulation of mucus, formation of diphtheritic membranes, necrosis and sloughing. Petechial haemorrhages were observed sporadically in the duodenal mucosa but were not consistent (Table 3; Figure 3(a)–(e)).

Discussion

Seven species of *Eimeria* are recognized from the turkey but, of these, the three included in this study (*E. adenoeides*, *E. gallopavonis*, and *E. meleagrimitis*) are considered the most pathogenic (Lund & Farr, 1965; Chapman, 2008). *E. adenoeides* develops in the rectum and caeca, *E. gallopavonis* in the ileum, rectum, and caeca, and *E. meleagrimitis* principally in the duodenum and jejunum extending in heavy infections to the lower intestine (Chapman, 2008). Their prevalence is difficult to determine because of the lack of clear-cut criteria for identification, but all three are considered to occur quite frequently in the field (Clarkson & Gentles, 1958; Edgar, 1986).
Figure 2. Appearance of macroscopic lesions of *E. gallopavonis* in the lower ileum and caecal neck of turkey poults observed 6 days following inoculation. Pictures labelled a (score 0), b (score 1), c (score 2), d (score 3), and e (score 4) represent various grades of lesions. Pictures b and c show ileum only. Arrow in b points to the small, white spots seen on ileal mucosa in score 1.

Figure 3. Appearance of macroscopic lesions of *E. meleagritis* in the duodenal mucosa of turkey poults observed 5 days following inoculation. Pictures labelled a (score 0), b (score 1), c (score 2), d (score 3), and e (score 4) represent various grades of lesions.
E. adenoeides included petechial haemorrhages in the lower small intestine, caeca and rectum, an accumulation of yellow–white mucoid material in the lower small intestine, and cheesy caseous clots in the caecum (Clarkson, 1958). According to Reid (1972), pathology and lesions of E. adenoeides include congestion, oedema, petechiae, strands of mucus, blood, and a “cottage cheese-like” material in the caeca, lower small intestine and rectum. More recently, the lesions were described as white caseous plugs in the caecal pouches and limited petechial haemorrhage without the severe blood loss that is usually observed in caecal coccidiosis in the chicken (El-Sherry et al., 2014a). Similar pathology and lesions were seen in the present study.

Pathology and lesions of E. gallopavonis

Although initially described by Hawkins (1952), the first descriptions of the pathology and lesions produced following infection with E. gallopavonis include marked inflammatory changes in the lower small intestine, rectum, and proximal portions of caeca with accumulation of soft, white, cheesy material in the lumen of the affected organs, oedema, epithelial sloughing, and lymphocytic infiltration (Farr et al., 1961; Wehr et al., 1962; Lund & Farr, 1965; Reid, 1972). Wall thickening in the distal ileum and rectum, petechiae, clotted blood in the ileum, and caseous plugs in the caecal neck have been reported (Vrba & Pakandl, 2014). According to El-Sherry et al. (2019), pathology and lesions caused by E. gallopavonis include necrosis, oedema, epithelial sloughing and white caseous material in the ileo-caecal junction, caecal neck and rectum. In heavy infections, the caseous material filled the lumen of the intestine from Meckel’s diverticulum through to the rectum. Many of these characteristics were observed in the present study.

Pathology and lesions of E. meleagrimitis

The first descriptions of pathology and lesions caused by E. meleagrimitis were oedema and congestion in the duodenum and upper small intestine with accumulation of brown necrotic material with streaks of blood (Hawkins, 1952; Clarkson & Gentles, 1958). Lesions described by Clarkson (1959) were an enlarged, congested duodenum with presence of a red-brown necrotic core, sometimes extending into the upper small intestine. Hein (1969) reported haemorrhagic enteritis, marked vascular congestion, and a mucosa covered with blood stained tissue debris in the proximal part of the small intestine. Similar descriptions were provided by Reid (1972) and Joyner (1973) including congestion and accumulation of a reddish brown mucoid necrotic core/exudate in the duodenum and jejunum. More recently, pathology and lesions caused by this species included necrosis, sloughing of the epithelium, ulceration, petechiae and mucoid casts mainly in the duodenum and jejunum (El-Sherry et al., 2014b). Another study reported petechiae, wall thickening, and a large amount of mucus in the duodenum and jejunum, as well as watery contents in the ileum (Vrba & Pakandl, 2014). Similar pathology and lesions were observed in this study.

Review of lesion scoring procedures for turkey coccidia

No lesion scoring system has been described for E. gallopavonis. Lesions in turkeys were evaluated following infection with a mixed coccidial culture based on a visual examination of intestinal damage, a score from 0 to 4 being assigned with 4 the most severe (Mathis, 1993). No descriptions of the actual lesions observed were provided. A procedure described by Abd El-Wahab et al. (2013) for E. adenoeides relies on the number of petechial haemorrhages and thickening of the caecal mucosa but does not take into account the accumulation of caseous material in the pouches, which is a major pathological lesion for this species. There are a few resources that describe a five-point scoring scale for E. meleagrimitis (El-Sherry et al., 2014b; Immucox® Turkey Lesion Scores originally developed by ANSES, France). Whereas the Immucox® procedure relies mainly on the presence of pseudo-membranes and blood in the intestine and minimal changes in mucosa, especially in lower scores, the method proposed by El-Sherry et al. (2014b) considers...
mucosal changes including congestion, the presence of petechiae, wall thickening, and necrosis.

**Development of a standardized scoring system**

Due to the lack of clear, standardized lesion score descriptions in the literature, a refined five-point scale scoring system with clear descriptions for different grades of pathology was developed based on observations from pathogenicity studies performed with pure cultures of *E. adenoeides*, *E. gallopavonis*, and *E. meleagrimitis*.

The lesion scoring system described here is one criterion that may be of value in determining the severity of *Eimeria* infection in turkeys. While a dose-dependent positive trend of lesion scores was observed in our study, it is important to carry out a preliminary experiment to determine an optimal dose of oocysts because of the time-consuming work involved. The procedure is inherently subjective and should be performed by an experienced worker familiar with the pathology caused by the parasites and blinded to treatments. As in the chicken, the method is of particular use for evaluating the efficacy of anticoccidial drugs in preventing coccidiosis and establishing the occurrence of drug resistance, evaluating coccidiosis vaccine efficacy and routine monitoring of turkey coccidiosis in the field. Caution should be exercised in utilizing lesion scores in experiments concerned with immunity since lesions may be present in partially immune or completely immune chickens even though weight gain is not depressed and the lesions contain few or no parasites (references in Chapman *et al.*, 2005). Some studies in chickens have demonstrated limitations to the use of lesions alone in assessing drug efficacy as there is a non-linear relationship between the magnitude of infection (oocyst dose) and lesion scores (Conway *et al.*, 1990). However, used in conjunction with other criteria, such as weight gain during the acute phase of infection, lesion scoring has proved the most widely employed procedure for evaluating the deleterious effects of coccidiosis upon galliform birds.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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