Pathology of Coccidiosis in Broilers Infected with Field Isolates of *Eimeria tenella* and Mixed Infection of *E. necatrix* and *E. maxima* in Makurdi, Benue State

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Abstract

Fifteen (15) commercial day old broiler chicks were experimentally infected at 14 days of age using field strains of *Eimeria species* in Makurdi. The experimental infection lasted for two weeks. At the commencement of the infection (day 14 [day 0 PI]), birds of group T1 (n=5) and M1 (n=5) were inoculated with 3x10^4 sporulated oocysts of *E. tenella* and a mixture of *E. maxima* and *E. necatrix* respectively. Birds of group X (n=5) served as the un-infected control group. The infected birds in both groups manifested clinical signs such as anorexia, depression, weakness, bloody diarrhoea and ruffled feathers. A mortality rate of 20 % (1/5) was observed in group T1. *Eimeria tenella* and mixed infection of *E. maxima* and *E. necatrix* caused a significant (p<0.05) reduction in the body weight and PCV of birds in groups T1 and M1 respectively. However, there was no significant difference (p>0.05) in the body weight and PCV between group T1 and group M1 birds. The gross lesions comprised of mild to severe haemorrhagic enteritis/typhilitis with necrosis of the intestinal mucosa and the thickening of the wall of the intestine and ballooning of portions of the intestines in some cases. Microscopically, there was necrosis and sloughing off of the epithelium, severe necrosis of the mucosa and severe hemorrhagic typhilitis with infiltration of mononuclear cells and eosinophils into the intestinal mucosa. Developmental stages of the parasite in the lamina propria and sub mucosa were prominent in *E. tenella* infection while the parasites of *E. maxima* and *E. necatrix* where restricted to the epithelium and lamina propria. In conclusion, the most prevalent species were found to be pathogenic.

Keywords: Field isolates, Pathology, *Eimeria species*, Coccidiosis, Makurdi.

1. Introduction

Coccidiosis is one of the major diseases of poultry farms worldwide (Amer et al., 2010). Both clinical and subclinical forms of the disease are responsible for huge losses in the poultry industry (Lilić et al., 2009). Coccidiosis in chickens is caused by intracellular protozoan parasites belonging to the genus *Eimeria* (Waldenstedt et al., 2001). Epidemiological studies have established coccidiosis as a major parasitic disease in Nigeria (Majaro, 1980; Majaro, 2001; Etuk et al., 2004; Jatau et al., 2012; Olanrenwaju and Agbor, 2014; Mohammed and Sunday, 2015).

Prior, to this study, we embarked on a study on the prevalence of coccidiosis in Makurdi which revealed a high prevalence rate of 40.1 % with *E. necatrix*, *E. tenella* and *E. maxima* being the most prevalent of all the species identified (Agishi et al., 2016). In order to comprehensively access the status of chicken coccidiosis in Makurdi, attention must be given not only to the prevalence of coccidiosis and *Eimeria species*, but also to the pathology caused by the most prevalent species in Makurdi. The high prevalence rate of coccidiosis also necessitates the need to study the pathology of the disease in Makurdi.

2. Materials and Methods

2.1 Birds and Management

Thirty (30) day old broiler chicks were purchased from a commercial hatchery (CHI Ibadan)
and reared in coccidia free wired floor cages for a period of two weeks to prevent accidental coccidial infection before the experimental infection. The birds were fed ad libitum using commercial feeds (Top feeds®). At two (2) weeks of age, birds of similar body weights were weighed and allocated to three properly disinfected deep litter pens (5 birds per pen). The routine vaccinations and anti-biotic treatments were administered in accordance with the guidelines of the National Veterinary Research Institute (NVRI) Vom, Nigeria.

2.2 Parasites

Field isolates of *E. tenella* and *E. maxima/E. necatrix* were obtained from intestinal segments of infected broilers at slaughter slabs in Makurdi, Benue State. *Eimeria tenella* oocysts were harvested from the caeca while *E. maxima* and *E. necatrix* oocysts were harvested from the middle one third of the intestine using the floatation method (Holdsworth *et al*., 2004). The oocysts were sporulated in 2.5 % potassium dichromate (K$_2$Cr$_2$O$_7$) solution for 48 hours in petri-dishes at room temperature. Oral suspensions were prepared and adjusted to 15,000 sporulated oocysts per ml using the method of Holdsworth *et al* (2004).

2.3 Experimental Design

The birds were divided into three groups (T1, M1 and X) comprising five birds each at 14 days of age. Birds of groups T1 and M1 were inoculated each with 2 ml of the oral suspension containing 30,000 sporulated oocysts of *E. tenella* and *E. maxima/E. necatrix* respectively. Group X birds were not inoculated (control group). A fourteen (14) day period after inoculation was used to record observations and parameters after which the birds were killed by cervical dislocation.

2.4 Parameters Evaluated

2.4.1 Clinical Signs and Body Weight

Birds of the various groups were monitored daily following infection for clinical signs of coccidiosis. The birds were weighed in grams (g) using a weighing balance at day 14 (day of infection), day 21 (7 days post infection) and day 28 (14 days PI).

2.4.2 Packed Cell Volume (PCV)

Half a millilitre (0.5 ml) of blood was collected from each bird by cardiac puncture using a tuberculin syringe. The blood was transferred into EDTA test tubes, placed on a rack and transported to the laboratory for processing. The PCV was determined using standard capillary method as described by Kelly (1979).

2.4.3 Gross Pathology and Histopathology

Post mortem examinations were carried out on birds that died during the experiment and on the birds sacrificed after the experiment. Sections from the caecum and the middle one third portion of the intestine were cut using a sharp knife and fixed in 10% formalin. The fixed sections were routinely processed and stained with Haematoxylin and Eosin (H and E) using the method of Drury and Wallington (1980).

2.4.4 Data Analysis

Data collected from the study were analyzed using Graph Pad Prism® software version 6.07. The results were subjected to a one way analysis of variance (ANOVA) and when significant (p<0.05), the means of variables (body weight and PCV) were compared by Tukey’s multiple comparison test - TMCT (q).

3. Results and Discussion

All chickens experimentally infected with *E. tenella* (group T1) and *E. maxima/E. necatrix* (group M1) showed clinical signs of depression, weakness, bloody diarrhoea, anorexia, ruffled feathers and death (group T1). The clinical signs observed were consistent with the clinical signs of coccidiosis as reported by Rabo *et al.* (2002); Shane (2005); Zulpo *et al.* (2007) and Dakpogan *et al.* (2012). A mortality rate of 20 % (1/5) was observed in group T1, indicating that *E. tenella* was more pathogenic than *E. maxima* and *E. necatrix* combined.

Table 1 shows the mean body weights of the infected groups and control groups on day 0 PI, day 7 PI and day 14 PI. There was no significant difference in mean body weights between groups T1, M1 and X at day 0 PI (F = 0.1441, P = 0.8672). However significant differences in mean body weight between groups T1, M1 and X were observed at day 7 PI and day 14 PI (F = 15.28, P = 0.0007 and F = 62.08, P <0.0001 respectively AVOVA). Experimental infection with *E. tenella* and *E. maxima/necatrix* elicited statistically significant reductions in the mean body weight of birds as from the 7th day PI, agreeing with the findings of Rabo *et al.* (2002) where *E. necatrix* was used to experimentally infect cockerels. There was no significant difference between the mean body weight of birds infected with *E. tenella* (T1) and birds infected with both *E. maxima/necatrix* (M1). This contradicts the theory by Gussem (2008) that *E. tenella* has less effect on growth and feed conversion rate than *E. maxima* and *E. necatrix* because its predilection site (the caeca) is less important in the process of digestion and absorption than the mid-intestine. No significant difference in mean PCV values between groups T1, M1 and X were observed at day 0 PI (F = 0.7126, P =
Table 1: Effect of *Eimeria tenella* infection and mixed infection of *E. maxima/E. necatrix* on mean body weight

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean Body Weight (g)</th>
<th>Day 14 (Day of Infection)</th>
<th>Day 21 (Day 7 PI)</th>
<th>Day 28 (Day14 PI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>199 ± 31.30</td>
<td>275.5 ± 22.2</td>
<td>430 ± 34.64</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>207 ± 24.90</td>
<td>316 ± 27.02</td>
<td>425 ± 20.92</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>207 ± 24.90</td>
<td>402 ± 28.64</td>
<td>590 ± 23.45</td>
<td></td>
</tr>
</tbody>
</table>

Different superscripts (*, **) within columns indicate significant difference (p<0.05) between the means of respective groups. T1: Infected with *E. tenella*, M1: Infected with *E. maxima* and *E. necatrix*, X: Uninfected (control).

Table 2: Effect of *E. tenella* infection and mixed infection of *E. maxima/E. necatrix* on PCV

<table>
<thead>
<tr>
<th>Groups</th>
<th>PCV (%)</th>
<th>Day 14 (Day of Infection)</th>
<th>Day 21 (Day 7 PI)</th>
<th>Day 28 (Day14 PI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>28 ± 1.9</td>
<td>18.2 ± 2.4</td>
<td>18.3 ± 4.6</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>29.2 ± 1.6*</td>
<td>19 ± 4.5</td>
<td>20.8 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>29 ± 1.6</td>
<td>29.6 ± 1.3</td>
<td>31.8 ± 0.8</td>
<td></td>
</tr>
</tbody>
</table>

Different superscripts (*, **) within columns indicate significant difference (p<0.05) between the means of respective groups. T1: Infected with *E. tenella*, M1: Infected with *E. maxima* and *E. necatrix*, X: Un-infected (control).

Anaemia due to blood loss is a consistent finding in *Eimeria* species infection of chickens (Anosa et al., 2011). The mean PCV values fell below the normal range of 22-35 % for chickens (Wakenell, 2010). This shows that *E. tenella* and *E. maxima/E. necatrix* infections were able to cause anaemia. It is expected that the haemorrhage caused by *E. tenella* should be more than that caused by *E. maxima* and *E. necatrix* (Morris and Gasser, 2006). However, in this study there was no statistically significant difference between the mean PCV values of birds infected with *E. tenella* (T1) and birds infected with *E. maxima/necatrix* (M1).

The lesions observed included moderate to mild haemorrhagic enteritis/typhilitis with necrosis of intestinal mucosa and the thickening of the wall of the intestine and ballooning of portions of the intestines in some cases (Fig 1). In *E. tenella* infections, the haemorrhagic inflammation with necrosis was confined to the caecum, while lesions of *E. maxima* and *E. necatrix* were restricted to the middle one third portion of the intestine (ileum). The carcasses of the infected birds were generally pale and the combs were also pale. In birds that lost significant amount of body weight, serous atrophy of coronary fat was observed in their hearts. Un-infected birds were found to be in good condition. The gross lesions observed in the intestine and caeca are all consistent with findings of previous reports (Shane, 2005; Zulpo et al., 2007; Dakpogan et al., 2012 and Jatau et al., 2014).

Microscopically, lesions observed in birds infected with *E. tenella* were necrosis and sloughing off of the epithelium of the caeca, severe necrosis of the mucosa and severe hemorrhagic typhilitis with ...
infiltration of mononuclear cells and eosinophils into the intestinal mucosa. There was also diffuse necrosis of mucosal glands. Developmental stages of the parasite in the lamina propria and sub mucosa were prominent (Fig 2). For birds infected with *E. maxima* and *E. necatrix*, the numerous developmental stages of the parasite were limited to the epithelium and lamina propria. There was massive infiltration of mononuclear cells in the epithelium and lamina propria with moderate to severe haemorrhage in most cases, necrosis and sloughing off of the mucosal epithelium (Fig 3).

In few cases, catarrhal exudate was seen in the intestinal lumen. These histopathological findings are consistent with findings of previous reports (Idris *et al.*, 1997; Shane, 2005; Zulpo *et al.*, 2007). Birds of group infected with *E. tenella* showed more signs of haemorrhage than birds infected with *E. maxima/E. necatrix*. *Eimeria tenella* parasites were predominantly located in the sub-mucosa and deeper parts of the lamina propria while the *E. maxima/E. necatrix* parasites were mainly located in the epithelium and upper parts of the lamina propria. This is consistent with the observations of Morris and Gasser (2006). Numerous mononuclear cells were seen alongside eosinophils in the intestinal tissues. This agrees with the findings of Jatau *et al.* (2014). Eosinophils play an important role against coccidiosis infection by releasing cytotoxins on the parasitic stages of *Eimeria* (Mescher, 2010) which are too large to be phagocytised by macrophages.

### 4. Conclusion

In conclusion, the field isolates of *E. tenella*, *E. maxima* and *E. necatrix* in Makurdi, Benue State were found to be pathogenic, causing severe pathology to younger broilers. Farmers should embark on proper sanitation of their farms. Emphasis should be laid on the early diagnosis and prophylactic treatment of coccidiosis in order to control its spread.

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**References**


