Isolation and Morphological Identification of *Eimeria Tenella* (Family:Eimeriidae) from Khartoum State (Sudan)

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(Received: October 17, 2015; Accepted: December 29, 2015)

**Abstract** - Caecal Coccidiosis is one of the most important diseases affecting poultry production. It is caused by *Eimeria tenella* (Family:Eimeriidae). The aim of this study was to identify *E. tenella* infection in broiler chickens using oocyst measurements and detection of different parasitic stages in the caecum tissue. Ten positive samples were histologically sectioned and stained with H&E (Haematoxylin and Eosin). The oocyst measurements recorded were 19.63 in length and 17.02 in width and the shape index of 1.16 was recorded for *Eimeria tenella*. The histopathological picture showed different parasitic stages in chickens' mucosa and glandular region.

**Index terms:** *Eimeria tenella*, Histopathology, Khartoum, Sporulated oocyst.

**I. INTRODUCTION**

Most vertebrates, as well as invertebrates, are parasatised by coccidia. Coccidia parasites are protozoan unicellular organisms of the phylum Apicomplexa. They are responsible for several of the most severe diseases known in animals and man. Coccidiosis is a common protozoan disease in domestic birds and other fowl, characterized by enteritis and bloody diarrhea. Clinically, bloody faeces, ruffled feathers, anaemia, reduced weight gained are observed.

*Eimeria tenella* infection causes a severe disease characterized by bleeding, hemorrhagic lesion development, high morbidity, mortality and reduced weight gain. Clinical coccidiosis is considered as the most significant parasitic infection affecting the commercial poultry industry. Initial sporadic hemorrhagic lesions occur together with hemorrhages in the cecal lumen and dysentery, which are often accompanied by oocysts formation, caseous cores, and clotted blood. This disease causes moderate to severe damage to the intestinal epithelium, resulting in reduced body weight gain, reduced feed efficiency, and often overt morbidity and mortality. Costs to the industry exceed US $1.5 billion annually worldwide.

Coccidiosis is enormously host specific, found in the gut, where it develops and multiplies intracellularly. Transmission of parasites among broilers is by faecal-oral route through the ingestion of sporulated oocysts. Many different factors affect the infectivity of sporulated oocysts, including the number of oocysts present in litter, chick density, susceptibility of birds and immunogenicity.

An infection is substantiated upon the ingestion of sporulated oocysts from contaminated feed or water by a poultry host and once inside the poultry stomach, the oocyst wall is changed by mechanical and chemical action releasing the sporocysts, which contain infective sporozoites. Excystation results in the opening of the anterior cap of sporocysts to release infective sporozoites. Interactions of the apical complex with the plasma membrane of epithelial cells allow sporozoites to penetrate the host cell. Within the cells, the sporozoites transform into merozoites, which multiply asexually for 2-7 generations, the exact number being species dependent. With each asexual generation, the host cell is destroyed to release merozoites that infect new cells.

After the final asexual division, the merozoites invade new epithelial cells and transform into gamonts, with the majority developing into macrogametes (i.e. female gametes), while a few undergo further division and develop flagella to become microgametes (i.e. male gametes). The resultant diploid zygote produced upon fertilization builds a heavy resistant oocyst wall around itself and enters the intestinal lumen. Sporulation occurs within the young oocyst to produce sporocysts, within which the infective sporozoites develop and contaminate the environment to begin a new cycle and begin a new cycle.
The intestinal lesions evoked by *Eimeria sp.*, are due to injury of the epithelial cells of the mucosa where the parasites are developed and multiplied. The oocysts exist in the litter in premises and are distributed by clothes, shoes, dust, insects etc. Pathoanatomically, dehydration, high degree of anaemia of the body and viscera are found. The wet litter and the heat in premises favours the sporulation and therefore, the outbreak of coccidiosis. The caecal wall is composed of four layers, mucosa, submucosa, muscularis and serosa. There is a progressive increase in the number of granulocytes, leukocytes and pyronophilic cells in the submucosa and lamina propria with *E. tenella*. The granulocytes response become most observed when the first generation merozoites had left the glandular tissue and migrate to the tunica propria. Loss of the mucosal epithelial tissue, shortening and atrophy of the villi is associated with coccidial infection. This will result in reduction of the surface area available for absorption and also reduction of the number of functional epithelial cells.

In Sudan, however, the exact losses cannot be determined since there is a lack of statistical indices. Among these indices are the medication costs, mortality rate, reduction rate in production and the prevalence of the common poultry diseases in Khartoum state showed that coccidiosis comes as the second most prevalent disease (14.7%) next to salmonellosis. The objective of this study is to identify *Eimeria tenella* species using oocyst measurement methods and histological screening of the parasite stages in the caecum to confirm the diagnosis.

**II. MATERIALS AND METHODS**

**Collection and processing of chicken guts**

Chicken caeca suspected to be naturally infected were collected from different poultry farms in Khartoum district. They were opened by giving longitudinal incision and the collected contents were examined by direct microscopy. The contents from positive samples were placed in 2.5 per cent potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) solution. Saturated sodium chloride (NaCl; 4:1) was used for flotation technique to isolate oocysts. The aspirated mixture was diluted with distilled water (4:1) to remove sodium chloride. The sediment obtained was subjected to sporulation.

**Sporulation of oocysts**

The washed samples were placed in 2.5 per cent potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) solution (6mm thickness) in petri dishes. The petri dishes were partially covered to allow the passage of oxygen and were incubated at 37 °C for 48 hours. The contents of petri dishes were stirred off and on to ensure the oxygenation of the oocyst. During sporulation 60-80 per cent humidity was maintained by placing water in 2 petri dishes in the incubator. The sporulation of the oocyst was confirmed by taking a drop of the mixture and examined for the sporocysts/sporozoites under themicroscope.

**Purification of sporulated oocysts**

The suspension having the sporulated oocysts was mixed thoroughly with equal quantity of 2.5 per cent potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) solution. The suspension was then filtered through a sieve followed by muslin cloth. The filtrate was centrifuged at 1500 rpm for 10 minutes. Almost 50 percent of the supernatant was discarded and the remaining portion of the supernatant in the centrifuge tubes was poured in a fresh tube and mixed with an equal quantity of sodium chloride (NaCl; 4:1) for flotation. The mixture was centrifuged at 1500 rpm for 10 minutes. The supernatant having sufficient number of sporulated oocysts was aspirated by pipetting system and collected separately in a tube. The sediment was processed in the same way until no sporulated oocysts remain in the supernatant. The palpatorium thus collected was mixed with water (1:5) in a falcon tube and kept undisturbed for overnight at 4 °C. The sporulated oocysts settled in the bottom were collected by removing all water (one inch above the bottom of the tube) through suction by pipetting system. The supernatant (3/4th portion) was removed and the remaining mixture at the bottom, having the sporulated oocyst, was centrifuged at 1500 rpm for 10 minutes.

**Parasitological examination**

The palpatorium containing sporulated oocysts was used for length measurements using a calibrated ocular micrometer at x400 magnification. Five, random oocysts were examined by measuring their length and width with light microscopy, armed with calibrated ocular lens as well as determination of the oocysts shape and length/width index. Oocysts were identified as *Eimeria tenella* using the key described by [18].

**Histopathology**

Infected caecal tissue was fixed in 10% buffered formalin. The specimens then were dehydrated in...
50%, 70%, 90%, 95% and absolute ethanol successively. Finally they were embedded in paraffin wax. 6µm, transversed sections were made by a rotary microtome and stained with Hematoxylin and Eosin (H&E) as described by. [22].

III. RESULTS AND DISCUSSION
Measurements obtained for *Eimeria tenella* oocysts recovered from the examined material are given in table (1). The table shows the length, width and shape index of *Eimeria tenella* oocyst. Unsporulated and sporulated oocysts isolated from infected birds, magnified x400 are shown in plate (1) and plate (2). The histopathological picture for the infection showing different parasitic stages in the chicken mucosa and in the glandular region are also studied as parameters for identification of *E. tenella* in the caecal region, as shown in plates (4-8).

### Table (1): *Eimeria tenella* oocyst measurements

<table>
<thead>
<tr>
<th>No.</th>
<th>Length (µm)</th>
<th>Width (µm)</th>
<th>Shape index (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.36</td>
<td>16.64</td>
<td>1.16</td>
</tr>
<tr>
<td>2</td>
<td>19.89</td>
<td>18.83</td>
<td>1.06</td>
</tr>
<tr>
<td>3</td>
<td>19.71</td>
<td>16.37</td>
<td>1.20</td>
</tr>
<tr>
<td>4</td>
<td>19.64</td>
<td>17.96</td>
<td>1.09</td>
</tr>
<tr>
<td>5</td>
<td>19.54</td>
<td>15.31</td>
<td>1.27</td>
</tr>
<tr>
<td>Average</td>
<td>19.63</td>
<td>17.02</td>
<td>1.16</td>
</tr>
</tbody>
</table>

Plate (1): Unsporulated and sporulated oocysts of *E. tenella* x1000

Plate (2): Unsporulated and sporulated oocysts of *Eimeria tenella* x400

Plate (3): Normal caecal tissue [23]

Plate (4); Sectioned caecal tissue showing sloughing, destruction and denudation of the villi compared to the control
In the present study, the mean values of *Eimeria tenella* oocyst measurements are 19.63 µm length, 17.02 µm width and 1.16 µm shape index. These findings are in agreement with William (1996) [24], Salim (2001) [25] and Amer (2010) [26] for the same species. The examined caecae showed histologically different stages in the life cycle of the parasite infecting the glandular region as well as the mucosal region. There is also infiltration of the epithelium with lymphocytes and macrophages. The folding of the villi was also observed. The liberation of merozoites resulted in severe tissue destruction, sloughing and denudation of the villi as observed in tissue sections. These results are in agreement with Hammond and Long (1973) [12], Pout (1967) [13] and Ali (1989) [16]. This is believed to affect food digestion and absorption and may lead to secondary bacterial infection. Commercial chicken flocks free of *Eimeria* infection are extremely rare [27]. However, development of anticoccidial resistance has threatened the economic stability of the broiler industry. Although there has been little effort by the pharmaceutical industry to develop new anticoccidials, the rising problem of drug resistance of *Eimeria* species has incited major research efforts to seek alternative means of control through increased knowledge of parasite biology, host response, and nutritional modulation.

In conclusion, the identification of local strains of *Eimeria tenella* and the study of their biology and molecular structure are central for their control.
More, research is needed in these lines to complete the whole picture to eradicate the parasite.

ACKNOWLEDGEMENTS
This study was supported by University of Bahri, who sponsored this work. We also thank the staff of the Genetics and Molecular laboratory, Zoology Department, University of Khartoum where this work was done.

REFERENCES