Studies on Coccidia in Experimental Infection with *Eimeria* spp in Rose-Cobb Broiler Chicken

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Abstract
The experimental completely randomized design was conducted on poultry coccidiosis of Rose-Cobb breed of one hundred broiler chicken from November 2015 to April 2016 in the Faculty of Veterinary Medicine at the premises of Tewodrose Campus, University of Gondar, Ethiopia. The objectives of this study were carried out to identify, diagnose and to assess potential effect of *Eimeria* spp in broiler chicken. The experimental animals (n=100) were randomly allocated into four equal groups, group one (G-I), group two (G-II), group three (G-III) and group four (G-IV) with 25 chickens in each group. The G-I, G-II, and G-III were treatments groups challenged by different *Eimeria* sporulated oocysts, while G-IV served as the control group. In this study, the infective dose of *E. tenella* (G-I), *E. acervulina* (G-II) and mixed *Eimeria* spp (G-III) was 2x10⁶ sporulated *Eimeria* oocyst inoculated orally at three weeks of age in broiler chicken and subsequent alterations in different haematological constituents were evaluated at interval of 5, 7 and 9 day of post inoculation. The study involved clinical sign and weight gain, morphology identification of *Eimeria* spp and oocyst count of *Eimeria* egg per gram of faces. The *Eimeria* species identified from the natural infected chickens were *E. tenella*, *E. acervulina*, *E. maxima* and *E. necatrix*. The clinical signs manifested mainly were loss of weight, severe anaemia and bloody watery diarrhoea. Morbidity in all three infected groups were 100%. *E. tenella* was more pathogenic than *E. acervulina* and mixed *Eimeria* spp. There was no statistical difference in the average body weight obtained at 7DPI between the experimental infected and control group. Oocyst counts per gram of faeces in pure (*E. tenella* and *E. acervulina*) and mixed *Eimeria* spp. infection showed marked increase from the 5 to 8DPI. Pure single infection of *E. acervulina* and *E. tenella* showed highest oocyst excretion on 6 DPI (62,395 oocyst/g of faeces) and 7 DPI (86,535 oocyst/g of faeces) respectively. Whereas mixed *Eimeria* spp. infection reached a maximum oocyst count of 51,270 oocyst/gm of faeces on 7DPI.

Keywords: Broiler chicken, Coccidiosis, *Eimeria*

Introduction
The main parasites found in poultry are caused by protozoa of the genus *Eimeria*, also known as coccidia, being responsible for serious economic losses, mainly due to diarrhoea and deaths in young poultry. The annual worldwide cost is estimated at about $800 million. These estimates include the costs of prophylactic in feed medication for broilers and broiler breeders, alternative treatments if medication fails and losses due to mortality, morbidity, impaired growth rate, temporary reduction of egg production in layers and poor feed conversion of chickens that survive outbreaks (Asadi et al., 2015) [5]. Poultry coccidiosis is prevalent in various parts of Ethiopia and affects mostly young growing birds (Mersha et al., 2009) [19]. The main constraints for development of commercial poultry is development of disease conditions and management problem which can have a devastating effect in intensive production. Commercial poultry consist of exotic breed selected for their capacity in producing eggs and meats, because of this selection these animals are more susceptible to disease than local traditional backyard poultry (CIRAD, 2005) [9]. *Eimeria* spp. are frequently described by the morphology of the oocyst, a thick walled zygote shed in faecal material by the infected host. Infective oocyst contains four sporocysts, which in turn contain two sporozoites (Saif et al., 2003) [21]. Majority of *Eimeria* oocysts; *E. tenella*, *E. maxima*, *E. acervulina*, *E. hagani* and *E. brunetti* are ovoid while, *E. necatrix* is oblong-ovoid shape. *E. maxima* (30.5 x 20.7μm) is the largest and *E. mitis* (15.6 x 14.2μm) is the smallest as compared to other *Eimeria* species. Oocyst size, shape, zone of intestine parasitized, nature of macroscopic lesions, minimum sporulation time, minimum prepatent period and location of the parasite within the intestinal
lesions and histopathological characteristics are the characteristics useful in species identification (Arabkhazael et al., 2011) [4]. Diagnosis by faecal examination may lead to quite erroneous results. In some instances the major pathology is produced before oocysts are shed in the faces and conversely the presence of large number of oocysts may not necessarily indicates a serious pathogenic condition. Diagnosis of coccidiosis in chicken is best accomplished by post mortem examination of representative number of birds. Size of oocysts, sites of infection, prepatent period and sporulation time are the morphological and biological features used for the diagnosis of coccidiosis. E. maxima can be easily identified based on oocyst size but E. tenella and E. necatrix produce confused lesions (Hadipour, et al., 2011) [13]. Therefore, this study was carried out to identify and diagnose parasitological effect of Eimeria spp. in broiler chicken.

Material and Methods
Study area
The experimental study was conducted in the Faculty of Veterinary Medicine at the premises of Tewodrose Campus, University of Gondar. The area is found in Amhara National Regional State, located in the north-western part of Ethiopia, (12.3° to 13.38° north latitudes and 35.5° to 38.3° east longitudes) (NMA, 2011) [20]. The research was conducted from November 2015 to April 2016.

Experimental animals and grouping
One hundred day-old Ross broiler breed chickens were purchased for this experiment from Alema, a private commercial broiler farm, Debre Zeit, Ethiopia. Unsexed day old chickens were randomly and equally allocated into four groups. All groups were maintained at the same management system. The chickens were reared for eight weeks in separate room under strict biosecurity measures and no vaccine was used during the study period. On the start of the experiment the birds were tagged with identification numbers on their wing and leg in each group.

Housing and management of experimental chickens
In this study, day-old chickens were kept with floor housing system. The house, feeder, water utensils were thoroughly cleaned, disinfected prior to stocking of chickens. The utensils were also cleaned daily to avoid reinfection and contamination. Thus, the chickens were reareded under strict coccidia free conditions through repeated cleaning and disinfection. Chickens were fed ad libitum on a commercial broiler starter, grower and finisher diet based on their ages throughout the period of the experiment. Continuous heating program with 120 watt bulbs were suspended at head height of the birds. The amount of temperature present in the house was measured and recorded by thermometer and the heat released from brooder was adjusted based on the age of chickens from suspended height. The temperature was maintained at 29-31°C for the first week and was reduced by 1-3°C on weekly basis. Bio-safety of chickens was maintained in study area through fenced farm, protected against wild animals and using footbath. The experimental house had gate and there were special store for feed, disinfectant, personal protective equipment’s and closing for the researchers and assistance. The current experiment was conducted with the approval of university of Gondar ethical review board. The experiment was conducted based on the international guidelines of animal experimentation and handling where they were fed ad libitum. The board approved the protocol to be done. Attached here with is the clearance copy.

Experimental design and inoculation of sporulated oocysts
The experimental design used for this research was completely randomized design. The experimental animals (n=100) were randomly allocated into four equal groups: group one (G-I), group two (G-II), group three (G-III) and group four (G-IV) with 25 chickens in each group. The G-I, G-II, and G-III were treatments groups challenged by different Eimeria sporulated oocysts, while G-IV served as the control group. All chickens were maintained until the 10th day of age the experiment with a ration containing anticoccidial additives following the recommended producer. After the 10th days of age, the chickens were fed on a ration without anti-coccidial additives until the end of experiment. Adlibitum provision of feed and water were maintained. Faecal material from each group was examined at 10th and 21th days of age before the infection, to ensure that the chickens were free from coccidia or other parasitic diseases. Additionally, blood examination was conducted for the detection of pathogenic bacterial agents. One bird from each group was sacrificed and examined to confirm the absence of any parasitic stage of Eimeria species and other pathological lesions at 21th day of age.

The treatment groups of chickens (G-I, G-II, G-III) were infected artificially infected with sporulated Eimeria oocysts at the age of three weeks as described by You (2014) [23]. They were infected orally with infective dose of 2x10⁴ sporulated oocysts of E. tenella, E. acervulina and field isolated mixed Eimeria oocysts. The G-I chickens were infected with E. tenella, G-II with E. acervulina and G-III with mixed Eimeria spp. (E. acervulina, E. tenella, E. necatrix and E. maxima). G-IV was remained as uninfected control groups.

Preparation of infective Eimeria species for the experiment
Pure culture E. tenella, E. acervulina and mixed identified Eimeria species were used for this experiment. The pure cultures of E. tenella and E. acervulina were acquired from India. The mixed Eimeria species were identified from the naturally infected chickens. For identification of these mixed species, oocysts were collected from a total of 22 local and koeykoey breed chickens of different sex and ages of clinically coccidiosis suspected chickens were purchased from Gondar town and donated from Kombolcha poultry research and multiplication centre. The chickens were sacrificed in the laboratory at post-mortem room by cervical dislocation using the technique described by Zander (1999). The gastrointestinal tract was grossly examined carefully. Intestinal contents from the respective sections of intestine with lesions were collected. The floatation technique using sodium chloride solution was applied to harvest oocysts (Bowman, 2003) [8]. The harvested Eimeria oocysts were identified by a combination of oocyst size, location in the gut and appearance of the lesions (Conway and Mckenzie, 2007; McDougald and Fitz-Coy, 2008) [10, 18]. The different species of Eimeria were identified according to the length, width and shape index of the individual oocyst after measuring 50 oocysts in each positive sample using a calibrated ocular microscope (McDougal, 2003) [17]. The identified Eimeria spp. oocysts were spread out in shallow Petri dish containing 2.5% potassium dichromate (K₂Cr₂O₇) solution and incubated with a temperature 29°C, with adequate oxygen and humidity to allowed sporulation as
describe by Conway and McKenzie (2007) [10]. The sporulation of the oocyst was confirmed by taking a drop of the mixture starting from the second day of incubation and examined for the presence of sporocysts under the microscope. The sporulated oocysts were collected and preserved in 2.5% K₂Cr₂O₇ and stored at 4 °C. The sporulated oocysts were counted using the McMaster method (Holdsworth et al., 2004) [14].

**Observation of Prepatent Period and Clinical Signs**

The animals from each experimental group were monitored closely for possible observable clinical signs throughout the course of the experiment. The predominant clinical signs were recorded in each infected groups, daily faecal samples were collected starting on the 3 DPI from each group of the experimental chickens. Detect the presence of oocysts using a simple floatation test to determine the prepatent period of the infection in each group. Body weight of the chickens was measured on 7DPI using a weighing balance (made in China by Hana) in the morning prior to feeding.

**Oocyst Count**

The faeces from each group were collected twice a day starting from day 3 to 11 DPI to count oocyst per gram of faeces. Excreted oocysts were counted using a McMaster counting chamber method. The oocysts counts were expressed as the mean number oocysts per gram of faeces (Holdsworth et al., 2004) [14].

**Results**

An experimental trial for *Eimeria* spp. infection in broiler chickens was conducted to evaluate the clinical sign, *Eimeria* spp. identification and parasitological changes.

**Eimeria species identified from the natural infection**

One of the groups (group three) in this experiment was inoculated with *Eimeria* spp identified from the field. The *Eimeria* species identified through calibrated ocular microscope from the natural infected chickens were *E. tenella, E. acervulina, E. maxima* and *E. necatrix* (figure 1). The un sporulated oocysts contained zygote filling the entire space (figure 2) *E. tenella* was the most abundant species followed by *E. acervulina*. Culture of oocysts was made to sporulated in 2.5% K₂Cr₂O₇ solution maintained at 29 °C in the incubator and the sporulation time was varies from two to three days. The morphological characteristics of the oocysts of *Eimeria* species that was used in the identification the different species were summarized in (Table 1).

<table>
<thead>
<tr>
<th>Site of infection</th>
<th>Mean oocyst length (µm)</th>
<th>Mean oocyst width (µm)</th>
<th>Shape index</th>
<th>Identified Eimeria spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caecum</td>
<td>21.31</td>
<td>17.92</td>
<td>1.19</td>
<td><em>E. tenella</em></td>
</tr>
<tr>
<td>Duodenum</td>
<td>18.23</td>
<td>14.02</td>
<td>1.3</td>
<td><em>E. acervulina</em></td>
</tr>
<tr>
<td>Jejunum and ileum</td>
<td>29.80</td>
<td>23.69</td>
<td>1.24</td>
<td><em>E. maxima</em></td>
</tr>
<tr>
<td>Jejunum and ileum</td>
<td>20.02</td>
<td>16.75</td>
<td>1.2</td>
<td><em>E. necatrix</em></td>
</tr>
</tbody>
</table>

Note: µm=micro meter

**Table 1:** The morphological identification of mixed infection of *Eimeria* species oocyst in natural infected chickens.

<table>
<thead>
<tr>
<th>Zygote</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
</tbody>
</table>

**Fig 1:** Un- sporulated *Eimeria* oocyst of *E. tenella* (A), *E. acervulina* (B), *E. necatrix* (C) and *E. maxima* (D) (40X).

**Figure 2.** Sporulated *Eimeria* oocyst,*E. tenella* (A), *E. acervulina* (B), *E. necatrix* (C) and *E. maxima* (D) (40X).
Prepatent period and clinical signs in the experimentally infected chickens

The prepatent period, mean body weight and the clinical sings observed in the different experimental groups were summarized in (Table 2). The clinical signs manifested mainly were loss of weight, severe anaemia and bloody watery diarrhoea. In *E. tenella* infected groups 6 chickens, in *E. acervulina* infected group 4 chickens and 3 chickens in mixed *Eimeria* spp. infection were died from 7 to 9 DPI. Morbidity in all three infected groups were 100%. However, no other gastrointestinal parasites were detected by sedimentation and floatation techniques and normal bacterial colony were appreciated in appropriately prepared bacterial growth media.

Table 2: Illustration of prepatent period, body weight and clinical signs of experimentally infected chickens

<table>
<thead>
<tr>
<th>Group</th>
<th>Species</th>
<th>Prepatent period</th>
<th>Bodyweight (Mean±SD)</th>
<th>Clinical sings</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-I</td>
<td><em>E. tenella</em></td>
<td>5 days</td>
<td>820±208.36*</td>
<td>Weakness, inappetance, dropping of wing and whitish diarrhoea showed on 4 DPI. By the 5 DPI the faeces became bloody and more number of <em>E. tenella</em> oocysts were presented in the faeces. Severe depression, decreased body weight, straining, somnolence, pale wattle and combe. Mortality occurred from 7 to 9DPI.</td>
</tr>
<tr>
<td>G-II</td>
<td><em>E. acervulina</em></td>
<td>4 days</td>
<td>884±117.84*</td>
<td>Whitish and bloody diarrhoea, wing drop, depression, muffled feathers, closed eyes and inappetance, pale wattle and combe, decrease body weight, straining, mortality occurs from 7 to 9 DPI.</td>
</tr>
<tr>
<td>G-III</td>
<td>Mixed <em>Eimeria</em> spp.</td>
<td>5 days</td>
<td>932±139.47*</td>
<td>Decrease body weight, watery, whitish and bloody diarrhoea, wing drop and inappetance, mortality occurs from 7 to 10 DPI.</td>
</tr>
<tr>
<td>G-IV</td>
<td>-</td>
<td>-</td>
<td>1065±198.5*</td>
<td>No clinical signs of the disease</td>
</tr>
</tbody>
</table>

Note: Mean ± SD= mean plus or minus standard deviation. *= Non statistical difference between groups

The body weight of the experimental group were recorded at 7 DPI. There was no statistical difference in the average body weight obtained at 7DPI between the experimental infected and control group. Although, G-IV chickens demonstrated the higher body weight (table 2).
Oocyst count per gram of faeces

In the present study, oocysts count in faeces demonstrated different kinetics shedding rate in different infected groups. Oocyst counts per gram of faeces in pure (E. tenella and E. acervulina) and mixed Eimeria spp. infection showed marked increase from the 5 to 8 DPI. Pure single infection of E. acervulina and E. tenella showed highest oocyst excretion on 6 DPI (62,395 oocyst/g of faeces) and 7 DPI (86,535 oocyst/g of faeces) respectively. Whereas mixed Eimeria spp. infection reached a maximum oocyst count of 51,270 oocyst/gm of faeces on 7DPI. After the oocyst count reaches its highest level it was gradually reduced and only a few number were seen on 11DPI (figure 6).

Fig 6: Oocysts shedding in chickens infected in G-I, G-II, G-III and G-IV

Discussion

In the present experiment, there were successful developments of infection with Eimeria species. The disease coccidiosis observed in all three experimental groups were discussed with regard to sporulation of oocysts, identification of Eimeria spp., prepatent period, clinical signs, body weight, oocyst count examination. In the present study, oocyst length, width, shape index and shape, were an indicative of the oocyst count examination. In the present study, oocyst length, width observed could be due to variation of species. However, G-IV recorded higher average body weight. These values agreed with Fagner (2014) [12] who described that there is no significant difference in weight gain but always lower average for the infected group. The non-significant decrease of body weight observed could be the short period of experimental infection. On the contrary, Danladi et al. (2014) [11] and Arabkhazaeli et al. (2011) [4] opined that by using a single dose of \(2\times10^4\) oocysts of E. acervulina, E. tenella and \(3\times10^5\) mixed isolated Eimeria species showed a significant decrease in weight gained compared to the control group. This
difference in results might be associated to several factors such as genetics of the birds, route of administration, amount of infective oocysts administered and strain of *Eimeria* spp. In the present study, oocyst count was markedly increased from 5 to 8 DPI. Similar observations were reported by you (2014) [24] and Luciana da et al. (2014) [10] who stated that mean oocyst counts of *E. tenella*, *E. maxima* and *E. acervulina* species increased more quickly on the 5 to 8 DPI. Among the infected groups, *E. tenella* infected groups showed highest mean oocyst per gram of faces on 7 DPI. Similar findings are recorded by earlier workers Zulpo (2014) [24] and Luciana da SB, Elder NP, Augusta AS, Vinícius BC, Fagner LC. Experimental Infection with Sporulated Oocysts of *Eimeria maxima* in broiler chicken. J Vet. Med., 2014; 7:273.


