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Gross and Histopathological Observations of Commercial Broiler Chicks experimentally Infected with Pure Eimeria tenella, E. acervulina and mixed Isolated Eimeria spp

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Abstract

An experimental study was conducted at University of Gondar from November 2015 to April 2016. The objective of this study was to evaluate the pathogenicity, gross and histopathological lesion of coccidiosis in broiler chickens.One hundred commercial one day old broiler chicks, unvaccinated against coccidiosis, was used during this experiment. At day 14, chickens of G1 (n=25), G2 (n=25) andG3 (n=25) were infected with 2 x 104 sporulated oocysts of E. tenella,E. acervulina and mixed Eimeria spp. respectively; G4 (n=25) served as the uninfected control group. Fifteen birds were sacrificed in each group with seven days after infection. The prepatent period for E. tenella (G1) and mixed Eimeria spp(G3) was five days, however, E.acervulina (G2) had prepatent period of four days. The observed pathological lesion score ranged from +1 to +4 were recorded in infected chickens. Chickens infected in E.tenella showed sever lesion score when compared to other infected groups. The histopathological findings infected chickens were showed different stages of parasite, villous atrophy, severe inflammation, mononuclear inflammatory cell infiltrated, sever haemorrhagic areas.

Keywords: Broilers, Coccidiosis, Eimeria, Pathogenicity

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Introduction

Coccidiosis is essential disease of poultry under intensive management condition caused by a protozoan parasite known as Eimeria. A mild coccidian infection due to non-pathogenic or low dose of pathogenic Eimeria oocyts is not harmful and creates flock immunity against coccidiosis. A severe attack of coccidiosis can however cause weight losses, morbidity and mortality (Sharma et al., 2013). Coccidiosis in chickens is one of the most costly diseases affecting the poultry industry worldwide. It is an intestinal parasitic disease caused by intracellular protozoan parasites of the genus Eimeria (Nematollahi et al., 2009). The infection inflict severe economic loss due to extensive destruction of the enteric epithelium resulting in reduction of feed conversion, body weight gain and egg production (Morris & Gasser, 2006).

The disease is endemic in most of the tropical and subtropical regions where ecological and management conditions favour an all-year round development and propagation of the causal agent (Obasi et al., 2006). This disease is caused by Eimeria parasites, which infect epithelial cells of the intestines of birds. Clinically, coccidiosis is manifested by bloody diarrhoea and listlessness; economic losses are primarily due to impaired feed conversion, depressed growth, loss of pigmentation, downgrading at processing, and mortality.In this study, the gross and histopathological observations of pre E. tenella, E.acervulina, and mixed Eimeria spp were evaluated in experimentally infected commercial broilers chicks (Ali tipu et al., 2002).

The characteristics of post-mortem lesions of coccidia infected chicken are based on location of the intestinal tract, appearance and severity, the nature of intestinal contents and other associated gross changes which can be useful in establishing a diagnosis (Conway and Mckenzie, 2007). Lesions of the intestinal mucosa and loss of pigmentation may also become apparent during the latter stages of infection (Amer et al., 2010). The purpose of this study was to study the pathogenicity, gross and histopathological lesion of coccidiosis in broiler chickens infected with *Eimeria spp.*

Materials 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). 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 reared 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 adlibitum. The board approved the protocol to be done.

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 treatment 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 anticoccidial 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 oocyts at the age of three weeks as described by You (2014). 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 specieswere 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).

The harvested Eimeria oocysts were identified by a combination of oocyst size, location in the gut and appearance of the lesions (McDougald and Fitz-Coy, 2008). 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 (McDougald, 2003).

The identified Eimeria spp oocysts were spread out in shallow Petri dish containing 2.5% potassium dichromate ($K_2Cr_2O_7$) solution and incubated with a temperature 290C, with adequate oxygen and humidity to allowed sporulation as describe by Conway and McKenzie (2007). 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% $K_2Cr_2O_7$ and stored at 40C. The sporulated oocysts were counted using the McMaster method (Holdsworth et al., 2004).

Gross lesion examination and lesion scores

Randomly selected 10 chickens from each group were sacrificed through cervical dislocation. On the 7 DPI, post-mortem examination was conducted on the sacrificed birds for the presence of gross lesions consistent with coccidiosis. The intestinal tract was thoroughly examined for gross pathological changes as described by (Lobago et al., 2005; Gari et al., 2008). The observed lesions were assigned lesion scores from 0 to +4. The lesion score zero represents absence of lesion and lesion score +4 is for very severe intestinal/cecal mucosa lesion due to coccidia infection (Duffy et al., 2005).

Histopathological examination

Intestinal and cecal segments were thoroughly examined and the segment which revealed lesion tissue samples were taken and fixed in 10% buffered formalin solution and processed for histopathological evaluation The tissues were trimmed and processed by automatic tissue processor dehydrated in different chambers containing different alcohol concentrations The processed tissues were cleared in xylene and embedded in paraffin for preparation into fine blocks. Finally blocks with a 5 µm size, transverse and longitudinal tissues section were made and stained with H and E stain (Talukder, 2007).

Results

An experimental trial for Eimeria spp. infection in chickens was conducted to evaluate the gross and histopathological changes and its gross lesion score.

Post mortem lesion and its score

Gross pathological examination of infected groups showed different lesions in different intestinal segments depending on the type of inoculated sporulated Eimeria spp. On 7 DPI extremely ballooned and petechial haemorrhages were easily seen on the serosal surface of intestine. Any pathological changes were not observed in the intestines and caecum contents of all non-infected control chickens. E. acervulina, E. tenella and mixed Eimeria spp. caused different lesion in the duodenum, caecum, duodenum and mid gut respectively. The observed pathological lesion score ranged from +1 to +4 were recorded in infected chickens. Among the three experimentally infected chickens G-I showed sever lesion score when compared to their infected groups. The gross lesion and lesion score of chickens during the experimental period were illustrated in (figures 1).

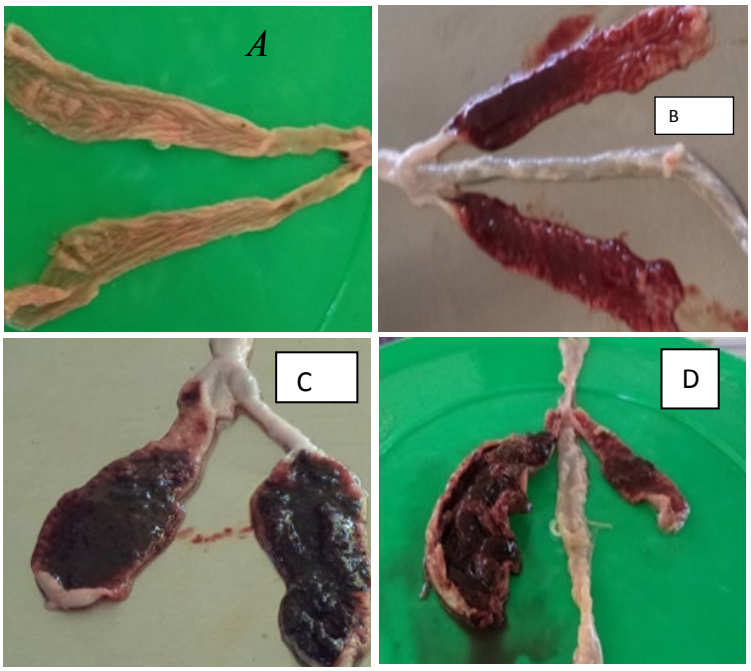

Figure 1. Gross pathological lesions of caecum in E.tenella infected chickens showing different lesions and lesion score. Normal caecum, lesion score 0(A), Haemorrhagic and thickened wall of caecum with grooves, lesion score +1(star) (B), Thickened caecal wall with blood, lesion score +3 (star) (C), Club-shaped, distended lumen filled with clotted blood, lesion score +4 (star) (D).

The post mortem lesion in E. tenella infected chickens revealed extremely ballooned intestine, and petechial haemorrhages easily seen while looking grossly without opening the gut. However, discrete haemorrhagic spots were also observed on the mucous membrane of caecum when opened. Caecum was often found, thickened, showing necrosis and sloughing of epithelium, enlargement of caecum with clotted blood, haemorrhagic corrugated mucosa, dilation of caecum with consolidation of caecal contents were observed. Lesion score ranged from + 1 to + 4 on 7DPI. The gross lesion in E. acervulina infected chickens (G-II) showed, ballooning and hypermic duodenum, creamy contents in the lumen, pin point necrotic foci, whitish transverse streak (ladder like lesion) in the mucosa and serosa, thickened and bright red mucosal wall, lumen filled with blood were seen. The lesion score ranged from + 1 to +4 based on the severity of the lesion on 7 DPI but the majority of the lesion scored under lesion score +2.

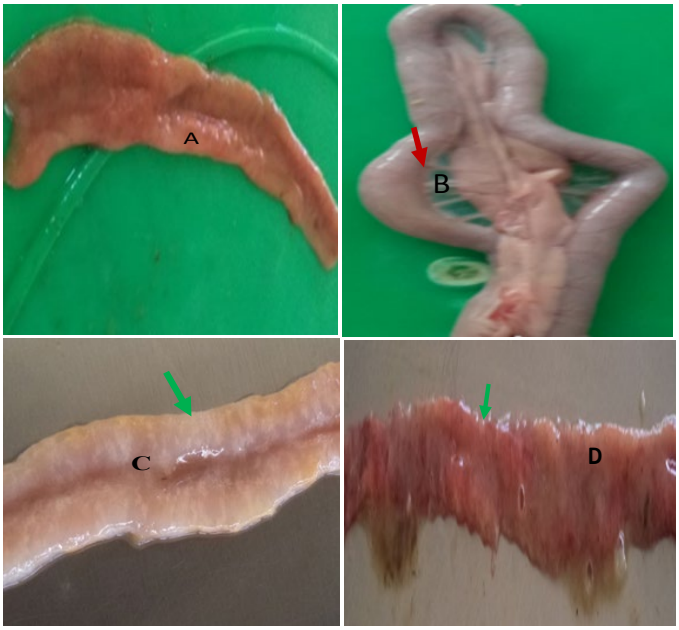

Figure 2. Gross pathological lesion of E.acervulina infected in chickensshowing different lesions and its lesion score. Control chicken duodenum (lesion score 0) (A), extrim ballooned duodenal loops (arrow) (B), white pinpoint ladder like mucosal lesion, lesion score +3(arrow) (C), haemorrhagic enteritis, bloody exudate, necrotized duodenal lumen, lesion score +4 (arrow) (D).

The gross lesion in mixed Eimeria species infected group on 7DPI post mortem revealed petechial haemorrhagic mucosa in the mild gut, discrete haemorrhagic spots on the jejunum mucosa, ballooned with orange like mucus mixed fluid in the mid gut and distended duodenum with blood. Distended caecum with bloody faeces and mucoid debris along with haemorrhages on the mucosa, blood field caecal lumen and thick caecal mucosa was observed. The lesion score in mixed infection were mild compared with other infected groups, it ranged from +1 to +3. G-IV, in this study no gross lesion were seen on 7 DPI post mortem.

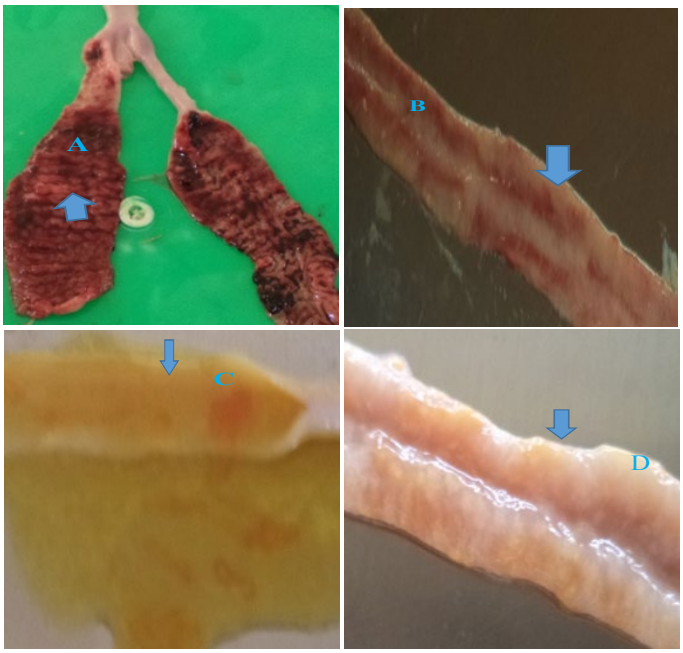

Figure 3. Gross pathological lesions of small intestine in infected with mixedEimeria Spp. showed different lesions with its lesion score. Corrugated, thickened and haemorrhagic cecal mucosa and its lesion score +3 (arrow) (A), ecchymotic haemorrhagic jejunum mucosa, lesion score +2 (arrow) (B), petechial haemorrhage, orange like fluid in the lumen and thickened ilium mucosa (arrow) (C), Very thickened and pi point white lesion in duodenal mucosa (D).

Group	No	Species	Lesion score				
			0	+1	+2	+3	+4
G-I	10	*E. tenella*	0	1	2	4	3
G-II	10	*E. acervulina*	0	2	4	2	2
G-III	10	Mixed *Eimeria* spp	0	4	3	3	0
G-IV	10	Control	10	0	0	0	0

Table 1. Gross lesion score in G-I, G-II, G-III and G-IV chickens sacrificed on 7 DPI

Histopathological examination

Histopathological examination of tissue sections on 7 DPI in G-I, G-II and G-III experimentally infected with different Eimeria spp. which revealed the developmental stages Eimeria spp. in cecum, duodenum, jejunum and ilium, respectively. Histopathological lesions of all experimental infected chickens were examined on the 7DPI. The major tabulated histopathological findings in G-I (E.tenella) infected chickens were showed different stages of parasite, villous atrophy, severe inflammation, mononuclear inflammatory cell infiltrated, marked proliferation of epithelial cells of intestinal crypts, dilation, necrosis of submucosal glands, multifocal areas of sever haemorrhagic areas, plenty of oocysts and schizonts in lamina propria and desquamation of epithelium, thickened mucosa and sub mucosal layer with slightly congestion of blood vessel which indicated disruption followed by leakage of blood, severe muscular oedema.

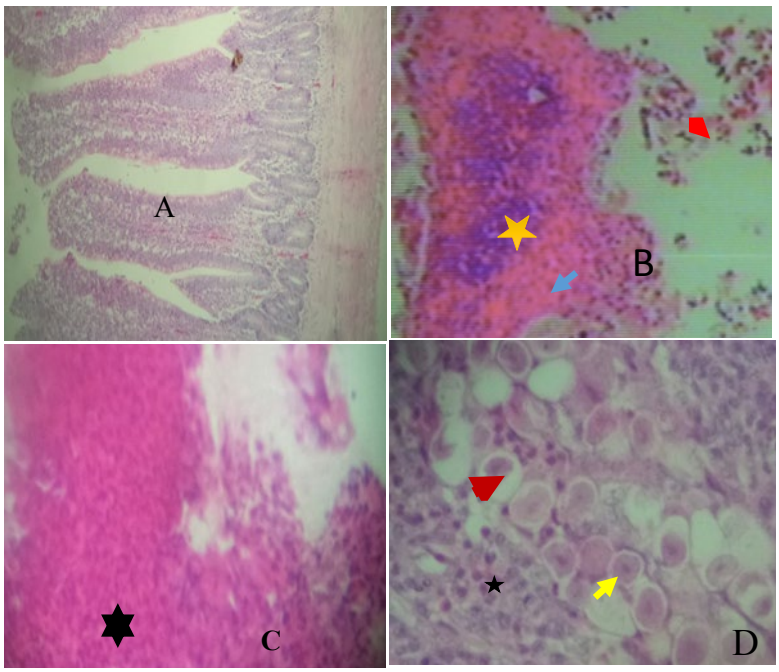

Figure 4. Histopathological observations on 7 DPI in E. tenella infection chickens. Control group caecum (A), inflammatory cells aggregation (yellow star), sever haemorrhage (arrow) and disintegrated villi (arrow head) (B). Sever haemorrhagic area in mucosa on 7 DPI (star) (C), Great number of oocysts (red arrow head) and schizonts (yellow arrow) and aggregated inflammatory cell (star) on 7 DPI (D) (H-E 100x).

Microscopically, lesions induced by E. acervulina (G-II) were principally characterized by hyperplastic epithelial mucosa with dilation of goblet cells, epithelial desquamation, infiltrated inflammatory cells in lamina propria, moderate villous atrophy and fusion, discrete haemorrhage, foci of concentrated mononuclear infiltrate at the submucosa layer, discrete oedema at the submucosa muscularies associated with various intralesional forms of the parasite within epithelial cells (figure 5).

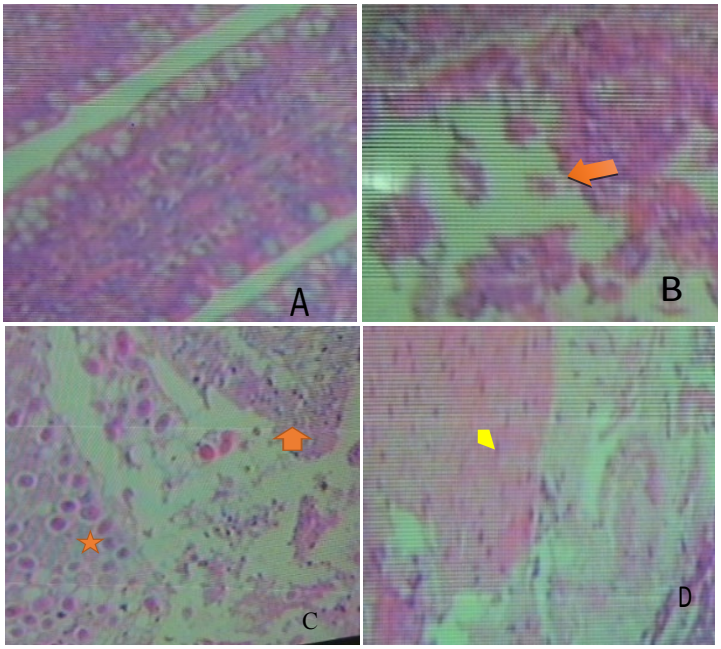

Figure 5. Histopathological observations on 7 DPI with E. acervulina infectionin chickens. Control chicken duodenum (A). Disintegrated of villi (arrow) (B), atrophied villi (red arrow) and plenty of schizonts and oocysts with disintegrated villi (star) (C), necrosis of tissue in mucosal layer (arrow head) (D).(H-E 100x).

Microscopic lesion of mixed infection were discrete villous atrophy, proliferation of epithelial cells, focal haemorrhagic areas, presence of parasite at the villi, congestion in the blood veins, infiltration of mononuclear cells and dilation of mucosal glands associated with parasite in various stages of development in the intestine (duodenum, jejunum and cecum).

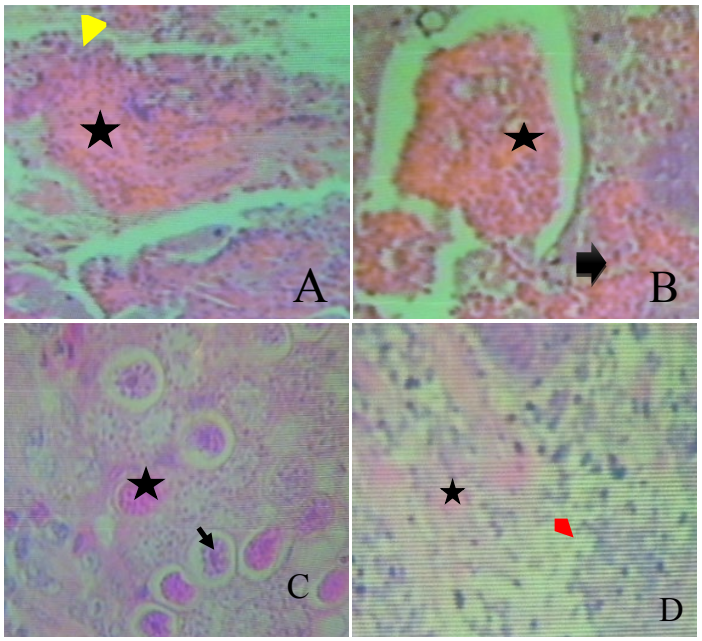

Figure 6. Histopathological lesion in small intestine in mixed Eimeria spp infected chickens at sacrificed on 7 DPI. Haemorrhage (star) and infiltration of inflammatory cells (arrow head) in the sub mucosa (A) Congestion(star) and focal haemorrhage (arrow) in the muscularies (B).Several number of oocyst(arrow) and schizonts(star) in the mucosa of ileum (C)(400x), Necrosis(star) and oedema (arrow head) in the sub mucosal layer of ilium (D)(H-E 100x).

Discussion

In the present study, the most common post mortem lesions of E. tenella infectionwere manifested by petechial to sever haemorrhage in the cecal wall, ballooning and thickened caecal loops of mucosa, enlargement of caecum with clotted blood, caecum filled with blood, complete ballooning with blood cores formation, haemorrhagic corrugated mucosa. This characteristic feature of E. tenella infection is in agreement to the report of Patra et al. (2010) and Chapman (2014) who stated that in case of caecal coccidiosis, enlarged and distended caeca filled with blood and petechial haemorrhages were observed in E. tenella infected chickens.

Gross post mortem lesions caused by E. acervulina were ballooned and hypermic duodenum, creamy contents in the lumen, bloody exudate in the lumen, pin point necrotic foci, whitish transverse streak (ladder like lesion) in the mucosa and serosa, thickened and bright red mucosal wall and lumen filled with blood. This finding is support by Chapman (2014) and Shohrehet al. (2014) who stated that E. acervulina affected duodenum characterized by white transversely oriented mucosal streaks, petechiation and thickening of the mucosa and watery content.

The most common post mortem lesions in mixed infection were manifested by enlarged and distended caeca filled with blood and petechial haemorrhages in the whole length of intestine and caecum of challenged group. Similar observation is record by Jenkins et al. (2009)and Chapman (2014) who reported that E. maxima,E. tenella and E.necatrix infected birds which have been characterized by petechiae on the jejunum serosa, mucosal hyperaemia, orange coloured intestinal content, intestinal wall thickening and distention.

The categories of lesion score in infected groups were depend on the infected Eimeria spp. E. tenella infected groups revealed the highest lesion score (lesion score +3, n= 4 and lesion score +4, n=3) followed by E. acervulina (lesion score +3, n=2 and lesion score +4, n=2) and mixed Eimeria spp. (lesion score +3, n=3 and lesion score +2, n=4). Similar findings are report by Conway and Mckenzie (2007) who stated that E. tenella infection showed very severe lesions, those induced by E. acervulina were moderate, while E.maxima lesions were discrete. Also this results is in accordance with Duffy et al. (2005) and Hussain (2010). The present observation disagreement with Zulpo et al. (2007) who described as equal challenges of 2x104 sporulated oocysts (E.tenella, E. acervulina and E. maxima were used) in experimental groups, this concentration was not sufficient to promote lesions above 2.

In the current study, histopathological lesions were more extensive lesion (E. tenella), extensive (E. acervulina) and medium (mixed Eimeria spp) on 7 DPI. Similar findings are reports by Karim and Trees (2003) identified Eimeria species based on a lesion examinations of naturally infected birds, and similar lesions seen in experimentally infected chickens. The difference histological lesions observed could be due to destruction of the epithelial lining of the infected intestine by different Eimeria spp. which results in reduced ability for the digestion and absorption of nutrient by chickens.

E. tenella infected grouprevealed loss of epithelial tissue, congestion of blood vessels which indicated disruption followed by leakage of blood, necrosis of submucosa, loss of villi, disruption of caecal mucosa, cluster of oocysts, schizonts and marked haemorrhage, necrosis of caecal mucosa considerable numbers of oocyst in lamina propria of caecum, presence of different developmental stages of parasite, villous atrophy, severe inflammation and mononuclear inflammatory cell infiltrate, sever haemorrhage and desquamation of epithelium. Similar findings were reported by Soomro et al. (2001), McDougald and Fitz-Coy (2008) and Meskerem et al. (2013). Histopathological findings showed more extensive lesionswithinflammatory cells in E. tenella infectionthan E. maxima. This is in agreement with the work of You (2014) who identified histological lesions in E.tenella, E. maxima and E. necatrix naturally infected chickens. The histopathological lesion occurrence of affected caeca in the present study could be due to the most pathogenic stage of E. tenella as the second generation schizonts, which caused excessive tissue damage, bleeding, disruption of the caecal glands and destruction of the mucosa and muscularis layer.

The histopathological changes in E. acervulina infected group revealed, presence of oocysts and schizonts with characteristic inflammatory cells in duodenal part of intestine, focal to sever haemorrhage. Similar occurrence have been reported by Asaduzzaman et al.(2011) who stated that E. acervulina showed presence of oocyst and schizonts with the characteristic of inflammatory cells in duodenal part of intestine.

Histopathological lesions in mixed Eimeria spp.infceted group on 7 DPI showed discrete villous atrophy, proliferation of epithelial cells, focal haemorrhagic areas, presence of parasite at the villi, infiltration of mononuclear cells and dilation of mucosal glands associated with parasite in various stages of development in the intestine (duodenum, jejunum and cecum)mild villous atrophy, epithelial cell proliferation, and mild haemorrhage in various stages of parasite development, congestion in the muscularies layer. The result is in agreement with Zulpo (2007)who reported that mild histopathological lesions observed in chickens induced 2 × 104 sporulated oocysts ofE. tenella, E. acervulina and E. maxima at 7 DPI.

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