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RESEARCH ARTICLE

Lesion scores, oocysts output, hematological and histopathological changes of the 7 days life cycle of *Eimeria tenella* in broilers

Tippayaporn Nonkookhetkhong[1*], Thanyakorn Chalalai[1]

[1]*Stress and Oxidation Stress in Animals Research Unit, Faculty of Veterinary Sciences, Mahasarakham University, Thailand*
[*]***Corresponding author*** *E-mail: tippayaporn.n@msu.ac.th*

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Abstract

Objective: *Eimeria tenella* is a protozoan which is a causative agent of avian cecal coccidiosis. The disease results in bleeding, diarrhea, weight loss, high morbidity and mortality in chickens. This study investigated lesion scores, oocysts output, hematological and histopathological changes of the 7 days life cycle of *E. tenella*.

Materials and Methods: Fifty-six, broilers were randomly divided into 2 groups of which group 1 was uninfected and group 2 was infected with $2x10^4$ *E. tenella* oocysts. Blood samples were collected from four chickens of each group and birds were then euthanized at 1, 2, 3, 4, 5, 6 and 7 days post infection (dpi). Lesion scores, oocyst output and hematological data were evaluated and compared between group.

Results: Lesion scores and oocyst output of the infected group were significantly higher than in the uninfected group at 5, 6 and 7 dpi ($p \leq 0.05$). PCV was significantly lower than in the uninfected group at 3 to 7 dpi ($p \leq 0.05$). WBC count was significantly higher than in the uninfected group at 4 dpi ($p \leq 0.05$). Lymphocytes and heterophils were significantly higher compared to the uninfected group at 4 dpi ($p \leq 0.05$). Monocytes were significantly higher than in the uninfected group from 3 to 5 dpi ($p \leq 0.05$). The developmental stages of *E. tenella* in cecal tissue during the 7 days life cycle included trophozoite at 2 dpi, first generation of schizonts at 3 dpi, secondary generation of merozoites and schizonts at 4 and 5 dpi and gametocytes and oocysts at 6 and 7 dpi.

Conclusion: These findings suggest that monocytes were the most effective white blood cell and secondary generation schizonts were the pathogenic developmental stage of *E. tenella* which effective on lesion scores, hematological and histopathological changes was on *E. tenella* infection.

Keywords: *Eimeria tenella,* lesion scores, oocysts output, hematology, histopathology, broilers

Introduction

Coccidiosis is the most common and costly disease of poultry production worldwide, including in Thailand. Economic losses are due to a high percentage of the mortality and increased feed conversion ratio (Györke et al., 2016). The parasites multiply in the intestinal tract, cause tissue damage, interruption of feeding and digestive processes, bloody diarrhea, reduced weight gain, increased morbidity and sometimes mortality (James et al., 2009; Hector et al., 2020). The species of coccidian infecting chickens belong to the genus *Eimeria*, each species causes separate and distinct, recognizable diseases, independent of the other species (Hector et al., 2020). Coccidia species that cause clinical infections include *Eimeria necatrix*, *E. brunette, E. acervulina, E. maxima* and *E. tenella* (Donal and Conway 2007). *Eimeria tenella* causes cecal coccidiosis, in which the cecum become distended with clotted blood and paled liver has been reported. There are weight losses, high morbidity and mortality (Kaewthamasorn et al., 2015; Sharma et al., 2015). *Eimeria tenella* infection results in a change in the number of circulating red and white blood cells (Adamu et al., 2013; Akhtar et al., 2015). Differential identification of each species is dependent on the part of intestine that is parasitized, the gross appearance of the lesion, minimum prepatent time, schizont location of development and location of parasite in the host intestinal epithelium (Donal and Conway, 2007). Knowledge of the hematological and histopathological changes in each day of the life cycle of *Eimeria tenella* will be of benefit for differential diagnosis, treatment and control the coccidiosis in chickens. The objective of this study was to investigate lesion scores, oocysts output, hematological and histopathological changes of *Eimeria tenella* infection in broilers from 1 to 7 days post infection (dpi).

Materials and Methods

Ethical approval

The experiment was reviewed and approved by the institutional Animal Care and Use Committee, Mahasarakham University (approval number: 033/2019).

Parasites

Eimeria tenella oocysts were collected from infected broiler chicken's cecal contents from broiler farms in Roi Et province, Thailand, and maintained at the Faculty of Veterinary Sciences, Mahasarakham University, Thailand. The oocysts underwent sporulation process in 2.5% (w/v) potassium dichromate at 30ºC for 48 hours (Donal and Conway, 2007). The sporulated oocysts were propagated in chickens; five commercial broiler chickens at 21 days old were inoculated with $2x10^4$ sporulated oocysts (Donal and Conway, 2007). Then the cecal contents were collected at 7 dpi.

Animals and experimental design

Fifty-six, one-day-old commercial broiler chickens (Cobb) were obtained from a commercial hatchery. They were housed in the metal cages, under the open house system and fed *ad libitum* before and during the experiment. The chickens were randomly divided into 2 groups of 28 chickens each. The chickens in group 1 were not inoculated and group 2 were inoculated with 2x104 sporulated oocysts at twenty-one-day-old. Blood samples were collected from four chickens of each group and birds were then euthanized by cervical dislocation at 1, 2, 3, 4, 5, 6 and 7 dpi. Cecal tissues were collected from euthanized chickens.

Lesion scoring

The lesion scores of *Eimeria tenella* were obtained by examining the intestinal lesions of euthanized chickens at 1, 2, 3, 4, 5, 6 and 7 dpi. Scoring of lesions was recorded as described previously of Johnson and Reid (Johnson and Reid, 1970). 0 = no gross lesions. 1 = mild lesions; very few scattered petechiae on the cecal wall. 2 = moderate lesions; lesions more numerous with blood in the cecal contents. 3 = severe lesions; large amounts of blood or cecal cores, cecal wall thickened. 4 = extremely severe lesions; cecal wall greatly distended with blood or large caseous cores.

Oocyst counting

The pooled fecal samples of each group were collected at 1, 2, 3, 4, 5, 6 and 7 dpi. The oocysts were processed in saturated sodium chloride solution and counted (oocysts/gram of feces) by using a McMaster chamber under a light microscope.

Hematological analysis

Blood samples were collected from brachial wing vein and transferred immediately into a sterile tube containing the anticoagulant (EDTA). Packed cell volume (PCV) was measured by using microhematocrit tubes and a hematocrit centrifuge. Total White Blood Count (TWBC) was performed in a 1:20 dilution of blood in Natt and Herrick's solution. Differential white blood cell counts were made by preparation of thin blood smears stained with Wright's stain and identified as lymphocytes heterophils eosinophils monocytes and basophils (Samour, 2006).

Histopathological examination

Cecal tissue samples of infected chickens were fixed in 10% buffer formalin, then embedded in paraffin and trimmed to 3-5 µm using a microtome. Tissue sections were prepared according to histological techniques, stained with hematoxylin and eosin (Awad et al., 2008) and examined under a light microscope.

Statistical analysis

The lesion scores, oocyst per gram of feces and hematological data were analyzed using T-test and Mann-Whitney U tests with SPSS for windows (SPSS Inc, Chicago). Statistical difference was considered significant at $p \leq 0.05$.

Results

Clinical signs and lesions

The uninfected group showed no clinical signs and lesions of disease. After being inoculated with $2x10^4$ sporulated oocysts of *Eimeria tenella*, all chickens of the infected group had bloody diarrhea at 6 to 7 dpi. The ceca were distended with clotted blood, but there was no resulting mortality.

Lesion scores

The Lesion scores of the infected group were significantly higher than the uninfected group at 5, 6 and 7 dpi ($p \leq 0.05$) and were highest at 6 dpi. In the uninfected group no coccidia lesions were seen. (Fig. 1).

Oocyst output per gram of feces

The oocyst output of the infected group was significantly higher than in the uninfected group at 5, 6 and 7 dpi ($p \leq 0.05$), the number of oocyst output was highest at 7 dpi. In the uninfected group, there was no oocyst output in feces. (Fig. 2).

Hematological value

The packed cell volume of the infected group was significantly lower than the uninfected group at 3, 4, 5, 6 and 7 dpi ($p \leq 0.05$) and it was lowest at 7 dpi. (Fig. 3).

The total white blood cell count of the infected group was significantly lower than in the uninfected group at 1, 2 and 7 dpi ($p \leq 0.05$) but at 4 dpi was significantly higher than those of the uninfected group ($p \leq 0.05$). (Fig. 4).

In differential white blood cell counts, the number of lymphocytes was significantly higher than in the uninfected group at 4 dpi ($p \leq 0.05$). The heterophils count was significantly lower than the uninfected group at 2 dpi but significantly higher than the uninfected group at 4 dpi ($p \leq 0.05$). The number of monocytes was significantly higher than the uninfected group from 3 to 5 dpi ($p \leq 0.05$). Eosinophils were significantly lower than in the uninfected group at 5 and 7 dpi ($p \leq 0.05$) but higher than the uninfected group at 6 dpi and basophils were significantly lower than the uninfected group at 5 and 7 dpi ($p \leq 0.05$). (Fig. 5).

Histopathological examination

At 1 dpi, the cecal tissue of chicken showed mild degeneration of villi epithelium but none of the stages of *Eimeria tenella* were present in cecal tissue (Fig. 6-i). At 2 dpi, the cecum showed trophozoites in the intestinal glands of cecum (Fig. 6-ii). At 3 dpi, the cecum exhibited the first generation of schizonts in the lamina propria and the first generation of merozoites in the first generation of schizonts in the intestinal gland (Fig. 6-iii). At 4 dpi, the cecum showed large clusters of a secondary generation of schizonts and a number of small secondary generation of schizonts in the lamina propria (Fig. 6-iv). At 5 dpi, the cecum revealed loss of villi, necrosis and hemorrhage of the cecal mucosa, there were clusters of secondary generation of schizonts and ruptured secondary generation schizonts releasing secondary generation merozoites (Fig. 6-v) which resulted in hemorrhage of the cecal mucosa at 5 dpi. At 6 dpi, the cecum showed macrogametocytes and microgametocytes and oocysts in intestinal glands (Fig. 6-vi). At 7 dpi, the cecum showed hemorrhage, infiltration of inflammatory cells as macrophages and clusters of oocysts in submu-

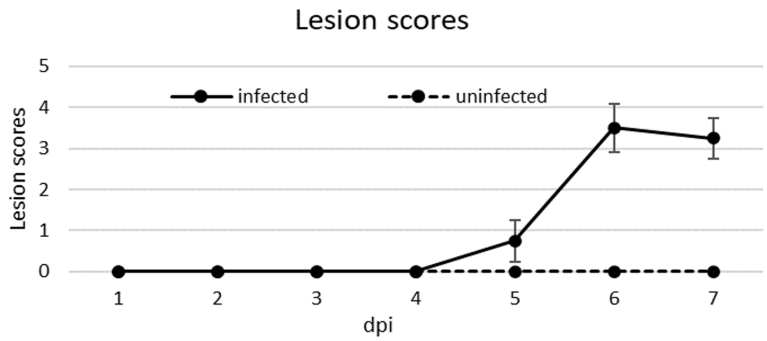


Fig. 1. The mean (±standard deviation) of lesion scores. Y-axis=the mean of lesion score, X-axis=dpi.

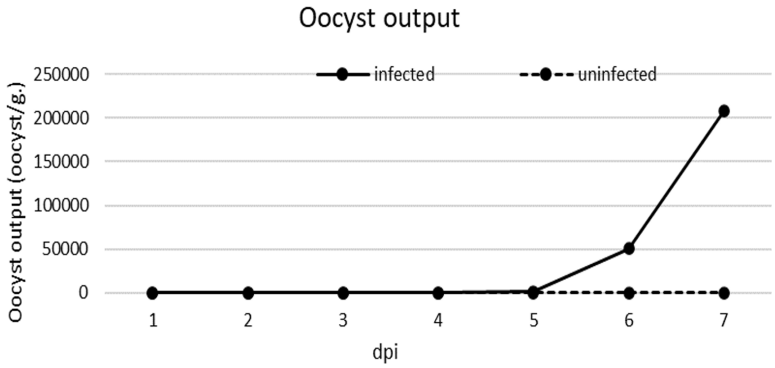


Fig. 2. The mean numbers (±standard deviation) of oocyst output. Y-axis=the mean numbers of oocysts output (oocyst/g.), X-axis=dpi.

cosa (Fig. 6-vii), and clusters of gametocytes and oocysts in intestinal glands (Fig. 6-viii).

Discussion

The infection of *Eimeria tenella* in broiler chickens manifested clinically as bloody diarrhea at 6 dpi as previously reported (Kaewthamasorn et al., 2015). Lesion scores and oocysts output were differentially increased from 5 to 7 dpi, so the prepatent period of these parasites is 5 dpi. This result different from a previous report (Awad et al., 2018) that oocyst shedding started at 6 dpi. There were differences of oocyst shedding because of different reproductive rates, differences in the number of schizont stages and the rate at which the stages develop (Cha et al., 2018).

The packed cell volumes of the infected group were significantly lower than in the uninfected group at 3, 4, 5, 6 and 7 dpi ($p \leq 0.05$) because blood vessels were disrupted when the schizonts matured and the merozoites were released (Hector et al., 2020), which was related to lesion scores that increased on those days. Blood loss from bloody diarrhea caused reduced erythrocyte count and hematocrit value (Dial, 2010) with maximum effect at 7 dpi. The reduction of total white blood cell count in infected chickens at 1 and 2 dpi, was due to the decline of lymphocytes, heterophils and monocytes. Improper sample handling may have caused an artifactual decrease in white blood cell count due to blood clotting (Mitchell and Johns, 2008). Circulating concentrations of leukocytes were depressed in birds maintained in captivity or stress, due to decreases in both the percentage and number of heterophils (Scanes, 2015). Leukocytosis at 4 dpi was due to the increase of lymphocytes and heterophils. Leukocytosis is often associated with inflammatory diseases, infectious diseases or stress (Jones, 2015). Heterophils are the first

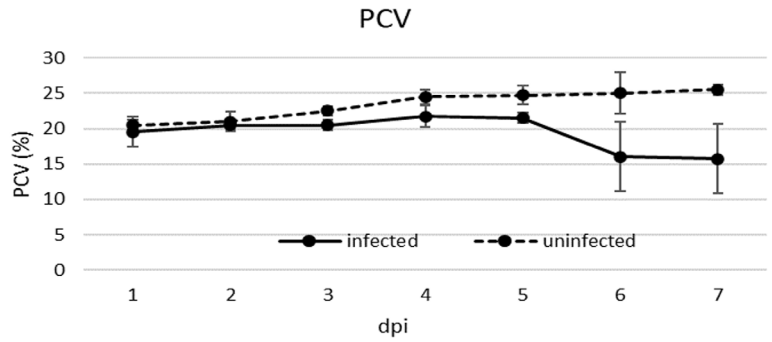


Fig. 3. The mean (±standard deviation) of PCV. Y-axis=The mean of PCV (%), X-axis=dpi.

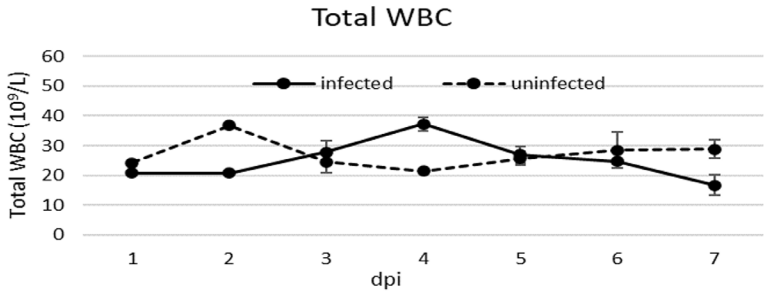


Fig. 4. The mean (±standard deviation) of Total WBC count Y-axis=The mean numbers of Total WBC (10^9/L), X-axis=dpi.

leukocytes to migrate from the blood to infected sites for killing pathogens (Rosales et al. 2016; Sukumaran et al., 2005). Heterophils exhibit superior ability to monocytes, both to phagocytize and to kill bacteria. Moreover, the severity of the leukocytosis associated with heterophilia may correlate with the severity of the disease (Scanes, 2015). Lymphocytes play important roles in both humoral and cell mediated immunity (Scanes, 2015). Lymphocytosis may result from antigenic stimulation of the immune system associated with infectious or inflammatory conditions (Jones, 2015) from large secondary generation of schizonts in lamina propria of cecum. Monocytes play an important role in the early immune response, enhanced oxidative burst and phagocytic activities during Eimeria infection (Taubert et al., 2009). Therefore, the most pathogenic stage is the secondary generation schizonts which were found at 4 dpi in histopathological lesions and this finding agrees with a previous report (Hector et al., 2020). After 4 dpi the total white blood cell counts were reduced from 5 to 7 dpi, because they migrate to the tissues to reparation and regulation of the immune response (Dial, 2010). This result correlates with the infiltration of inflammatory cell as macrophages in cecal tissue. *Eimeria tenella* infection rapidly and locally induced at the site of the parasite development in an increased proportion to the CD4+ cells on day 8 post infection and CD8+ cells on days 6 and 8 post infection in cecal intraepithelial lymphocytes (Bessay *et al*., 1996). Monocytes are phagocytic and once they enter into tissues they are transformed into macrophages (Mitchell and Johns, 2008). Moreover, monocytes and macrophages function via chemotaxis, which involves migration toward an inflammatory gradient (Qureshi, 2003). Moreover, Avian macrophages isolated from *E. tenella* and *E. maxima* infected animals showed enhanced IL-1 and TNFa production (Byrnes et al., 1993). Eosinophilia is rare in many avian species but common in others. When an eosinophilia is present, it may be due to marked tissue damage, parasitic diseases (Jones, 2015) but for *Eimeria*

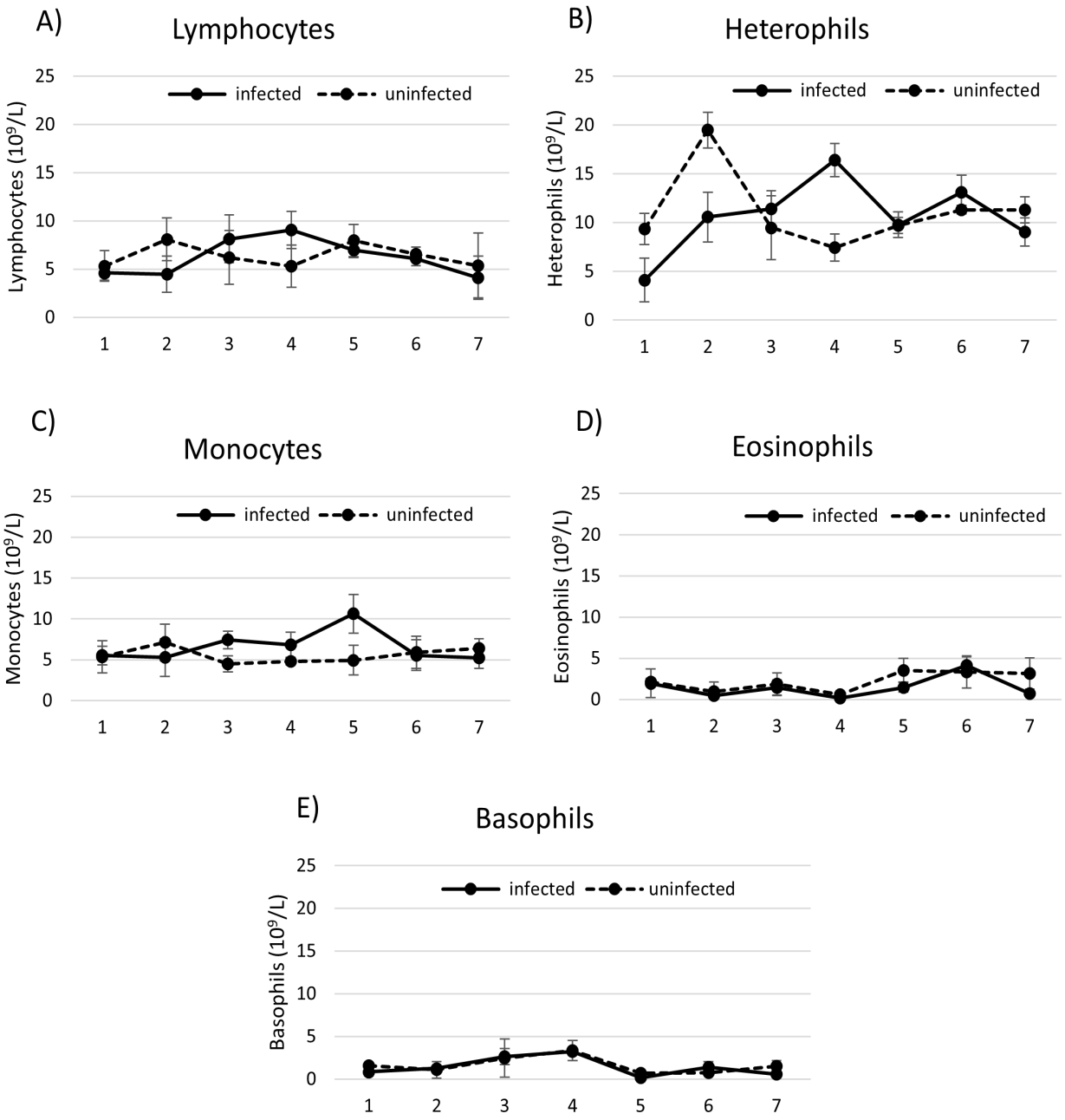


Fig. 5. Differential white blood cell counts of experimental chickens from 1-7 dpi. The mean numbers (±standard deviation) of lymphocytes (A), heterophils (B), monocytes (C), eosinophils (D) and basophils (E). Y-axis=the mean numbers of white blood cell (10^9/L), X-axis=dpi.

tenella infection, the number of eosinophils does not change. The number remains the same as the number of basophils.

The major difference present of leukocytes was the numbers of monocytes. These results suggest that monocytes were the most effective white blood cells on *Eimeria tenella* infection and monocytosis associated with *Eimeria tenella* infection. This agrees with another report on *Eimeria bovis* infection (Taubert et al., 2009).

Histopathological lesions in this study showed loss of cecal villi, necrosis and hemorrhage of cecal mucosa as has been reported in previous studies (Sharma et al.,

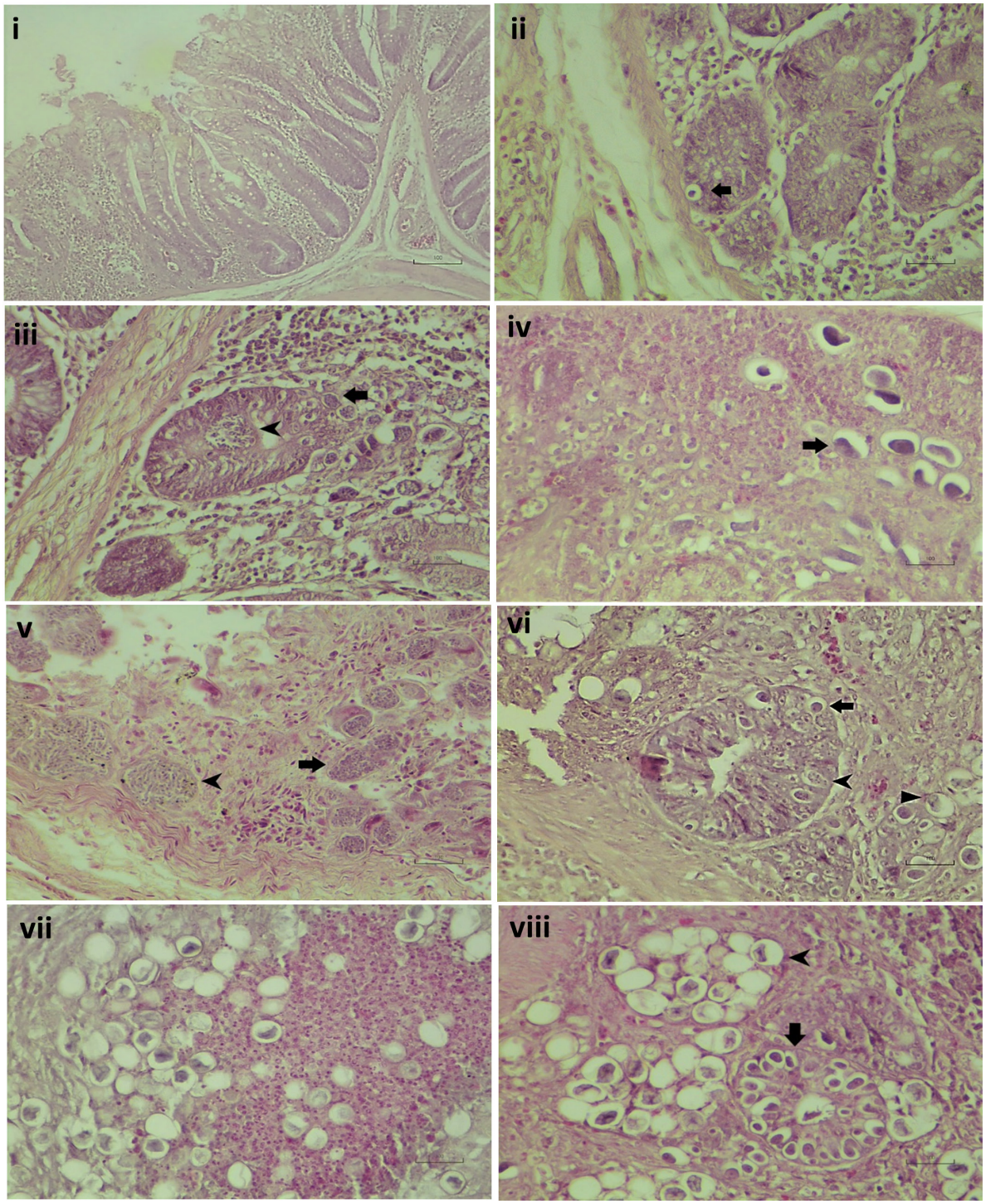


Fig. 6. Section of the cecal tissue of chicken from 1 to 7 days after E. tenella infection. (i) 1 dpi, there were no any stages of E. tenella in cecal tissue (10x), (ii) 2 dpi, trophozoites in intestinal glands of cecum (arrow) (40x), (iii) 3 dpi, first generation of schizonts in lamina propria (arrow) and first generation of merozoites in first generation of schizonts in intestinal glands of cecum (pointed arrow) (40x), (iv) 4 dpi, clusters of large secondary generation of schizonts (arrow) and the numbers of small secondary generation of schizonts in lamina propria (40x), (v) 5 dpi, clusters of secondary generation of schizonts (arrow) and ruptured secondary generation of schizonts releasing secondary generation merozoites (pointed arrow) (40x), (vi) 6 dpi, macrogametocytes (arrow), microgametocytes (pointed arrow) and oocysts in intestinal glands (arrow head) (40x) and (vii-viii) 7 dpi, hemorrhage, infiltration of macrophages and cluster of oocysts in submucosa, clusters of gametocytes (arrow) and oocysts (pointed arrow) in intestinal glands (40x).

2015; Adamu et al., 2013; Abdelrazek et al., 2020). The developmental stages of *Eimeria tenella* in cecal tissue during its 7 day life cycle includes, trophozoite at 2 dpi, first generation of schizonts at 3 dpi, secondary generation of merozoites and schizonts at 4 and 5 dpi and gametocytes and oocysts at 6 and 7 dpi. These findings were similar to the generalized life cycle of *Eimeria* spp. in chickens reported in (Donal and Conway, 2007). Clusters of large schizonts develop deep in the lamina propria, due to the blood vessels disruption when the merozoites are released (McKenzie et al., 1985), in agreement with our results of lesion scores.

The developmental stage of *Eimeria tenella* which affected the packed cell volume and circulating white blood cells was the secondary generation schizonts, of which the most pathogenic stage is when mature at 4 dpi (Hector et al., 2020). Macrophages significantly infiltrate the cecal mucosa to phagocytosis of the pathogen at 7 dpi. Similar results have been reported for Eimerial infection in calves (Taubert et al., 2009) and were related to the reduction of white blood cell count because of loss of white blood cells into cecal tissues (Abdelrazek et al., 2020).

Conclusion

In this study, monocytes were found to be the most effective white blood cell during *Eimeria tenella* infection. The most pathogenic developmental stage of *Eimeria tenella* which was effective on lesion scores, hematological and histopathological changes, was secondary generation schizonts.

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Conflict of interest statement

The authors declare that we have no conflict of interests in this work.

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