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Caprine coccidiosis: the effects of induced infection on certain blood parameters

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ABSTRACT

The objective of this experiment was to elucidate the aspects of differential circulatory cell responses during experimental coccidiosis in goat. A total of twenty newborn kids were selected; ten of them were infected with sporulated oocysts of the most pathogenic species of *Eimeria* and the remainder served as control. Blood samples were taken at 0 (before inoculation), 3, 7, 14, 17, 21, 24, 28 and 35 days post infection (dpi) and some hematological and fecal related parameters were measured. According to our data, except for a significant decrease at 24 dpi, packed cell volume (PCV) and hemoglobin (Hb) concentrations evidenced no substantial changes in the infected kids compared to controls. The percentage of the circulatory neutrophils showed remarkable increases from 7 to 24 dpi. Unlike neutrophils, the level of lymphocytes represented marked decreases from 7 to 24 dpi. In addition, circulatory eosinophils evidenced no statistical changes during the infection; however monocytes revealed a significant elevation only at 17 and 24 dpi. Substantial activations of neutrophils and lymphocytes indicate that these cells have key roles in either preventing or establishing primary infections with *Eimeria* in goats.

Key words: coccidiosis, hematological parameters, cellular responses, goat

Introduction

Coccidiosis of goats is an enteric parasitic disease caused by multiple protozoan parasite species of the genus *Eimeria*. In recent years, high mortalities of kids due to coccidiosis have been identified as one of the major constraints of goat production (KOUDELA and BOKOVÁ, 1998). Clinical coccidiosis may affect 100% of kids within

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the age range of 4-10 week, and cause severe economic losses by affecting animal health and profitability of the goat industry (RUIZ et al., 2010). In goats, 16 species of *Eimeria* have been described, of which *E. christenseni*, *E. arloingi*, *E. caprina*, and *E. ninakohlyakimovae* are of major concern (NORTON, 1986).

The diagnosis of coccidiosis depends on the clinical findings (diarrhea, dehydration, and progressive emaciation) and the presence of large numbers of oocysts in the feces (GEORGI and GEORGI, 1990); However, because the life cycle of *Eimeria* comprises intracellular, extracellular, asexual, and sexual stages, it is clear that the host immunity response is also complex (LILLEHOJ, 1998). Thus, investigating the population of blood cells circulating after challenge with coccidiosis in the susceptible animals may help to characterize the immunity generated and associated with the pathogenic effects caused by this protozoan (YUN et al., 2000).

The pattern of alterations in the hematological parameters has been investigated in natural and experimental infections with different species of *Eimeria* in sheep. According to the literature, recorded documents generally indicated a reduction in erythrocytic count (RBCs) and hemoglobin (Hb) concentration (RAMA et al., 1978; HAYAT et al., 1990; GHANEM and ABD EL-RAOF, 2005). Moreover, the disease could cause haemoconcentration indicated by an increase in packed cell volume (HAYAT et al., 1990) and has also been reported to be associated with leucocytosis, eosinophilia and neutrophilia (DEGHIDY et al., 1984). In contrast to characterized hematologic changes in eimerian infections in sheep, coccidiosis in goats have been neglected in the past and only limited numbers of studies conducted on this topic. Owing to the current lack of investigations on cellular immune responses against *Eimeria* spp, this study was undertaken to evaluate the patterns of oocyst shedding and some hematological parameters among experimentally-infected kids.

Materials and methods

Parasites. In order to prepare the infectious material, from January to April 2012, several distinct small ruminant rearing areas were inspected, located in Fars province in the south of Iran, to find cases of clinical coccidiosis. Kids with clinical symptoms of the disease (mainly light-green diarrhea) as well as *Eimeria* positive fecal samples were selected. Approximately 5 gram of feces of each *Eimeria* positive animal were collected and filtered through different sieves covered with folded gauze. The filtered material was then centrifuged at 250 g for 10 min and the supernatant was removed. The resulted suspension was placed into a shallow layer of 2.5% (w/v) potassium dichromate solution in separate Petri dishes. The sporulation of oocysts was achieved by leaving the suspensions in a wet chamber (26-28 ^{o}C) and stirring the oocyst suspensions daily to infuse air. After about 1 month, samples with moe than 90% of sporulate were mixed to

form the stock suspension and stored at 4 °C for about 3 weeks. The suspension was freed of potassium dichromate solution by serial washing with normal (physiologic) saline (0.9% NaCl) before inoculation.

Parasitological assays. The sporulated oocysts were counted per 1.0 mL of stock solution using the hemocytometer method as described earlier (HOLDSWORTH et al., 2004). The species identification of oocysts was performed based on sporulation time and morphological characteristics (size, shape, color, shape index, presence or absence of micropyle and its cap, the presence or absence of residual, polar and stieda bodies) of the oocysts and sporocysts examined at 400× magnification as previously described (JALILA et al., 1998). The species of *Eimeria* identified in the stock suspension were E. caprina (65%), E. ninakohlyakimovae (33%) and E. arloingi (2%) which are considered to be the most pathogenic species (JALILA et al., 1998).

Experimental design. Twenty newborn Iranian crossbred kids, at the ages of 1-5 days, were selected from an area with no previous reports of the infection with *Eimeria* parasites. The kids were reared artificially under parasite-free conditions in individual cages and fed with a milk replacer. All kids remained uninfected for 14 days, as determined by conducting routine parasitological tests on several samples of feces (using sedimentation-floatation technique in sucrose saturated solution) and blood (using Giemsa staining method) from each animal during this period. Ten kids (diseased group) were infected by oral inoculation of 2×10^3 sporulated oocysts per animal. The other kids remained uninfected as the control.

Sample preparation and fecal examination. Blood samples were taken from the jugular vein into EDTA containing tubes at 0 (before inoculation), 3, 7, 14, 17, 21, 24, 28 and 35 days post infection (dpi). Thin blood smears were prepared from all samples, fixed by absolute (three minutes) methanol and stained with 10% Giemsa solution (30 min). Differential blood counts were performed manually by analyzing 200 leukocytes in blood smears. In order to trace the potential formation of the anemia, packed cell volume (PCV) and the concentration of hemoglobin (Hb) were determined according to standard procedures as described by JAIN (1993).

Fecal samples were collected daily from 7 dpi and examined for the presence of oocysts, using the sedimentation-floatation technique in a sucrose saturated solution. In positive samples, the number of oocysts per gram (OPG) of feces was estimated by the modified McMaster method (HENRIKSEN and CHRISTENSEN, 1992). In addition, fecal consistency was assessed on the basis of a scoring system (1, normal (solid); 2, semi liquid to liquid; 3, watery; 4, hemorrhagic and/or with tissue).

Statistical analysis. Student's *t* test was applied to calculate statistical differences between hematological measurements in the diseased and control groups. Also, the Pearson's correlation coefficients were calculated to determine the relationships among

the oocyst excretion (OPG) and hematological parameters in the diseased group. All values were expressed as mean and standard error of mean (SEM) and P values of less than 0.05 were considered significant.

Results

Hematological parameters. The statistical analysis and the patterns of alterations in hematological parameters in both non-infected and experimentally infected kids are presented in Figs 1 and 2. The data clearly evidenced that the packed cell volume (PCV) and the hemoglobin concentration displayed no remarkable changes during the experiment, although a significant decrease was observed for both at 24 dpi (Fig. 1 parts a and b). This probably indicated transient anemia at that point of the infection.

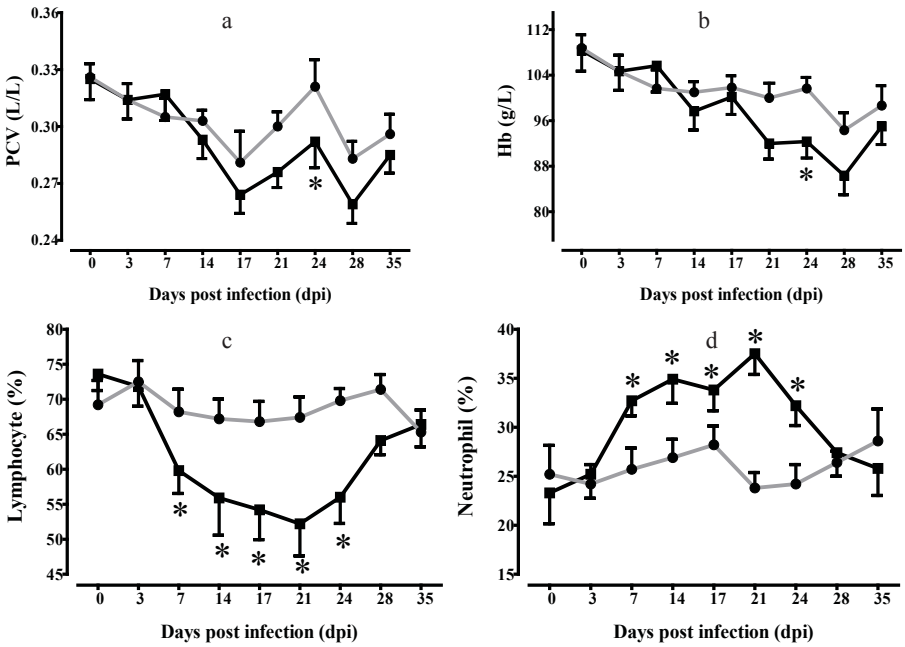

Fig. 1. Mean ± SD of hematological parameters, (a) packed cell volume (PCV), (b) hemoglobin (Hb) concentration, (c) percentage of the circulatory lymphocytes and (d) percentage of the circulatory neutrophils in non-infected (control, ●) and Eimeria infected (■) kids. (*) Shows significant differences ($P < 0.05$) between two groups

As depicted in Fig. 1 (parts c and d), the total counts of lymphocytes and neutrophils showed some significant changes during the study. The total lymphocyte count remarkably decreased from 7 dpi until 24 dpi with a maximum decrease of 23% at 21 dpi. In contrast, parallel to lymphocyte declining, the total neutrophil count showed remarkable elevations from 7 dpi to 24 dpi. Blood neutrophil counts reached a peak (about 1.5 times more than that of control) at 21 dpi, and then gradually decreased to normal values from 28 dpi.

The alterations of band neutrophils indicated a similar pattern as that of total neutrophils (Fig. 2a). A substantial elevation in band neutrophils emerged from 7 dpi and reached about 3 times normal values at 21 dpi. The values remained at high levels to 28 dpi (comparing to controls) and resumed theirnormal state on the last day of the experiment.

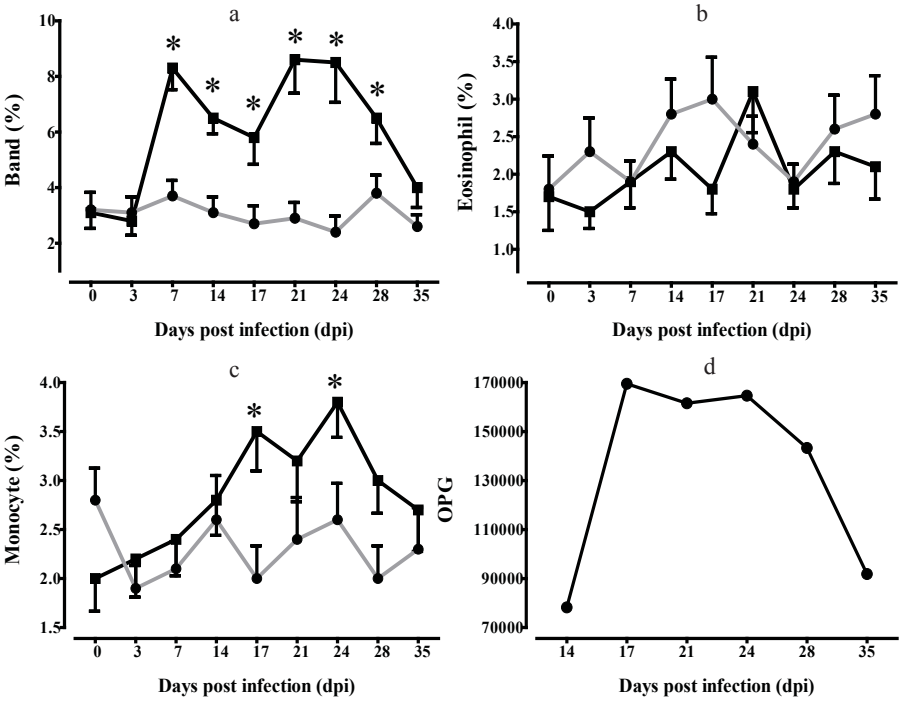

Fig. 2. Mean ± SD of hematological parameters, (a) percentage of the circulatory band neutrophils (b) percentage of the circulatory eosinophils, (c) percentage of the circulatory monocytes in non-infected (control, ●) and Eimeria infected (■) goat kids. (d) oocyst per gram of feces (OPG) in the infected animals. (*) Shows significant differences ($P < 0.05$) between two groups.

The level of blood eosinophils revealed no significant alteration in infected animals compared to the controls (Fig. 2b). Similarly, the blood monocyte counts resumed at normal values, although significant increases were observed in monocyte percentage of the infected kids at 17 and 24 dpi (Fig. 2c).

Oocyst excretion (OPG) and clinical features. The onset of oocyst excretion (prepatent period) was observed at 13-14 dpi (Fig. 2d). Oocyst shedding reached the highest levels at 17-18 dpi and subsequently decreased gradually, to the end of the experiment (35 dpi). The most common fecal consistency recorded was 2 (semi liquid); however, liquid or watery feces (diarrhea) were sporadically recorded in the infected kids from 14 to 24 dpi. No hemorrhagic diarrhea was observed.

Significant correlations were seen between OPG and the percentage of neutrophils ($r= 0.65$, $P<0.05$) at 17dpi, and PCV ($r= 0.73$, $P<0.05$), Hb ($r= 0.73$, $P<0.05$) and lymphocyte percentage ($r= -0.63$, $P<0.05$) at 28 dpi. Also, OPG was significantly correlated with the percentage of monocytes ($r= 0.7$, $P<0.05$) at 24 dpi.

Discussion

Infection with *Eimeria* parasites induces complex interactions involving several cell types and signals (parasite associated or host-derived), which certainly regulate and activate different cellular compartments (YUN et al., 2000). This study demonstrated that the increase in the blood levels of neutrophils and also the decrease in circulatory lymphocytes can be seen as the major primary cellular response to intestinal invasion of *Eimeria* parasites in kids; however, eosinophils and monocytes levels did not respond significantly during the course of the infection.

Despite the emergence of clinical signs, mainly diarrhea, the levels of PCV and Hb remained unchanged. This indicates that coccidiosis of moderate severity does not induce anemia in affected kids. The transient decrease in PCV and Hb at 24 dpi in infected animals may be attributed to the negative influences of the pathological and clinical features of the disease (i.e. diarrhea, malabsorption and etc.), which distort protein synthesis in the liver and also production of erythrocytes in bone marrow. In line with our results, RUIZ et al. (2012) and DAI et al. (2006) investigated the infection characteristics of E. ninakohlyakimovae in kids. They showed no significant or rather moderate changes in red blood cell fraction, although a moderate increase in PCV was detected at 14-17 dpi in primary infected animals. They affirmed that the slight increase of PCV values observed in primary infected animals corresponded to the decrease of the total blood volume as a result of blood losses during the hemorrhagic phase of diarrhea. In contrast, GHANEM and ABD EL- RAOF (2005) and RAMA et al. (1978) reported a significant decrease in total erythrocyte count (RBC) and Hb concentration in lambs suffering from hemorrhagic

enteritis associated with coccidiosis. However, in our study, hemorrhagic enteritis was not a clinical feature of the disease, which explains the divergent observations.

In the present study, the blood levels of neutrophils, band neutrophils and lymphocytes were subject to significant alterations due to infection with the highly pathogenic *Eimeria*, but the percentage of eosinophils and monocytes remained unchanged. This may relate to the critical role of neutrophils and lymphocytes during intestinal epithelium invasion by *Eimeria*. Similar observations were made before in ovine (CHAPMAN, 1974) and caprine coccidiosis (RUIZ et al., 2012). RUIZ et al. (2012) found clear indications of eosinophilia in kids infected with E. ninakohlyakimovae. In addition, they demonstrated increased numbers of monocytes in kids experiencing primary infection at the age of 7 weeks. However, the leukocyte changes were not significant in younger goat kids. They suggest that the developing immune competence in older goat kids may be responsible for the significant increase in the level of leukocytes. On the other hand, some previous studies (RAMA et al., 1978) reported an increase in total lymphocyte counts in lamb coccidiosis, and it was claimed that this was attributed to the phagocytic role of these cells. In contrast, in accordance with our data, ANWAR et al. (1999) found an increase in neutrophils and a decrease in the eosinophil count, along with marked lymphopeniak in buffalo calves infected with some *Eimeria* species. They suggest that the decrease in lymphocyte counts could be attributed to their role in the supply of globulins, which is under the control of adrenocortical hormones upon lymphoid tissues and lymphocytes, resulting in increased dissolution of the cells during the disease. In addition, they emphasized that increased levels of circulatory eosinophils may be related to their important role in neutralizing histamine, which is released from damaged intestinal cells. In contrast, the unchanged levels of eosinophils in our work revealed that damage to the intestinal cells resulting from primary moderate coccidiosis could not induce a significant eosinophil-mediated response.

In our study, monocyte counts showed normal values, however, significant increases were observed at days 17 and 24 post infection. According to the literature, the percentage of monocytes usually increases in chronic infections, whereas coccidiosis is as an acute infection and consequently no striking increases in monocyte counts were expected to occur in our study.

The pattern of the oocyst excretion in this work is in accordance with previous observations (HASHEMNIA et al., 2011; RUIZ et al., 2012). According to our data, except for significant correlations between OPG and the levels of neutrophils (at 17 dpi), lymphocytes (at 28 dpi) and monocytes (at 24 dpi), no substantial relationships were recorded between the number of expelled oocysts and the levels of circulatory leukocytes. These results may imply that the circulatory cell response is not in line with oocyst excretion during the course of the infection.

Overall, our study revealed that the levels of neutrophils and lymphocytes, but not eosinophils and monocytes, were significantly altered during caprine coccidiosis. The former could play a key role in the control of primary infections with pathogenic *Eimeria* species in goats, whereas the latter appears to play a negligible role during moderate primary cocidiosis.

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SAŽETAK

Cilj pokusa bio je rasvijetliti različite odgovore stanica u krvnom optjecaju tijekom pokusno uzrokovane kokcidioze u koza. U pokus je bilo uključeno 20 novoojarene jaradi od čega je 10 bilo zaraženo sporuliranim oocistama najpatogenije vrste roda *Eimeria*, a ostalih 10 su poslužili za kontrolu. Uzorci njihove krvi bili su uzeti 0. (prije zaražavanja), 3., 7., 14., 17., 21., 24., 28. i 35. dana nakon zaražavanja te su bili pretraženi na neke hematološke pokazatelje. Parazitološki su bili pretraženi i uzorci izmeta. Vrijednosti hematokrita i koncentracije hemoglobina nisu pokazivale bitne promjene u zaraženih u odnosu na kontrolne životinje, osim značajnog smanjenja 24. dana nakon infekcije. Postotak optjecajnih neutrofila bio je značajno povećan od 7. do 24. dana nakon infekcije. Za razliku od neutrofila, razina limfocita bila je znantno smanjena od 7. do 24. dana poslije infekcije. Povrh toga, statistički značajne promjene ustanovljene su u broju optjecajnih eozinofila dok je broj monocita bio značajno povišen 17. i 24. dana nakon infekcije. Znatna aktivacija neutrofila i limfocita upućuju na zaključak da te stanice imaju ključnu ulogu u sprječavanju primarne infekcije kokcidijama roda *Eimeria* u koza.

Ključne riječi: kokcidioza, hematološki pokazatelji, stanični odgovor, koza