Blood Profile of Rabbits Infected with *Eimeria magna*

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Abstract. The research aimed at determining the blood profile of local rabbits infected with different dose of *Eimeria magna* oocysts. This research used 45 male rabbits with the age of 4 month old, range from 1.5 to 1.8 kg, clinically healthy and free from coccidiosis. The rabbits were randomly divided into 3 groups, group I as control (K-0) was given 1.0 ml distilled water/rabbit orally, group II (K-10) was infected with single dose of $10x10^6$ oocysts of *E. magna*/rabbit orally, and group III (K-20) was infected with single dose of $20x10^6$ oocysts of *E. magna*/rabbit orally. After infection, rabbits were examined for clinical signs, body weight and temperature daily for five days. Blood samples were drawn from the *vena marginalis* to examine the number of erythrocytes, hemoglobine, packed cell volume (PCV), leukocytes and its deferent, total protein plasma (TPP) and fibrinogen, activities of alkaline phosphatase (ALP), alanine amino transferase (ALT), and aspartat aminotransferase (AST). The data were statistically analyzed by two-way anova using factorial design. The results of this research showed that the infection of *E. magna* in rabbits caused fever and weight loss, accompanied by normochromic microcytic anemia (at doses of $10x10^6$ oocysts), macrocytic normochromic (at doses of $20x10^6$ oocysts), leukocytosis, lymphocytosis, hiperfibrinogenemia, and increased of ALP activity. There were correlations between clinical symptoms and blood profile of rabbits infected with *E. magna* for five days. The higher the dose and the longer the infection of *E. magna* in rabbits caused weight loss, increased body temperature, MCV (microcytic to macrocytic), leukocyte, fibrinogen and ALP activity. These findings were useful to have a better understanding of pathophysiology of *E. magna* infection in rabbits.

Key Words: *Eimeria magna*, oocyst, rabbit, blood profile

Introduction

Coccidiosis is one of the fatal diseases in rabbits caused by infection of *Eimeria sp*. Fifteen species of Eimeria in rabbits have been identified. *Eimeria (E)stidea* is the only one that invades the liver. The other species, namely, *E. perforans, E. piriformis, E. exigua, E. media, E. magna, E. coecicola, E. vejdovskyi, E. flaverscens, E. roobroucki, E. intestinalis, E. agnotsa, E. nagpurensis, E. irresidua, E. matsubayashi,* and *E. oryctolagi,* parasitize the small intestine (Soulsby, 1986).

Eimeria magna is one of the pathogenic species in rabbits (host specific), located in the small intestine. *Eimeria sp*. more often infects young rabbits of 6 week to 5 month old. The older rabbits can acquire immunity after healing, and be the carriers of infection (Kulisic et al., 2006; Jithendran, 2000). Clinical symptoms in rabbits infected with *Eimeria sp*. include loss of appetite, weight loss, diarrhea which can be watery and bloody, and dehydration (Pakandl et al., 2003; Jithendran, 2000). Infections of *Eimeria sp*. can change the blood profiles of hosts, characterized by anemia (decreasing of red blood cell and hemoglobin), increasing leukocytes (Polijicak-Milas et al*.,* 2009; Kulisic et al., 2006; Bhat et al., 1996), increasing packed cell volume (PCV), no change of eosinophils and decreasing lymphocytes (Kulisic et al., 2006).

In the early of *Eimeria sp.* infections, there are no clinical signs but followed by sudden diarrhea or death. Anemia affected with *E. magna* infection depends on the dose and duration of infection. Correlation between clinical symptoms and the alteration of blood profiles is necessary to determine the development of *E. magna* infection in rabbits.

Materials and Methods

This research used 45 male rabbits, 5 month old, range from 1.5 to 1.8 kg body weight, clinically healthy and free from coccidiosis. Rabbits were obtained from the group of husbandry from Cangar, Kota Batu, Malang, East Java. All rabbits were adapted to the environmental conditions for 7 days with normal foods (CP 86 pelleted diet and cabbage leaves) and water *ad libitum* in individual hutch ($40x50x50cm^3$ size).

Feces and ingesta of 10 rabbits full of coccidiosis (based on clinical symptoms and fecal examination) were collected and strained sequently with 100, 200 and 325 mesh strainer. Straining result was added with aquadest and incubated for two nights at room temperature (27^0C). Faecal pellete was added with 2% bichromate kalium and incubated overnight at room temperature (27^0C). After sporulation, oocyst solution was centrifugated and washed three times. Oocysts were separated from the debris by adding 13% hypochlorite sodium and being centrifugated. Infective oocysts in the supernatant were collected and washed three times. Oocysts were then counted using haemocytometer to dicide the dose of infection (Guimaraes et al., 2007, Sumartono et al., 2005).

All rabbits were randomly divided into 3 groups, group I as control (K-0) was given 1.0 ml distilled water/rabbit orally, group II (K-10) was infected with single dose of $10x10^6$ oocysts of *E. magna*/rabbit orally, and group III (K-20) was infected with single dose of $20x10^6$ oocysts of *E. magna*/rabbit orally. After infection, rabbits were examined for clinical signs, body weight and temperature, every day until the day fifth. Blood samples were drawn through the *vena marginalis* to be examined for blood profiles (RBC, Hb concentration, PCV, WBC, defferential of WBC, TPP and fibrinogen), and blood enzyme activities (ALP, ALT, AST).

The data were statistically analyzed by two-way anova using a factorial design. The first factor was the time (day-1 until day-5), while the second factor was the treatment of oocyst infection of *E. magna* with dose of $0x10^6$, $10x10^6$, $20x10^6$ (Montgomery, 1984).

Results and Discussion

Clinical symptoms of rabbits infected with $10x10^6$ of oocysts (K-10) and $20x10^6$ of oocysts (K-20) were marked by loss body weight and elevation of body temperature (Table 1-3). According to Bhat and Jithendran (1995), there were no effects of the single dose of $2x10^4$ oocysts *E. magna* infected orally for 6 weeks in Angora German rabbit (8-10 week old). However, the infective dose of $10x10^4$ oocysts caused weight loss, diarhhea and death. Coccidiosis in postweaned rabbit could cause loss of body weight significantly. The clinical symptoms of *Eimeria sp.* infections depended on the species and the number of oocysts ingesting in rabbit (Bhat et al*.,* 1996; Fioramonti et al*.,* 1981).

The loss of body weight was probably caused by the disruption of metabolism in the small intestine due to infection of *E. magna*. Oocysts were sporulated at the lumen of intestine. Sporozoites were then released from the host, penetrated, and multiplicated in the epithelial cells of intestine's mucosa. The disturbance of the metabolism of the small intestine resulted in the lack of sugar and weight loss (Zulpo et al., 2007). In this research, weight loss started two days after infection. It seemed that the higher was the dose of *E. magna* given, the faster was the rate of weight loss. The loss of body weight has been reported in cows, goats, horses, sheep, dogs and chickens infected with *Eimeria sp.* (Soulsby, 1986).

Table 1. Mean and standard deviation of clinical signs and blood features of rabbits of control-group (K-0) during five days

Parameter	Day-1	Day-2	Day-3	Day-4	Day-5
Body weight (g)	1668.0±100.70	1664.7±117.90	1671.3±120.10	1663.7±115.20	1642.7±150.10
Body temperature (^oC)	36.97±0.12	37.40±0.66	37.33±0.55	37.37±0.50	37.40±0.50
Erythrocyte ($10^6/mm^3$)	5.77±0.51	5.37±0.55	5.9±0.55	5.09±0.46	5.20±0.69
Hemoglobin (g/dl)	9.00±1.31	10.10±0.17	9.87±0.65	8.3±1.59	8.70±0.60
PCV (%)	38.00±6.50	39.33±5.77	39.00±5.20	36.67±4.73	34.33±2.00
MCV (fl)	66.01±11.48	74.12±16.00	72.41±5.87	72.27±9.74	66.86±10.25
MCH (pg)	15.71±2.84	18.96±2.15	18.36±0.70	17.05±1.69	16.87±1.81
MCHC (%)	2.38±0.22	2.60±0.33	2.55±0.21	2.40±0.49	2.54±0.12
Leukocyte ($10^3/mm^3$)	6.07±1.16	8.50±0.35	6.52±0.87	7.48±0.50	7.58±1.03
Neutrophil (sel/mm^3)	204.88±76.64	279.03±196.04	321.05±103.97	236.90±107.47	235.18±199.12
Eosinophil (sel/mm^3)	8.53±9.91	8.55±14.81	7.50±12.99	13.10±16.92	6.40±11.09
Monocyte (sel/mm^3)	38.33±34.85	41.72±23.62	41.68±25.63	44.32±31.94	44.32±31.94
Lymphocyte (sel/mm^3)	354.92±69.41	519.03±228.65	281.43±40.20	454.02±119.24	499.45±183.56
TPP (g/dl)	7.00±0.53	6.93±0.90	7.67±1.33	7.77±0.68	6.73±2.00
Fibrinogen (g/dl)	0.40±0.10	0.30±0.40	0.40±0.20	0.50±0.30	0.40±0.40
ALP (U/L)	6.12±2.65	6.12±2.65	4.59±0.00	6.12±2.65	4.59±0.00
ALT (U/L)	10.78±0.00	14.37±6.22	7.32±3.01	14.37±3.11	8.98±3.11
AST (U/L)	12.58±6.22	8.98±3.11	10.78±5.39	12.58±3.11	8.98±3.11

Tabel 2. Mean and standard deviation of clinical signs and blood features of rabbits infected with $10x10^6$ oocysts of *E. magna* (K-10) during five days

Parameter	Day-1	Day-2	Day-3	Day-4	Day-5
Body weight (g)	1643.3±81.30	1610.0±70.50*	1583.3±65.30*	1569.3±86.10*	1578.3±49.30*
Body temperature (^oC)	37.67±0.31	38.00±0.20	37.53±0.42**	37.83±0.80	38.07±0.40**
Erythrocyte ($10^6/mm^3$)	4.86±0.66	5.05±0.86	6.03±1.45**	5.84±0.69**	5.29±0.47
Hb (g/dl)	8.03±1.42	7.20±0.30	6.60±0.87**	8.20±2.17	9.00±0.52
PCV (%)	36.33±2.30	37.00±7.55	34.00±5.29	38.33±2.08	34.33±0.50
MCV (fl)	71.43±9.86	61.83±10.31*	59.46±16.37*	63.28±6.89	61.53±3.65*
MCH (pg)	16.55±2.48	14.49±2.16	11.34±2.78	14.10±3.82	17.15±2.39
MCHC (%)	2.21±0.34	2.01±0.48	1.96±0.29	2.12±0.47	2.62±0.19
Leukocyte ($10^3/mm^3$)	9.97±1.39	10.13±2.80	9.70±1.66	11.10±2.15**	10.52±1.11
Neutrophil (sel/mm^3)	201.57±58.51	131.30±62.23	250.80±115.83	287.10±102.02	167.80±63.21
Eosinophil (sel/mm^3)	7.67±13.28	0.00±0.00	2.83±4.91	4.40±7.62	7.32±6.38
Monocyte (sel/mm^3)	51.37±25.28	68.53±67.39	52.37±14,.98	70.23±40.08	42.07±4.44
Lymphocyte (sel/mm^3)	736.07±104.36	813.50±186.81	664.00+98.97	748.27±266.99	834.48±147.20
TPP (g/dl)	7.00±0.80	6.53±1.10	7.27±1.10	8.20±1.31	7.40±1.50
Fibrinogen (g/dl)	1.00±0.35	0.40±0.28**	1.33±0.50	0.80±0.87	0.67±0.50
ALP (U/L)	16.85±9.57**	4.59±0.00**	7.66±2.66	7.81±2.39	15.32±5.31**
ALT (U/L)	28.75±16.47	8.98±3.1	10.78±9.34	19.77±11.23	16.17±9.34
AST (U/L)	10.78±0.00	14.37±6.22	17.97±13.57	30.55±18.93	19.76±3.11

• : P<0.05 ; ** : P<0.01 compared to control group (K-0)

Tabel 3. Mean and standard deviation of clinical signs and blood features of rabbits infected with $20x10^6$ oocysts of *E. magna* (K-20) during five days

Parameter	Day-1	Day-2	Day-3	Day-4	Day-5
Body weight (g)	1691.7±51.1	1583.3±18.9*	1529.3±53.0*	1539.7±61.4*	1618.3±39.1*
Body temperature (^oC)	37.7±1.19**	37.9±0.62	38.4±0.51	38.6±0.56	38.9±0.40
Erythrocyte ($10^6/mm^3$)	4.02±0.08**	4.53±0.63	5.00±1.08	5.40±1.50	4.03±0.56**
Hb (g/dl)	8.10±0.52	8.40±1.50	8.30±0.96	7.30±1.71	8.10±1.08
PCV (%)	34.33±1.50	30.67±0.58	34.33±2.52	37.00±6.08	32.67±4.70
MCV (fl)	85.32±2.93*	68.50±8.69	70.04±9.32	71.66±22.23	82.57±19.04
MCH (pg)	20.12±1.00	14.24±11.11	17.33±5.02	14.31±5.94	20.55±5.17
MCHC (%)	2.36±0.17	2.74±0.49	2.44±0.43	1.96±0.19	2.51±0.47
Leukocyte ($10^3/mm^3$)	8.17±1.21	8.50±1.73	10.40±0.36	11.04±2.26**	9.07±1.32
Neutrophil (sel/mm^3)	95.97±35.44	278.30±107.36	197.10±26.55	143.83±99.76	230.70±89.96
Eosinophil (sel/mm^3)	6.20±10.74	0.00±0.00	3.50±6.06	3.37±5.83	6.13±5.47
Monocyte (sel/mm^3)	36.57±17.79	62.17±36.24	66.40±37.55	33.35±12.61	43.30±17.33
Lymphocyte (sel/mm^3)	677.93±7.63	509.53±116.43	773.00±26.85	923.45±250.97**	626.53±188.08
TPP (g/dl)	7.27±0.64	8.20±0.92	7.27±0.64	8.13±0.33	8.07±1.14
Fibrinogen (g/dl)	0.67±0.42	0.73±0.42	1.53±1.01	0.80±0.40	1.67±0.42**
ALP (U/L)	4.59±0.00**	7.65±5.39	12.25±5.3	7.66±2.66	10.72±2.65
ALT (U/L)	28.75±31.13	14.37±6.22	26.96±32.80	21.56±10.79	14.37±6.22
AST (U/L)	7.19±3.11	17.97±11.22	28.75±22.45	14.37±6.22	8.98±3.11

• : P<0.05 ; ** : P<0.01 compared to control group (K-0)

The fever caused by infection of *Eimeria sp*. was probably due to the response of inflammatory forming endogenous pyrogen that stimulates praeoptic area of hypothalamus leading to the release of local prostaglandin. The inflammatory response were characterized by the presence of polymorphonuclear leukocytes, monocytes, macrophages, and Kupffer cells. The thermoregulation mechanism occurred to maintain the body temperature at the above normal level (Dalal and Zhukovsky, 2006). The high temperature of rabbits in this research was indicated by the presence of leukocytosis. Other clinical symptoms of rabbits were weakness, gnashing teeth, dirty anus, and soft to watery feces.

This research showed that infection of *E. magna*'s oocysts in rabbits infected with $10x10^6$ of oocysts (K-10) and $20x10^6$ of oocysts (K-20) caused very significant decrease of erythrocytes and haemoglobine concentrations (P<0.01) as well as very significant increase of MCV (P<0.05), leukocytes (P<0.01), lymphocytes (P<0.01), fibrinogen (P<0.01) and ALP activity (P<0.01) compared to the control group (K-0) (Tabel 1-3). This data showed that rabbits infected with *E. magna* at dose of $10x10^6$ oocysts suffered from normochromic microcytic anemia and macrocytic normochromic anemia at dose of $20x10^6$ oocysts. Anemia that occured in rabbits in this study was probably due to the damage of the intestinal mucosal epithelium and blood vessels by *E. magna*. In dogs and sheep suffering from coccidiosis were reported a decrease in the number of erythrocytes and hemoglobin, with a form of normocytic normochromic anemia (Sahinduran et al., 2006; Kaymaz et al., 1999; Bhat et al., 1996). Kaymaz et al. (1999) reported that coccidiosis in dogs showed no change in PCV. Type of anemia in coccidiosis cases were probably influenced by the type of *Eimeria*, amount and duration of exposure of the infection. Erythrocytes contain a lot of hemoglobin, and the decrease of

erythrocytes can be followed by a decrease in hemoglobin and/or PCV and lack of oxygen in the body (Harcourt-Brown, 2003).

Leukocytosis is part of complex clinical intestinal coccidiosis feature, where most leukocytes will be transported to areas of inflammation to defend against *E. magna* infection. Leukocytosis was also reported in chickens, dogs and sheep, during acute phase of *Eimeria sp*. infection (Turk, 1986; Kaymaz et al., 1999; Sahinduran et al., 2006). Rabbits are very vulnerable to stress, and leukocytosis can be seen during stress due to illness (Lester et al., 2005).

Lymphocytes play an important role in the immunologic response. Functional tissue associated with immunity against coccidiosis is the gut associated lymphoid tissue (GALT) located in the intestine, along the mucosal layer and lamina propria. This lymphocyte forms an obstacle against the infection and takes part in the formation of antibody during the development of immunity against coccidiosis (Kulisic et al., 2006; Hartcourt-Brown, 2003; Talebi, 2001). The large amount of lymphocytes migrate actively to the mucosa of intestine confirms the presence of physiological response due to stimulation of sporozoites of *E. magna* that damages the intestine.

Fibrinogen plays an important part in blood-agglutination (Kogika et al., 2003), known as acute-phase reactant produced in the liver, acting as a good parameter for cases of inflammation. Stimulation of permeability of the capillary vessels can increase fibrinogen concentration in the tissue liquid or lymph in great amount for agglutination (Kogika et al., 2003). The increase of fibrinogen in this research might be caused by the destruction of small intestine's mucosa due to infection of *E. magna*. Inflammation of small intestine due to *E. magna* could produce histamine, protein, as well as fibrinogen to protect against infection.

In rabbits, ALP is found in almost all tissues, mostly in the epithelial cells of intestine, *tubulus renalis*, osteoblast, liver and placenta. In this research, infection of *E. magna* destroyed the epithelial cell of intestine. The inflammatory reaction caused an increasing cell activity and change in cell permeability; consequently, the ALP was released into the plasma and body liquid (Hartcourt-Brown, 2003).

Conclusions

The results of this research could be concluded that the infection of *E. magna* in rabbits caused fever and weight loss, accompanied by normochromic microcytic anemia (at doses of $10x10^6$ oocysts), macrocytic normochromic (at doses of $20x10^6$ oocysts), leukocytosis, lymphocytosis, hiperfibrinogenemia, and increasing of ALP activities. There were correlations between clinical symptoms (weight and body temperature), and blood profile of rabbits infected with *E. magna* for five days. The higher the dose and the longer the infection of *E. magna* could increase body temperature, lose weight, increase MCV (microcytic to macrocytic), leukocyte, fibrinogen, and ALP activities. These findings were useful to have a better understanding of pathophysiology of *E. magna* infection in rabbits.

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