J. Protozool. Res. 16, 42-50 (2006)

White blood cell differential count in rabbits artificially infected with intestinal coccidia

Zoran Kulišić[1*], Zoran Tambur[2], Živorad Maličević[2] Nevenka Aleksić-Bakrač[1] and Zorana Mišić[1]

[1]Faculty of Veterinary Medicine, Bulevar oslobodjenja 18, 11 000 Belgrade, Serbia.

[2]Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia.

*Corresponding author: Zoran Kulišić, E-mail: zkulisic@vet.bg.ac.yu

ABSTRACT

The aim of the present study was to examine the effect of intestinal coccidia upon the white cell count and differential leukocyte count of the infected rabbits. Two groups of rabbits were infected with various doses of intestinal coccidia oocysts. The first group (A) was infected with 2 x 10^5, and the second (B) with 4 x 10^5 of sporulated oocysts. The inoculum was composed of oocysts of the following intestinal coccidia: *Eimeria flavescens* (7%), *E. matsubayashii* (9%), *E. magna* (12%), *E. neoleporis* (19%), *E. perforans* (21%) and *E. media* (32%). The infective mix was dominated by moderately pathogenic species causing a mild form of intestinal coccidiosis in the rabbits. The third group (C) served as the control (uninfected rabbits). Immediately before the infection, on days 4, 7 and 10 post infection, the rabbits were bled and white cell count, blood smears and differential blood cell count were done. We found that in this form of intestinal coccidiosis, the number of white cells was increased. The number of monocytes was risen significantly only on day 10. Lymphocyte count decreased whereas the number of eosinophiles remained unchanged despite the fact that coccidiosis is a parasitic disease.

Key words: rabbit, coccidiosis, leukocyte differential count

INTRODUCTION

Coccidiosis is one of the most frequent and most prevalent parasitic diseases, accompanied by weight loss, mild intermittent to severe diarrhea with faeces containing mucus or blood and results in dehydration and decreased rabbit breeding (Bhat and Jithendran, 1995; Jithendran and Bhat, 1996; Peeters *et al*., 1984). In addition to this, certain changes in blood, urine and feces can often be revealed (Kulišić *et al*., 1998; Licois *et al*., 1978a; Licois *et al*., 1978b; Tambur *et al*., 1998a; Tambur *et al*., 1998b; Tambur *et al*., 1998c; Tambur *et al*., 1999).

It is difficult to find brood of rabbits without various species of coccidia oocysts. This infection attacks young rabbits, especially 2-6-month-old (Gres *et al*., 2003). Older animals, having recovered from the disease acquire immunity, but they are still important as carriers.

Many authors worldwide published studies on morphological characteristics of coccidia (Licois *et al*., 1992; Pakandl *et al*., 1996a, 1996b), but few of them examined changes in blood, urine and faeces caused by coccidia developing in rabbit's liver and intestines.

Intending to investigate the potential of rabbit's intestinal coccidia on leukocytes, we decided to monitor white blood cell count and differential blood cell count in artificially infected rabbits.

MATERIALS AND METHODS

In order to induce the sporulation of oocysts, potassium bichromate water solution (20 g/l) was added to faeces of rabbits infected with coccidia and incubated at 27°C for 5 days. In order to determinate the intestinal coccidia species, the following morphological characteristics were observed: the length, width, shape, colour and wall thickness of oocysts, as well as the presence of micropili and oocyst's residual body.

The material for artificial infection was prepared by standard methods as well as the artificial infection of rabbits itself.

The infective material consisted of the following species of rabbit's intestinal coccidias: *Eimeria flavescens* (7%), *E. matsubayashii* (9%), *E. magna* (12%), *E. neoleporis* (19%), *E. perforans* (21%) and *E. media* (32%).

The groups consisted of 12 52-day-old chinchilla rabbits (*Oryctolagus cuniculus*) weighing 1,200-1,300 g. Two groups were infected with various doses of sporulated oocysts of intestinal coccidia. The first group (A) was infected with 2×10^5 sporulated oocysts and the other one (B) with 4×10^5 oocysts. The inoculum was administrated to the animals by a stomach tube, after 12-hour starvation. The third group (C), the uninfected rabbits, was the control one. All the animals in the experiment were free of other parasites excluding the abovementioned coccidia used for artificial infection.

Immediately before inoculation (day 0) as well as on days 4, 7 and 10, rabbits were bled and white blood cell (WBC) count and differential leukocyte count were estimated as described further.

Venous blood was drawn to the 0.5 mark in a white blood cell diluting pipet (Davidson and Henry, 1974). The diluting fluid (2% acetic acid) was immediately drawn to the "11" mark while rotating the pipet to mix the specimen and diluent for 3-5 minutes. This resulted in dilution 1:20. Unmixed fluid from the capillary portion of the pipet was expelled. The chambers of the hemacytometer were filled completely. The cells are left to settle for about 3 minutes. The leukocytes were counted under low-power magnification and reduced light, focusing on the ruled area and counting the white blood cells in the four 1 mm^2 corner areas corresponding in each of the two chambers. Since, blood is drawn to the 0.5 mark and diluted to the 11 mark with WBC diluting fluid. All the blood is washed into the bulb of the pipet (which has a volume of 10). Since the depth of the counting chamber is 0.1 mm and the area counted is 4 mm^2 (4 squares are counted, each with an area of 1 mm^2 therefore, 4 x 1 mm^2 = a total of 4 mm^2). The volume counted is: area x depth = volume. Four mm^2 x 0.1 mm = 0.4 mm^3. The formula is as follows:

$$\text{WBC per mm}^3 = \frac{\text{Average number of chambers (2) x Dilution (20)}}{\text{Volume (0.4)}}$$

Blood smears were prepared on microscope slides, from a drop of capillary blood (from the rabbits' ears). Each blood drop was spread with another slide. The preparation is fixated for 3 minutes in May-Grünwald stain, diluted with an equal quantity of distilled water and stained for 3 minutes. The solution is poured off and the preparation is further stained with Giemsa solution for 15-20 minutes. After washing in tap water, the slides were air-dried and examined under 400 x magnification (Davidson and Henry, 1974).

Descriptive methods in statistical analysis (MV, SD) and Student's *t*-test for dependent samples were used.

RESULTS

After the infection rabbits developed mild coccidiosis, presenting the symptoms as polydipsia, bristling hair, decreased appetite and moderate body weight loss. Only three rabbits in B group fully developed clinical manifestations of intestinal coccidiosis with bloody diarrhoea and considerable body weight loss.

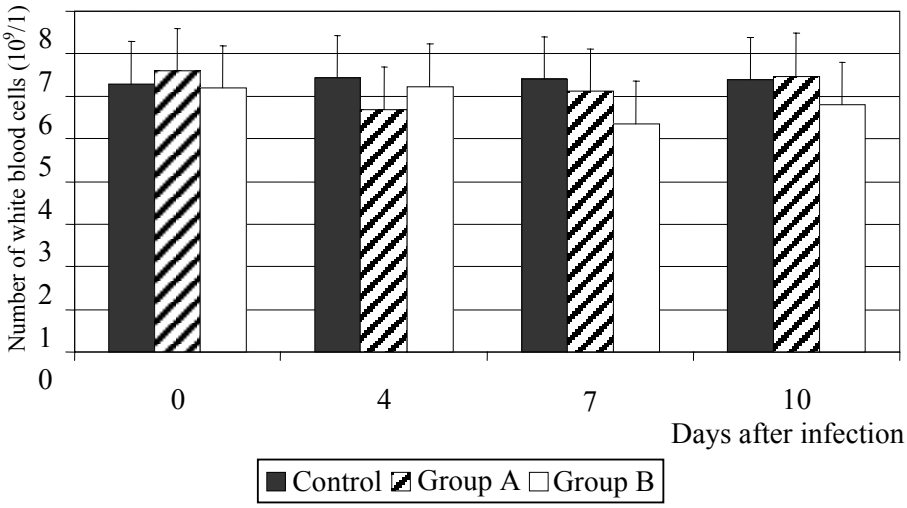

Figure 1. White blood cell numbers (MV, SD) in rabbits infected with intestinal coccidia (10^9/l)

White blood cell count (Fig. 1)

There was no statistically significant difference in WBC count between uninfected (Control) and infected rabbits (groups A and B), as well as between the groups of infected rabbits ($p>0.05$) neither on day 0, nor at any time during the experiment.

The average leucocyte number in the control group was 6.29 x 10^9/l on the day of infection, 6.42 x 10^9/l on day 4, 6.41 x 10^9/l on day 7 and 6.38 x 10^9/l on day 10.

In the group A, the number of white blood cells was initially 6.59 x 10^9/l (day 0), reaching 5.69, 6.11 and 6.48 x 10^9/l on days 4, 7 and 10, respectively.

Rabbits infected with higher number of oocysts had 6.2 x 10^9/l leucocytes on the day of infection. The range of WBC count persisted as following: 6.23 (day 4), 5.36 (day 7) and 5.81 x 10^9/l (day 10).

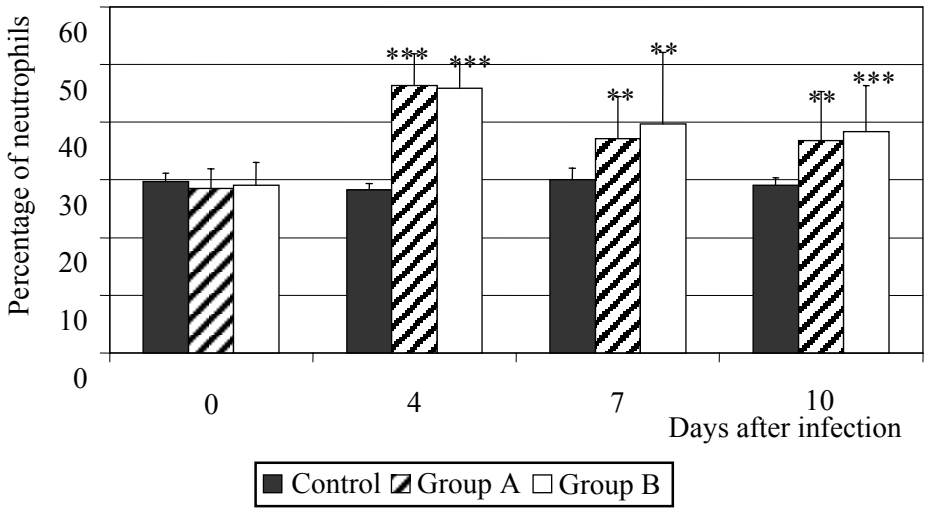

Figure 2. Neutrophil numbers (MV, SD) in rabbits infected with intestinal coccidia (%) ** $p<0.01$; *** $p<0.001$ – significance for intergroup statistical difference (C:A) and (C:B)

Neutrophil granulocyte count (Fig. 2)

In both infected groups of rabbits the number of neutrophil granulocytes increased significantly in comparison with the control.

In group A the percentage of neutrophils increased from initial 28.5% to 46.3% on day 4. The value calculated on day 7 was somewhat smaller, 37.2%, and similar to the value on day 10. In comparison with the control group neutrophil count, the difference was statistically significant ($p<0.001$) on day 4, as well as on days 7 and 10 ($p<0.01$).

Neutrophil count in rabbits from the group B was 29.00% at the beginning of the experiment and 45.90%, 39.70% and 38.30% 4, 7 and 10 days later, respectively. The difference was statistically significant compared to the control, the p value being $p<0.001$ on days 4 and 10, and $p<0.01$ on day 7.

The differences in neutrophil counts between groups A and B were insignificant ($p>0.05$) on days 4, 7 and 10.

The percentage of neutrophils in uninfected rabbits ranged from 28.3 % (day 4) to 30.8 % (day 7), thus the difference being without significance ($p>0.05$).

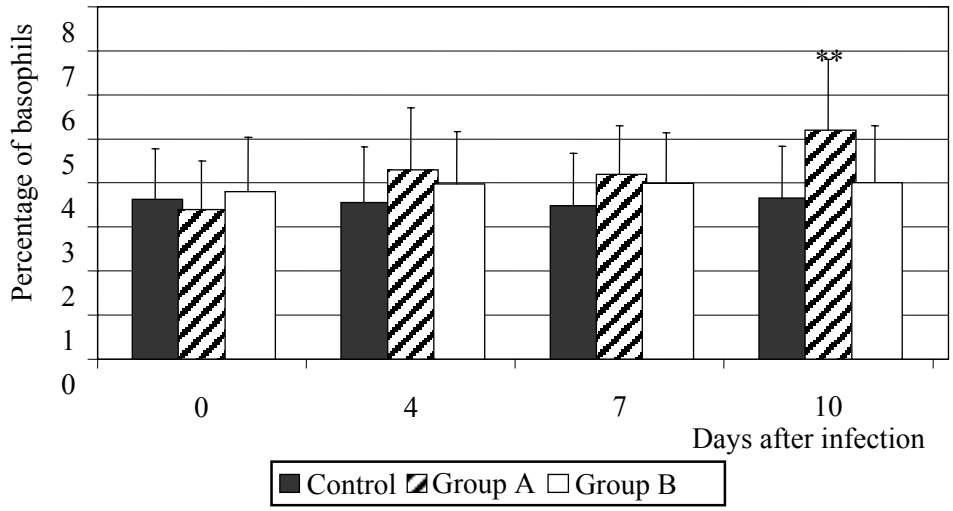

Figure 3. Basophil numbers (MV, SD) in rabbits infected with intestinal coccidia (%)

** $p<0.01$ – significance for intergroup statistical difference (C:A) and (C:B)

Basophils granulocyte count (Fig. 3)

The basophil granulocyte counts in the group A were 3.40%, 4.30%, 4.20% and 5.20% on days 0, 4, 7 and 10, respectively. The increase on day 10 was the only significant one ($p<0.01$).

In group B, the average numbers of basophils varied within the interval from 3.89 (day 0) to 4.00% (day 10), remaining insignificant ($p>0.05$).

The differences in basophil counts between the groups of infected animals were insignificant ($p>0.05$) on days 4, 7 and 10.

The percentages of basophils in uninfected rabbits were 3.62% (day 0), 3,55%(day 4), 3.48% (day 7) and 3.65% (day 10) and differed insignificantly ($p>0.05$).

Eosinophil granulocyte count (Fig. 4)

The percentages of eosinophils remained on similar levels throughout the experiment, with insignificant difference ($p>0.05$). In the control group the number was 1.25% the whole time, in group A the values ranged from 1.15% (day 0) to 1.35% (day 10), similarly to those in group B, in which the corresponding percentages fluctuated in limits from 1.28% (day 0) to 1.34% (day 4).

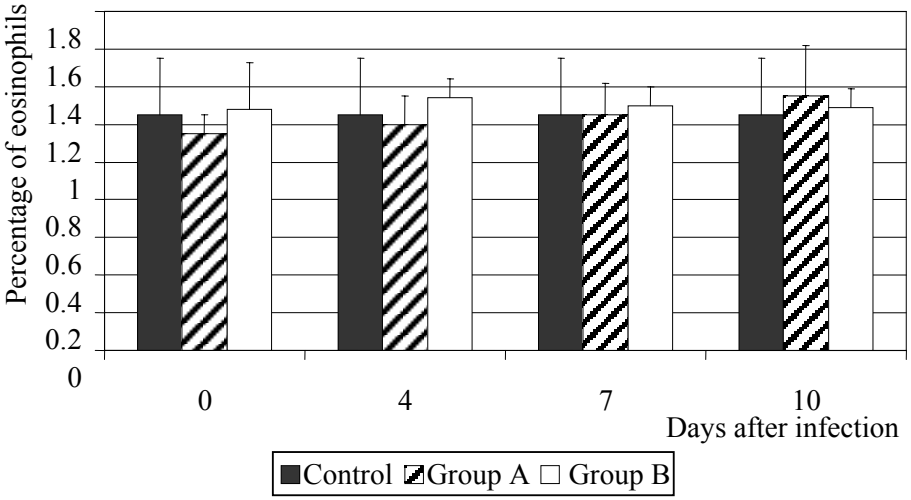

Figure 4. Eosinophil numbers (MV, SD) in rabbits infected with intestinal coccidia (%)

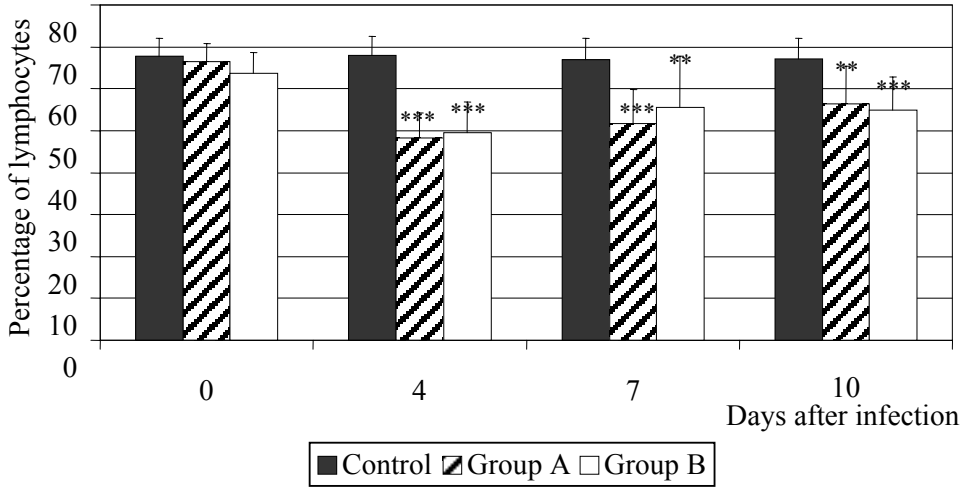

Figure 5. Lymphocyte numbers (MV, SD) in rabbits infected with intestinal coccidia (%)

** $p<0.01$; *** $p<0.001$ – significance for intergroup statistical difference (C:A) and (C:B)

Lymphocyte count (Fig. 5)

The number of lymphocytes in blood significantly decreased in rabbits infected with coccidia. In both infected groups, the numbers of lymphocytes were lowest on the day 4, significantly lower than in the control ($p<0.001$). On days 7 and 10 the numbers slightly increased. In animals infected with less oocysts, the percentages of these cells were 51.70% (day 7) and 56.40% (day 10). The decrease was significant in comparison to the control ($p<0.001$ on day 7, and $p<0.01$ on day 10).

In group B, the calculated lymphocyte percentages were 55.50% and 54.90% on days 7 and 10, respectively. By comparison with the control, the decreases were significant ($p<0.01$ on day 7 and $p<0.001$ on day 10).

The differences in lymphocyte counts between the two infected groups of rabbits was insignificant. The percentages of lymphocytes in uninfected rabbits remained on similar levels, with differences being insignificant ($p>0.05$).

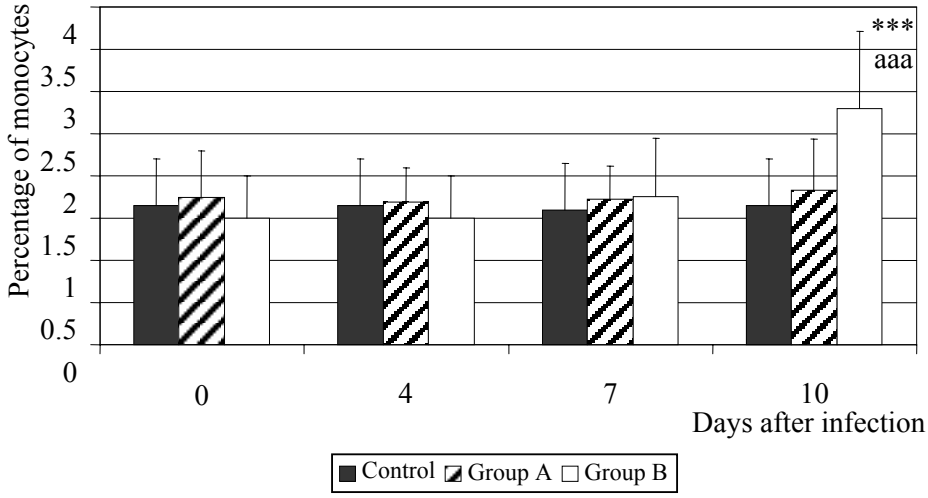

Figure 6. Monocyte numbers (MV, SD) in rabbits infected with intestinal coccidia (%)

*** $p<0.001$ – significance for intergroup statistical difference (C:A) and (C:B);

aaa $p<0.001$ – significance for intergroup statistical difference (A:B)

Monocyte count (Fig. 6)

In the control group, the numbers of monocytes in blood were almost constant, 1.65%, with the exception of the value on day 7 (1.60%), but still with no significant difference ($p>0.05$).

In group A, the average monocyte number varied slightly, from 1.75% at the begining of the experiment, to 1.83% on day 10, being insignificantly different in comparison with the control.

In group B, the percentages of monocytes were 1.50 on days 0 and 4, increasing insignificantly to 1.76% (day 7) and significantly ($p<0.001$) on day 10, reaching the peak of 2.80%, in comparison with the control and group A.

DISCUSSION

The causal agents of rabbit coccidiosis develop direct life cycle multiplying very fast and leading to massive infection, especially in young rabbits (Gres *et al*., 2003; Licois *et al*., 1992; Pakandl, 2005; Ryley & Robinson, 1976). Developing through more generations of meronts, these pathogens cause desquamation of intestinal mucosa, capillary rupture and bleeding into the intestinal lumen with catarrhal or haemorrhagic enteritis, consequently (Bhat and Jithendran, 1995; Castro and Duszynski, 1984; Peeters *et al*., 1984). Besides damaging intestinal mucosa, coccidiae cause general reaction of the organism with consequent changes in blood, urine and faeces (Licois *et al*., 1978a; Licois *et al*., 1978b; Jithendran and Bhat, 1996; Kulišić *et al*., 1998; Tambur *et al*., 1998a; Tambur *et al*., 1998b; Tambur *et al*., 1998c; Tambur *et al*., 1999). Biochemical investigations of blood taken from rabbits experimentally infected with coccidia revealed significant changes in the activity of GOT and alkaline phosphatase and in the amount of bilirubin (Sherkov *et al*., 1986).

Leukocyte count change could be considered as a part of clinical intestinal coccidiosis complex. Inflammatory process locally recruites leukocytes, so they are lost through damaged

intestinal mucosa in the next phase, which is the probable reason of their count decrease. Count increase in later phase should be attributed to haemoconcetration as a result of fluid loss.

All the time during the infection we have noticed neutrophil count increase. It was most remarkable on day 4 after the infection and coincidental with development of the second generation of meronts that cause serious damages of intestinal mucosa and blood vessels endothelium. Neutrophil count was mildly increased, because of inflammation (Castro and Duszynski, 1984).

It is interesting that during the experiment, there was no change in eosinophils count in coccidious rabbits.

According to Castro and Duszynski (1984), who reported on rats infected with *E. nieschulzi*, coccidia interfere with some phase in the directed migration of leukocytes to sites of inflammation and does not affect hematopoiesis. That is the reason why the granulocyte count in peripheral blood is normal or slightly increased in coccidious rabbits.

During the whole experiment there was a remarkable decrease in lymphocyte count due to the damage of lymphatic tissues covering the intestines, which was caused by coccidia. The total leukocyte count decreased insignificantly because of the damage of intestinal mucosa and toxic metabolic products (Peeters *et al*., 1984).

A monocyte cell count increase on day 10 post infection was noticed.

McQuistion & Schurr (1978) revealed that rats infected with *E. neischulzi* had significantly higher total leukocyte counts in comparison with the control. Relative and absolute neutrophil counts increased concomitantly with a decrease in the relative lymphocyte levels in *E. nieschulzi*-infected rats on day 7 post-infection. Absolute and relative neutrophil counts in infected rats on days 7 and 8 were closely correlated with the host's total oocyst discharge. The *E. nieschulzi* infection had no significant effect on the relative or absolute levels of monocytes or eosinophils. The described changes in leukocyte levels were not accompanied by a significant change in the erythrocyte count.

The results of the present study indicated the influence of intestinal coccidia on white blood cell differential count in rabbits, which must be taken in consideration while investigating the reasons of changes in leukocyte counts.

ACKNOWLEDGEMENTS

This study is supported by the Ministry of Science and Environmental Protection of the Republic of Serbia (Project No. 143022).

REFERENCES

Bhat, T. K. & Jithendran, K. P. 1995. *Eimeria magna*: The effect of varying inoculum size on the course of infection in Angora rabbits. World Rabbit Sci. 3: 163-166.

Castro, G. A. & Duszynski, D. W. 1984. Local and systemic effects on inflamation during *Eimeria nieschulzi* infection. J. Protozool. 31: 283-287.

Davidson, J. & Henry, J. 1974. Clinical diagnosis by laboratory methods. 15th ed. Philadelphia, Pa: WB Saunders Co.

Gres, V., Voza, T., Chabaud, A. & Landau, I. 2003. Coccidiosis of the wild rabbit (*Oryctolagus cuniculus*) in France. Parasite 10: 51-57.

Jithendran, K. P. & Bhat, K. P. 1996. Subclinical coccidiosis in Angora rabbits – A field suvey in Himachal Pradesh (India). World Rabbit Sci. 4: 29-32.

Kulišić, Z., Tambur, Z., Maličević, Ž. & Radosavljević, M. 1998. Changes in the activity of enzymes primarily not synthesited in the liver following the infection rabbits with intestinal coccidia. J. Protozool. Res. 8:1-9.

Licois, D., Coudert, P. & Mongin, P. 1978a. Changes in hydromineral metabolism diarrhoeic rabbits. A study of the changes in water metabolism. Ann. Rech. Vet. 9: 1-10.

Licois, D., Coudert, P. & Mongin, P. 1978b. Changes in hydromineral metabolism in diarrhoeic rabbits. 2. Study of the modifications of electrolyte metabolism. Ann. Rech. Vet. 9: 453-464.

Licois, D., Coudert, P., Bahagia, S. & Rossi, G. L. 1992. Endogenous development of *Eimeria intestinalis* in rabbits (*Oryctolagus cuniculus*). J. Parasitol. 78: 1041-1048.

McQuistion, T. E. & Schurr, K. M. 1978. The effect of *Eimeria neischulzi* infection on leukocyte levels in the rat. J. Protozool. 25: 374-377.

Pakandl, M. 2005. Selection of a precocious line of the rabbit coccidium *Eimeria flavescens* Marotel and Guilhon (1941) and characterisation of its endogenous cycle. Parasitol. Res. 97: 150-155.

Pakandl, M., Eid Ahmed, N., Licois, D. & Coudert, P. 1996a. *Eimeria magna* Perard, 1925: study of the endogenous development of parental and precocious strains. Vet. Parasitol. 65: 213-322.

Pakandl, M., Gaca, K., Licois, D. & Coudert, P. 1996b. *Eimeria media* Kessel 1929: comparative study of endogenous development between precocious and parental strains. Vet. Res. 27: 465-472.

Peeters, B. E., Charlier, G., Antoine, O. & Mammericlex, M. 1984. Clinical and pathological changes after *Eimeria intestinalis* infection in rabbits. Zbl. Vet. Med. B 31: 9-24.

Ryley, J.F. & Robinson, T.E. 1976. Life cycle studies with *Eimeria magna* Perard, 1925. Z. Parasitenkd. 12: 257-275.

Sherkov, Sh. N., Khalacheva, M., Kostova, T., Malchevski, M. & Arnaudov, D. 1986. Etiopathogenesis and epizootiology of coccidiosis in rabbits. Vet. Med. Nauki. 23: 11-17.

Tambur, Z., Kulišić, Z., Maličević, Ž. & Mihailović, M. 1998a. The influence of intestinal coccidia upon the activity of liver enzimes. Acta Vet. 48: 139- 146.

Tambur, Z., Kulišić, Z., Maličević, Ž. & Mihailović, M. 1998b. Influence of intestinal coccidia infection of rabbits upon plasma and fecal proten levels, and plasma and urinary urea and creatinins levels. Acta Vet. 48: 147-156.

Tambur, Z., Kulišić, Z., Maličević, Ž. & Mihailović, M. 1998c. The influence of intestinal coccidia of rabbits upon plasma and urine electrolyte concentrations. Acta Vet. 48: 225-234.

Tambur, Z., Kulišić, Z., Maličević, Ž. & Mihailović, M. 1999. Blood glucose, plasma osmolarity and urea and creatinine clearance in rabbits artificially infected with intestinal coccidia. Acta Vet. 49: 171-176.