EFFECT OF PARASITIC NEMATODES *TRICHOSTRONGYLUS COLUBRIFORMIS* ON HAEMATOLOGICAL PARAMETERS OF MODEL HOST (*ORYCTOLAGUS CUNICULUS* F. *DOMESTICA*)

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Groups of 5 rabbits were infected with a single dose of 10 000 infective larvae of *Trichostrongylus colubriformis* per rabbit or lower multiple infections with or without immunosuppression. Another groups were uninfected control with or without immunosuppression. All rabbits were killed 20 days after last infection and before their killing 2 ml of their blood were taken from each rabbit, then mixed with 0.1 ml EDTA. Haematological parameters of infected rabbits and uninfected control were analysed by means of the apparatus Coulter CBC-5. RBC – red blood cell count (T/l), HCT – haematocrit (%), MCV – mean corpuscular volume of erythrocyte (fl), HGB – haemoglobin (g/100 ml), WBC – white blood cell count (G/l) were determined. In addition, differential leucocyte counts (% neutrophils, eosinophils and basophils) were made. The nematode infections altered the neutrophil level to high degree, especially in groups with immunosuppression. In uninfected animals the mean value of neutrophils ranged from 9.33 to 39.33%. Eosinophils were found only in uninfected groups. No basophils were found. Values of erythrogram (RBC, haemoglobin and haematocrit) were mostly reduced in the present work.

rabbit; *Trichostrongylus colubriformis*; red blood cell count; haematocrit; mean corpuscular volume of erythrocyte; white blood cell count; haemoglobin; differential leucocyte counts

INTRODUCTION

Trichostrongylus colubriformis is a common pathogenic nematode of ruminants. Control of *Trichostrongylus colubriformis* infection is currently being attempted using anthelmintic drugs, but in future it is anticipated that immunological control by vaccination may be achieved. In order to work towards this goal, it is important to characterise parasitic antigens. However, the use of substantial numbers of large farm animals in experimental studies can be very costly and in laboratories this factor has a limiting effect on research into diseases of sheep and cattle. There are obvious important advantages in identifying laboratory animal host systems for such research. Studies of laboratory trichostrongylidosis, and among others of the parasite on the blood picture, may throw comparative light on other, more economically important models, such as sheep and their gastro-intestinal nematodes.

The course of *Trichostrongylus colubriformis* infection in rabbits resembles that in the ruminants. The aim of the present study was to examine the haematological changes in rabbits (*Oryctolagus cuniculus* f. *domestica*) exposed to various single or multiple infections of *Trichostrongylus colubriformis*.

MATERIAL AND METHODS

Host

The rabbits (New Zealand White race) of similar age (3 months) and weight, both sexes were randomly allotted to 5 groups of 5 rabbits. They were kept in cages with wire-netting floors to prevent autoinfection, and were fed on pellet-form diet KKK POLT with anticoccidium for the duration of the study. Faecal examinations were made before infection to ensure that animals were free from natural infections.

Parasite

Acquisition of parasites was described in earlier paper "Arrested development of *Trichostrongylus colubriformis* in experimentally infected rabbits. Effect of decreasing photoperiod, low temperature and desiccation" (Langrová, Jankovská, 2002).

Immunosuppression of rabbits

Immunosuppression of animals was performed with continual administrations of prednisolone (Prednison 80 mg per rabbit and day). Start of immunosuppression was one week before inoculation L_3 to rabbits and end of immunosuppression was 3 days before killing of rabbits.

The immunosupression on the rabbits with continuous administration of prednisolone showed no significant increase in the propensity of *Trichostrongylus* larvae for arrested development (Schmid, 1986).

Inoculation

Group 1: Rabbits with immunosuppression were infected with 10 000 *T. colubriformis* infective larvae. The capture was 2000 worms (20%).

Group 2: Rabbits with immunosuppression infected with multiple infection: a) 5 x 150 larvae; b) 2 x 750 larvae. The capture was 220 worms (21%).
Group 3: Non infected rabbits with immunosuppression.
Group 4: Rabbits without immunosuppression were infected with 10 000 *T. colubriformis* infective larvae. The capture was 2200 worms (22%).
Group 5: Non infected rabbits without immunosuppression (control group).

Investigation of blood of rabbits

The blood samples (2 ml of blood) were taken from rabbits of part B before their killing (20 days after infection) and were mixed with 0.1 ml EDTA. Haematological parameters of infected and uninfected rabbits with or without immunossuppresion were investigated by means of the apparatus Coulter CBC-5 and those parameters were found out: RBC – red blood cell count (T/l), HCT – haematocrit (%), MCV – mean corpuscular volume of erythrocyte (fl), WBC – white blood cell count (G/l), HGB – haemoglobin (g/100 ml). In addition, differential leucocyte counts was made.

Statistical methods

Haematological parameters were compared by non-parametric Kruscal–Wallis ANOVA. Subject to the ANOVA indicating that significant difference existed between the means, the least squares means were then compared by LSD (least significant difference) test ($P < 0.05$).

RESULTS

Table 1 shows haematological parameters of infected rabbits and uninfected controls with or without immunossuppresion. The nematode infections altered the neutrophil level to the high degree, especially in groups with immunosuppression (groups 1 and 2). In uninfected animals the mean value of neutrophils was 9.33% (uninfected without immunosuppression, group 5) and 39.33% (uninfected with immunosuppression, group 3). Values of erythrogram (erythrocyte counts /RBC/, haemoglobin and haematocrit) of infected animals were mostly reduced.

The average values of individual components of blood picture are shown in Fig. 1. The effect of immunosup-

Table 1. Haematological parameters

	RBC	HCT	MCV	WBC	HGB	LYM	NEU	EOS
1) Rabbits with immunosuppression infected with 10 000 *T. colubriformis* infective larvae. The capture was 2000 worms (20%)								
Mean	3.29	26.33	66.50	5.35	8.23	24.40	75.20	0.00
SMODCH	0.66	5.45	3.84	1.25	1.83	16.89	16.47	0.00
Min	3.07	20.30	61.00	4.10	6.50	6.00	44.00	0.00
Max	4.80	33.50	71.00	6.60	11.30	56.00	92.00	0.00
2) Rabbits with immunosuppression infected with multiple infection (5 x 150 and 2 x 750 *T. colubriformis* infective larvae) groups. The capture was 220 worms (21%)								
Mean	4.89	32.80	76.50	4.13	8.87	36.00	63.33	0.00
SMODCH	1.09	7.50	13.35	0.90	1.31	9.93	8.99	0.00
Min	3.80	25.30	66.00	3.10	7.20	22.00	56.00	0.00
Max	5.99	40.30	99.00	5.30	10.40	44.00	76.00	0.00
3) Non-infected rabbits with immunosuppression								
Mean	5.31	33.56	57.67	5.83	11.90	52.33	39.33	0.67
SMODCH	0.97	3.19	2.05	0.66	1.39	21.77	23.71	1.49
Min	3.96	29.10	55.00	4.90	10.10	24.00	12.00	0.00
Max	6.54	36.40	60.00	6.30	13.50	88.00	76.00	4.00
4) Rabbits without immunosuppression infected with 10 000 *T. colubiformis* infective larvae. The capture was 2200 worms (22%)								
Mean	3.41	24.47	71.00	2.57	7.82	79.00	19.00	0.00
SMDOCH	1.58	11.02	1.58	1.53	3.34	2.55	0.71	0.00
Min	1.87	13.20	69.00	1.00	4.10	76.00	18.00	0.00
Max	5.07	37.40	73.00	5.10	11.30	82.00	20.00	0.00
5) Non-infected rabbits without immunosuppression (control group) – KB4								
Mean	4.32	32.75	75.00	6.45	10.40	89.00	9.33	2.67
SMDOCH	0.43	4.15	2.00	0.35	0.90	3.42	3.94	0.94
Min	3.89	28.6	73.00	6.10	9.50	84.00	4.00	2.00
Max	4.74	36.90	77.00	6.80	11.30	94.00	16.00	4.00

Table 2. Statistical evaluation of haematological parameters on the 5% level calculated ($P < 0.05$)

Tested groups	RBC	HCT	MCV	WBC	HGB	LYM	NEU	EOS
1 : 2	0.006	NS	NS	0.02	NS	NS	0.05	NS
1 : 3	0.005	0.03	0.002	NS	0.007	0.05	0.02	NS
1 : 4	NS	NS	0.04	0.01	NS	0.0001	0.0001	NS
1 : 5	NS	NS	0.002	NS	0.04	0.00001	0.00001	0.002
3 : 5	NS	NS	0.0001	NS	NS	0.006	0.02	0.03
4 : 5	NS	NS	0.008	0.006	NS	0.008	0.006	0.002

NS = non-significant difference between tested groups

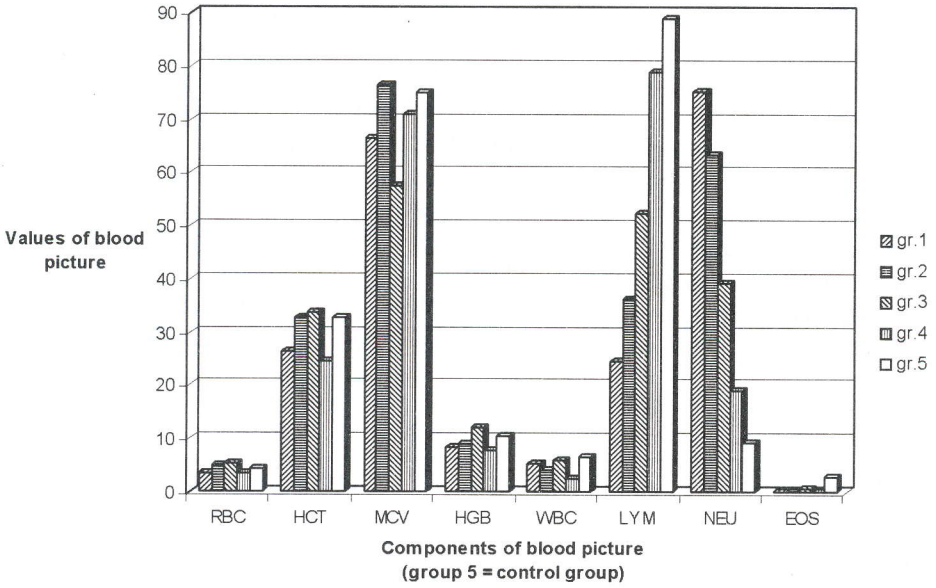

Fig. 1. Blood picture of individual groups of rabbits

pression manifested only in case of lymphocytes, where lymphopenia was observed in immunosuppressed groups. Neutrophilia occurred in both infected animal groups with immunosuppression, particularly in group 1 (75.2%).

DISCUSSION

Statistically significant changes during the course of infection were found in the neutrophills in present study. The fluctuations in the numbers of these leucocytes were more considerable in animals exposed to infective larvae and immunosuppression than in other groups of animals. Turner et al. (1960) claim that during sheep strongyloidosis neutrophils migrate most rapidly and that they actively attack larvae. The occurrence of neutrophilia, in the present study, can be explained by the fact that neutrophils posses a wide range of hydrolytic enzymes as well as cationic proteins, which have been shown elsewhere to be lethal for the developing parasite (Incani, McLaren, 1983). Secondly, neutrophils may actively secrete or generate products to activate macrophages.

The differential leucocyte counts were carried out 20 days after infection in rabbits exposed to a single infection with 10 000 infective larvae or lower multiple infections and only neutrophils showed increased levels. Purvis and Sewell (1971) reported that in rabbits infected with a single dose of 15 000 *Trichostrongylus colubriformis* larvae eosinophilia and basophilia occurred between 14 and 28 days after infection. In present work no basophils were found out. Eosinophils were found out only in blood of uninfected rabbits. Doligalska et al. (1990) found out changes in percentage of neutrophils, too. Increased counts of eosinophils were found out by Doligalska et al. (1990) only 21 days p.i. However, in present work rabbits were killed and blood samples were taken 20 days p.i. Eosinophilia is most often connected with infections with parasites which complete migration in the host organism (Stankiewicz, 1969; Bezubik, 1970) and to a lesser extent in animals infected with parasites whose location is limited to the alimentary tract (Bezubik et al., 1977).

Also statistically significant lymphopenia were observed in this work as well as Bezubik et al. (1988) did. Significant lymphopenia occurred in all infected rabbits, and particularly in those exposed to immunosuppression. Lymphopenia was also observed by Bezubik et al. (1977) and Sinski (1975) in sheep

ostertagiosis, but in sheep haemonchosis Silverman et al. (1970) and Bezubik et al. (1980) did not obtain significant changes in blood leucocyte levels. Many authors claim that peripherial lymphopenia in parasitic diseases is connected with local leucocytosis. Bezubik et al. (1988) describe during *Obeliscoides cuniculi* infection, the occurrence of lymphopenia was directly associated with lymphocyte migration from vessels to the gastric mucosa. Lymphopenia is also associated with increase in neutrophil percentages in peripherial blood. It is generally believed that polymorphonuclears, neutrophils and basophils, play – besides macrophages – a part in phagocytosis of antigens (Chapes, Haskill, 1983).

Haematological changes in rabbit obeliscoidosis were studied by Russell et al. (1966). These authors did not find changes in the red (haematocrit, haemoglobin) and white (differential leucocyte counts) blood picture after exposure of rabbits to 2500 to 25 000 larvae of *Obeliscoides cuniculi*. Values of RBC, haemoglobin and haematocrit were in present work mostly reduced, especially in groups infected with 10 000 infective larvae. Also Hayat et al. (1996) found out values of RBC, haemoglobin and haematocrit of sheep infected with trichostrongylids (*Trichostrongylus colubriformis, Haemonchus contortus*) significantly reduced.

The present experiments were carried out in order to investigate the effect of the size of single and multiple infections upon the course and pathogenicity of *T. colubriformis* infection in rabbits.

Haematological parameters in present study showed only neutrophils increased levels in rabbits exposed to a single infection with 10 000 infective larvae (the mean value was 75.20%) or lower multiple infections (the mean value was 63.33%). The mean value of neutrophils in uninfected animals (control groups) was 9.33%.

Trichostrongylus colubriformis is not highly pathogenic for rabbits, if measured by the changes in the blood picture. Immunosuppression affected only in case of lymphocytes.

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Vliv parazitické hlístice *Trichostrongylus colubriformis* na krevní obraz modelového hostitele králíka domácího (*Oryctolagus cuniculus* f. *domestica*).

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Skupiny po pěti králících byly infikovány buď jednorázově dávkou 10 000 infekčních larev hlístice *Trichostrongylus colubriformis* na jednoho králíka, nebo nižší opakovanou dávkou spolu s přítomností nebo absencí imunosupresiva. Další králíci sloužili jako neinfikované kontrolní skupiny s přítomností nebo absencí imunosupresiva. Všechna zvířata byla zabita 20 dní po poslední infekci a před zabitím jim byly odebrány 2 ml krve, které byly smíchány s 0,1 ml EDTA. Krevní obraz byl vyhodnocován pomocí přístroje Coulter CBC-5. Hodnoceny byly tyto parametry: RBC – počet červených krvinek (T/l), HCT – haematokrit (%), MCV – střední objem erytrocytu (fl), HGB – hemoglobin (g/100 ml), WBC – počet bílých krvinek (G/l). Navíc byl spočítán diferenciální krevní obraz (leukogram), který udává početní zastoupení jednotlivých druhů bílých krvinek (procento neutrofilů, eosinofilů a bazofilů). U infikovaných králíků byla zjištěna zvýšená hladina neutrofilů, zejména u skupin s imunosupresí. U neinfikovaných zvířat se hodnota neutrofilů pohybovala v rozmezí 9,33 až 39,33 %. Eosinofily byly zjištěny jen u neinfikovaných zvířat. Bazofily nebyly zjištěny vůbec. Hodnoty erytrogramu (RBC, hemoglobin a haematokrit) byly většinou snížené.

králík; *Trichostrongylus colubriformis*; počet červených krvinek; hematokrit; střední objem erytrocytu; počet bílých krvinek; hemoglobin; diferenciální krevní obraz (leukogram)

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