Bulgarian Journal of Veterinary Medicine (2005), 8, No 2, 141–148

CLINICAL AND HAEMATOLOGICAL STUDIES IN DOGS, EXPERIMENTALLY INFECTED WITH *TRICHURIS VULPIS*

Z. KIRKOVA, P. PETKOV & D. GOUNDASHEVA

Faculty of Veterinary Medicine, Trakia University,
Stara Zagora, Bulgaria

Summary

Kirkova, Z., P. Petkov & D. Goundasheva, 2005. Clinical and haematological studies in dogs, experimentally infected with *Trichuris vulpis. Bulg. J. Vet. Med.*, **8**, No 2, 141–148.

The experiment was performed on 9 mixed-breed dogs (6 infected and 3 non-infected controls). The experimental infection was provoked with *Trichuris vulpis* (10000 eggs/kg). The clinical signs and the following haematological parameters were monitored: haemoglobin, packed cell volume, red blood cell counts and morphology, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), haemoglobin index (HbI), erythrocyte sedimentation rate (ESR), total and differential white blood cell counts.

A typical clinical signs of the disease was the diarrhoea that resulted in full exhaustion and cachexia. In infected dogs, elevated haemoglobin and MCH values were observed between post infection days 60 and 207, decreased MCV by day 15 and 35 and no changes in PCV, HbI values and red blood cell morphology. Also, a leukocytosis with eosinophilia, neutrophilia (on the account of segmented neutrophils' elevation), lymphocytopenia and enhanced ESR were found out.

Key words: dogs, *Trichuris vulpis*, trichurosis

INTRODUCTION

Trichurosis is a nematodosis of carnivores, caused by *Trichuris vulpis* Froelich (1789) belonging to the Trichuridae family. The most typical clinical signs of trichurosis are: diarrhoea (often chronic and bloody mucoid), abdominal discomfort (from unclear distress to acute pain), pica, dehydratation, weight loss and dry, dirty hair coat (Miller, 1941, Smith, 1954, Prelesov & Grosev, 1994), depression, rapid exhaustion (Rubin, 1954, Cardani *et al.*, 1978). Several cases, in which the disease was manifested by alternating episodes of diarrhoea and constipation (Widmer & Van Kruiningee, 1974; Ewing & Bull, 1996), neurological and behavioural disorders as fits and hysteria (Emmerson, 1941) were also reported. The reported clinical manifestations are related by some authors (Smith, 1954) to the intensity of invasion (the infection with hundreds to thousands of parasites in the large intestine is accompanied by bloody diarrhoea, dehydratation, anaemia and death whereas the infection with up to 12 parasites is asymptomatic), to the location of parasites and to the individual traits of the host (age, body condition and pre-sence of other parasitic infections).

Burrows & Lillis (1964) have provided evidence that *T. vulpis* is haematophague, but there are no studies upon the haemotological changes in infected dogs. The opinions about anaemia as a sign ac-

companying trichurosis, are contradictory. In dogs with mixed *Ancylostoma caninum* and *T. vulpis* infection, anaemia with neutropenia, eosinophilia and monocytosis was reported (Cardani *et al.*, 1978). The authors stated that the anaemia was caused by *A. caninum* that is a considerably more active haematophague than *T. vulpis*.

The reviewed data showed that the pathogenesis of trichurosis is not adequately studied. The aim of the present study was to follow out the clinical signs and the haematologial changes occurring in dogs, experimentally infected with *T. vulpis*.

MATERIALS AND METHODS

Animals

The studies were performed on 9 mixed-breed dogs (2 male and 7 female) at the age of 6–12 months, weighing 3–5 kg.

After a 10-day period of adaptation, the dogs were vaccinated and revaccinated with Novibac DHP (Intervet, Holland). They were twice treated against parasites at 14-day intervals with the combination praziquantel, pyrantel embonate and febantel (Drontal plus, Bayer, Germany) at a dose of 1 tablet/10 kg. A disinsection was performed with permethrin and carbaryl (Tapilan B, Dorvet, Israel). The animals were divided into 2 groups: experimental – infected (1 male and 5 females) and control – non-infected (1 male and 2 females). For prevention of any accidental infection, the animals were housed in cages, previously cleansed and disinfected with warm 2% NaOH solution.

Experimental infection

The experimental infection was provoked by oral administration of *T. vulpis* eggs (10000 eggs/kg).

Blood sampling

Blood samples for erythrocyte sedimentation rate (ESR) determination were aseptically obtained from *vena cephalica anterior* by sterile syringes and needles. For analysis of red blood cells (RBC), white blood cell counts (WBC), haemoglobin (Hb), packed cell volume (PCV) and mean corpuscular volume (MCV) and for preparation of blood smears, 6 mL blood with EDTA were obtained from the same vein and analysed immediately. Blood was sampled prior to the infection and by post infection days 15, 35, 60, 112 and 207.

Haematological studies

Red blood cell counts, total WBC counts, Hb, PCV and MCV were determined by an automated haematological analyser (Haematology System 150). The differential WBC counts were determined on a blood smear stained with May-Grünwald-Giemsa stain. The ESR was determined by the micromethod of Panchenko. The following RBC indexes were calculated:

Mean corpuscular haemoglobin (MCH) as

$$\text{MCH [pg]} = \text{Hb [g/L]} / \text{RBC [}10^{12}\text{/L]};$$

Mean corpuscular haemoglobin concentration (MCHC) as

$$\text{MCHC [g/L]} = \text{Hb [g/L]} / \text{PCV [L/L]};$$

Haemoglobin index (HbI) as

$$\text{HbI} = \frac{\text{RBC}_c[10^{12}/\text{L}] \times \text{Hb}_e[\text{g/L}]}{\text{RBC}_e[10^{12}/\text{L}] \times \text{Hb}_c[\text{g/L}]}$$

where the index "c" = "control" and "e" = "experimental".

Morphological changes in RBC were assessed microscopically (Laboval 4 microscope, Zeiss, Austria).

Parasitological studies

The degree of infection was determined post mortem by counting of parasites. The infection with < 12 parasites was considered as weak, with > 12 and < 100 parasites – as medium and with > 100 parasites – as heavy.

Statistical analysis

The data were statistically processed by the one-way analysis of variance (ANOVA) at the $P<0.05$ level.

RESULTS

Clinical studies

In control dogs, no clinical signs of disease or deviations in the general conditions were observed during the 275-day period of the study.

In experimental dogs, a short-term diarrhoea occurred 10–15 days after the infection, characterized by discharge of soft faeces with yellow colour. After the 60th day, the diarrhoea became permanent and resulted in full exhaustion and cachexia. The faeces were soft, yellow and with increased mucus content. After the 120th day, mucoid and blood appeared in faeces of experimental dogs. Their appetite and the thirst were preserved until the death of animals.

In 3 of infected dogs, a gradual hair loss and red skin papules occurred between post infection days 60 and 120. Afterwards, the hair coat was slowly renewed, but the skin was with decreased elasticity, rough and scaly.

Between days 220 and 275, the disease was lethal for all dogs. The necropsy revealed sexually mature *Trichuris* located predominantly in the region of the ileocecocolic valve. The degree of invasion was from 154 to 1070 *Trichuris* organisms. According to the criteria of Smith (1954) it was determined as heavy (Table 1).

Table 1. Degree of infection in dogs, experimentally infected with *T. vulpis* (10000 eggs/kg) at the different post infection intervals

Dog No.	Post infection day	Number of parasites
7	220	175
3	220	182
1	232	547
5	238	1070
6	265	163
2	275	154

Haematological studies

The results of haematological studies are presented in Tables 2, 3 and 4.

In the experimental group (Table 2), Hb increased between days 60 and 207. This elevation was statistically significant vs both baseline and controls by day 60 ($P<0.01$) and by day 207 ($P<0.001$ vs baseline and $P<0.05$ vs controls). RBC counts in infected animals tended to be slightly elevated and were statistically significantly higher than baseline values by days 112 and 207 ($P<0.01$). A similar increase occurred in PCV values from the 15th day onward only vs baseline ($P<0.01$; $P<0.001$ by day 207).

The RBC indexes in the infected groups were characterized by elevation in MCH by post infection day 60 ($P<0.05$ vs baseline), significantly higher MCV values by days 15 and 35 ($P<0.001$ compared to baseline and $P<0.01$ and $P<0.05$ compared to controls by days 15 and 35 respectively). The ESR was considerably enhanced in experimental dogs by day 35 ($P<0.05$).

Table 2. Dynamics of changes in haemoglobin (Hb), red blood cells (RBC), packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and erythrocyte sedimentation rate (ESR 1h) in control dogs (n=3) and dogs, experimentally infected with *T. vulpis* (10 000 eggs/kg). Data are presetned as mean ± SEM

Post infection days	Hb (g/L)	RBC (× 10^{12}/L)	PCV (L/L)	MCH (pg)	MCHC (g/L)	MCV (fL)	ESR 1 hour (mm/h)
Control group							
0	121 ± 6.35	5.6 ± 0.13	0.32 ± 0.01	21.6 ± 0.7	375 ± 10.6	57.5 ± 0.35	6.3 ± 2.0
15	125 ± 2.3	6.9 ± 0.49	0.54 ± 0.04 [2]	18.2 ± 1.1	235 ± 15.1 [2]	77.2 ± 0.4 [3]	7.0 ± 1.5
35	121.7 ± 4.9	6.7 ± 0.5	0.47 ± 0.03 [2]	18.3 ± 1.0	259.9 ± 11.5 [2]	70.3 ± 1.3 [3]	6.7 ± 0.3
60	133 ± 1.1	6.1 ± 0.1 [1]	0.38 ± 0.01 [2]	21.7 ± 0.1	346.7 ± 8.9	62.8 ± 1.8 [1]	12.3 ± 1.2
112	128.3 ± 2.2	7.1 ± 0.5 [1]	0.45 ± 0.03 [1]	18.2 ± 1.4	288.7 ± 23.8 [1]	63.2 ± 0.8 [2]	8.33 ± 1.6
207	142.7 ± 6.6	7.4 ± 0.6 [1]	0.45 ± 0.04 [1]	19.6 ± 1.9	324.5 ± 30.2	60.3 ± 1.3	3.66 ± 0.9
Experimental group							
0	120 ± 6.25	6.25 ± 0.2	0.35 ± 0.02	19.2 ± 1.0	348 ± 17	55.4 ± 1.5	12.3 ± 2.7
15	125 ± 6.19	6.75 ± 0.5	0.46 ± 0.04 [2]	18.7 ± 0.7	276.1 ± 15 [2]	68.4 ± 1.4 [3b]	10.7 ± 2.1
35	130 ± 2.1	7.10 ± 0.35	0.46 ± 0.02 [2]	18.6 ± 0.8	284.2 ± 11 [2]	65.9 ± 0.9 [3a]	14.5 ± 1.9 [a]
60	146.8 ± 2.6 [2b]	6.70 ± 0.2	0.41 ± 0.01 [2]	21.9 ± 0.3 [1]	358 ± 4.2	61.4 ± 0.7 [2]	18.2 ± 5.6
112	132.5 ± 6.6	7.50 ± 0.3 [2]	0.47 ± 0.02 [3]	17.9 ± 1.2	281.4 ± 14 [2]	63.5 ± 1.6 [2]	15.3 ± 4.2
207	164.6 ± 5.1 [3a]	7.90 ± 0.5 [2]	0.48 ± 0.04 [2]	21.4 ± 2.0	360.6 ± 41.2	60.2 ± 1.7	6.4 ± 2.4

[1] P<0.05; [2] P<0.01; [3] P<0.001 vs hour 0; [a] P<0.05; [b] P<0.01; [c] P<0.001 vs controls at each time interval.

Table 3. Color index (HbI) values in dogs, experimentally infected with *T. vulpis* (10000 eggs/kg) prior to and after the challenge

Post infection days	HbI
Day 0 (prior to infection)	175
Day 15	182
Day 35	547
Day 60	1070
Day 112	163
Day 207	154

The HbI is presented in Table 3. No significant changes in this parameter were observed. The RBC morphology was not altered – the RBC size (6–10 μm) and staining were unchanged, no inclusions were present. In two of infected dogs, a slight poikilocytosis was noticed.

The changes in total and differential WBC counts are given in Table 4. In experimentally infected dogs, total WBC counts increased statistically significantly between days 60 and 207 vs controls ($P<0.05\div0.01$). Higher percentages of eosinophils between post infection days 35 and 60 ($P<0.01$ vs controls) and of segmented neurophils by day 207 ($P<0.05$) were present. The band neutrophils and lymphocytes decreased compared to non-infected dogs by days 112 and 207 respectively ($P<0.05$).

DISCUSSION

The clinical signs observed by us are also communicated by Prelesov & Grosev (1994) and Widmer & Van Kruiningen (1974) in heavily infected dogs. The heavy *T. vulpis* infection caused large intestine inflammation manifested by permanent bloody mucoid diarrhoea that was probably responsible for the observed haematological changes.

The increased RBC counts was accompanied by increased values of Hb, PCV and RBC indexes, possibly as a result of the dehydratation following the continuous diarrhoea in infected dogs. Because of the lack of respective haematological data in dogs, infected with *T. vulpis*, we compared our results with data obtained in children infected with *T. trichura*. In 409 heavily infected children, Ramdath *et al.* (1995) observed a slightly reduced Hb concentrations, MCV and MCHC, but RBC counts were not changed. The data reported by Tsuyuoka *et al.* (1999), Grell *et al.* (1981), Greenberg & Cline (1979), King *et al.* (1997) and Atukorala & Lanerolle (1999) showed that the trichurosis in children was not associated with anaemia. The enhanced ESR could be interpreted with regard to intestinal tract inflammation.

The occurring changes in total WBC counts and the percentages of WBC classes during the different periods of the study could be explained by the activation of the mechanisms of systemic nonspecific defense against the parasitic infection. The early increase in eosinophils' percentages is indicative of their initiating role in the destruction of parasites. According to some authors (Tizard, 1996) their counts increased 10 to 30 times under the influence of interleukin-5 released by T-helpers. Eosinophils exert their phagocytic function via secretion of enzymes that injury or kill the parasites (Spry, 1985). The increase in segmented neutrophils in the later stages of the disease is probably determining the efficacy of the systemic antihelminthic response.

The reduced blood lymphocyte percentages observed by day 207 could be probably due to the migration of these

Table 4. Total and differential white blood cells (WBC) counts in control dogs (n=3) and dogs, experimentally infected with *T. vulpis* (10 000 eggs/kg). Data are presetned as mean ± SEM

Post infection days	WBC ($\times 10^9$/L)	Eosinophils (%)	Metamyelocytes (%)	Band neutrophils (%)	Segmented neutrophils (%)	Lymphocytes (%)	Monocytes (%)
Control group							
0	13.26 ± 2.3	3.00 ± 0.57	2 ± 0.57	3.66 ± 1.8	54.3 ± 7.2	36.0 ± 7.5	0.66 ± 0.66
15	15.53 ± 0.9	2.66 ± 0.33	1 ± 1	3 ± 0.6	53.6 ± 9.7	39.7 ± 9.6	0 ± 0
35	15.16 ± 2.3	2.0 ± 1.15	1 ± 0.57	4 ± 1.2	66.7 ± 4.1	26.3 ± 6.3	0 ± 0
60	14.2 ± 2.0	2.33 ± 0.33	0.33 ± 0.33	2 ± 0	64.0 ± 3.1	31.3 ± 2.9	0 ± 0
112	15.5 ± 1.1	2.66 ± 0.66	1 ± 1	7.3 ± 1.8	57.0 ± 5.1	34.7 ± 3.4	0.66 ± 0.66
207	7.7 ± 0.3	3.66 ± 2.18	1.66 ± 1.2	8.3 ± 1.9	53.0 ± 5.5	33.3 ± 4.9	0 ± 0
Experimental group							
0	14.14 ± 1.69	3.66 ± 1.24	1 .33 ± 0.49	5.5 ± 0.7	63.8 ± 2.7	24.5 ± 3.9	0.50 ± 0.22
15	12.55 ± 1.14	6.00 ± 1.3	0.50 ± 0.22	4.3 ± 1.5	57.5 ± 3.7	31.3 ± 3.6	0.33 ± 0.21
35	13.26 ± 1.23	6.50 ± 0.8 [b]	1.16 ± 0.4	4.3 ± 0.7	64.8 ± 1.8	22.7 ± 2.1	0.50 ± 0.22
60	20.95 ± 2.0 [2a]	8.16 ± 1.13 [1b]	1.33 ± 0.6	5.33 ± 1.68	58.2 ± 5.4	26.8 ± 4.5	0.16 ± 0.16
112	21.60 ± 1.6 [2a]	6.00 ± 1.03	0.50 ± 0.5	3.0 ± 0.5 [1a]	62.5 ± 4.2	27.7 ± 3.8	0.33 ± 0.21
207	11.80 ± 0.9 [b]	4.20 ± 1.06	2.00 ± 0.83	4.8 ± 0.8	66.6 ± 2.5 [a]	21.8 ± 2.4 [a]	0.60 ± 0.24

[1] P<0.05; [2] P<0.01; [3] P<0.001 vs hour 0; [a] P<0.05; [b] P<0.01; [c] P<0.001 vs controls at each time interval.

cells towards the intestinal tract. Unpublished data of ours evidence a profuse infiltration of lymphocytes into the caecal and colonic mucosa and submucosa. According to Tizard (1996), the lymphocytes mobilized into the intestines inhibit the activity of helminths that penetrate deeply into the intestinal mucosa.

CONCLUSION

The course of the experimental trichurosis in dogs in our study was chronic with a permanent bloody mucoid diarrhoea that results in dehydratation, cachexia and death. Consequently to dehydratation, blood parameters changed and were characterized by elevated Hb, RBC, PCV, MCHC and MCV values. Increased total WBC counts, eosinophilia and neutrophilia probably related to an activation of the non-specific systemic defense as well as lymphocytopenia were observed. The enhancement of ESR and the pathological leukocytosis were most likely manifestation of general systemic changes under the influence of *T. vulpis*-induced large intestinal inflammation.

REFERENCES

Atukorala, T. M. & P. Lanerolle, 1999. Soil-transmitted helmintic infection and its effect on nutritional status of adolescent schoolgirls of low socioeconomic status in Sri Lanca. *Journal of Tropical Pediatrics*, **45,** No. 1, 18–22.

Burrows, R. B. & W. G. Lillis, 1964. The whipworm – a blood sucker. *The Journal of Parasitology*, **50**, No 5, 675–680.

Cardani, G., A. J. Delgadillo & G. Mengozzi, 1978. Anaemia due to *Ancylostoma caninum* and *Trichuris vulpis* in the dog. I. Correlation between hemoglobin value and the worm burden. *Annali de Facoltad di Medicina Veterinaria*, **31**, 163–174.

Emmerson, E. D., 1941. Whipworm hysteria in dogs. *Veterinary Medicine*, **36**, 522–523.

Ewing, S. A. & R. W. Bull, 1966. Severe chronic canine diarrhea associated with *Balantidium–Trichuris* infection. *Journal of American Veterinary Medical Asociation*, **149**, No 5, 519–520.

Greenberg, E. R. & B. L. Cline, 1979. Is trichuriasis associated with iron deficiency anemia? *American Journal of Tropical Medicine and Hygiene,* **28**, No 4, 770–772.

Grell, G. A., P. Desai, E. Watty, R. Muller & G. R. Serjeant, 1981. A survey of parasites in primary schoolchildren in Dominica, West Indies. *Annals of Tropical Paediatrics*, **1**, No 3, 155–160.

King, J. E., M. Mazariegos, C. Valdez, C. Castaneda & N. W. Solomons, 1997. Nutritional status indicators and their interactions in rural Guatemalan elderly: A study in San Pedro Ayampuc. *American Journal of Clinical Nutrition*, **66**, No 4, 795–802.

Miller, M. G., 1941. Quantitative studies on *Trichocephalus* infection in dogs. *American Journal of Hygiene*, **33**, 58–70.

Prelesov, P. & Ts. Grosev, 1994. Trihurosa po domashnite kucheta [Trichurosis in domestic dogs]. Veterinarna sbirka, No 5, 18–19 (BG).

Ramdath, D. D., D. T. Simeon, M. S. Wong & S. M. Grantham-McGregor, 1995. Iron status of schoolchildren with varying intensities of *Trichuris trichiura* infection. *Parasitology*, **110**, 347–351.

Rubin, R., 1954. Studies on the common whipworm of the dog, *Trichuris vulpis*. *Cornell Veterinarian*, **44**, 36–49.

Smith, D. E., 1954. Two fatalities due to whipworms in the dog. *Journal of American Veterinary Medical Asociation*, **125**, 314–315.

Spry, C. J., 1985. Synthesis and secretion of eosinophil granule substances. *Immunology Today*, **6**, 332–335.

Tizard, I. R., 1996. Immunity to parasites., In: *Veterinary Immunology. An Introduction*, W. B. Saunders Company, Philadelphia, 315–330.

Tsuyuoka, R., J. W. Bailey, A. M. Nery Guimaraes, R. Q. Gurgel & L. E. Cuevas, 1999. Anemia and intestinal parasitic infections in primary school students in Aracaju, Sergipe, Brazil. *Cadernos de Saúde Pública*, **15**, No 2, 413–421.

Widmer, W. R. & H. J. Van Kruiningen, 1974. *Trichuris*-induced transmural ileocolitis in dog. *Journal of American Veterinary Medical Asociation*, **10**, 581–585.

Paper received 15.05.2003; accepted for publication 26.01.2005

Correspondence:

Dr. Z. Kirkova,
Department of Microbiology, Infectious and Parasitic Diseases,
Faculty of Veterinary Medicine,
Trakia University
Student's Campus,
6000 Stara Zagora, Bulgaria