

Changes in haematological parameters in wild ruminants experimentally infected with *Haemonchus contortus*

E. SESZTÁKOVÁ¹, A. KÖNIGOVÁ^{2*}, L. MOLNÁR¹, M. BABJÁK², P. MAJOR¹, Š. MEGYESI², Z. VASILKOVÁ², M. VÁRADY¹

¹Clinic of Birds, Exotic and Free Living Animals, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovak Republic; ²Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, 040 01, Košice, Slovak Republic, *E-mail: konig@saske.sk

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Summary

Our study describes changes in haematological parameters in wild ruminants with parasitic infection. Six European mouflons (*Ovis musimon*), six fallow deer (*Dama dama*) and six roe deer (*Capreolus capreolus*) were experimentally infected with the resistant strain of the model parasite 8000 L3 *Haemonchus contortus*. The blood samples were collected on Day 0, 16, 37, 58, 77, and 99 of the experiment. Mild anaemia was observed in mouflons and roe deer while red blood cells increased in red blood counts (total erythrocytes, haematocrit and haemoglobin). As for the white blood cells count, leucopenia with neutrophilia and lymphopenia was recorded in mouflons, in the fallow deer and roe deer leucocytosis with neutropenia and lymphocytosis were observed. Changes in the dynamics of haematological parameters were statistically insignificant.

Keywords: mouflons; fallow deer; roe deer; *Haemonchus contortus*; haematological parameters

Introduction

Parasitic infections in wild ruminants may cause high morbidity, and in the case of heavy infections, immunosuppression and poor body mass condition, they may even lead to death of the animal. The negative effect of parasitic infection depends on the host's nutritional status, gender – males have a weaker immune system than females, age – young and elderly individuals are more susceptible to infection than adults (Jégo *et al.*, 2014). Most studies have focused on the impact of parasites on the nutritional status of wild ruminants, but this has been confirmed as a nonspecific and slow indicator of parasitic infection (Irvine *et al.*, 2006; Jégo *et al.*, 2014). More specific and faster diagnostic indicator of the presence of parasitic infection may be host haematology parameters (Beldomenico & Begon, 2010). Changes in the health status of wild ruminants may also be reflected in haematological parameters (Ciberej *et al.*, 2007; Mašek *et al.*, 2009). Monitoring

their dynamics contributes greatly to diagnosis, differential diagnosis, assessment of the effect of therapy as well as prediction of a course of diseases in animals. However, blood analysis may also indicate an accurate status of nutrition, trauma, and environmental stressors (Peréz *et al.*, 2003). *Haemonchus contortus* is one of the hematophagous species that can cause haemorrhagic anaemia, especially in young and immunosuppressed animals. If the infection persists for a long time (chronic blood loss), it can lead to a rapid depletion of the erythrocyte stocks in the spleen and bone marrow and anaemia begins to develop, characterized by a decrease in all red blood component parameters (Doubek *et al.*, 2003; Kolodziej-Sobocińska *et al.*, 2016). Changes observed in the white blood cells in wild ruminants may result from an organism's response to stress (stress leukocytosis) and inflammation as a result of parasitic and allergic reactions (Ciberej *et al.*, 2007). The values of haematological parameters of both the red and white blood cells are dependent on many factors such as breed,

* – corresponding author

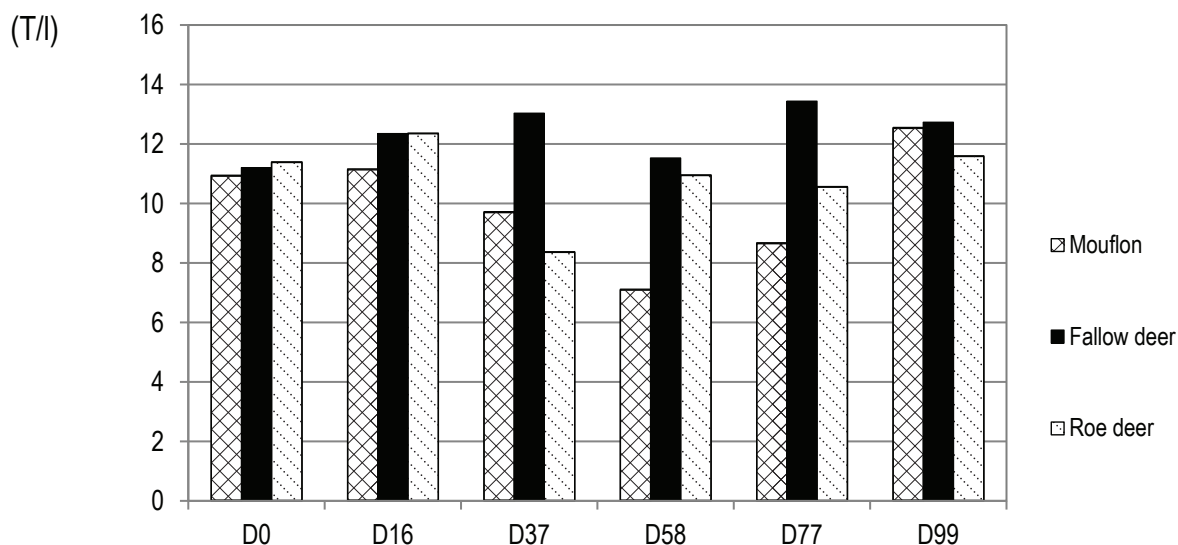


Fig. 1. Results of total number of erythrocytes (T/l) in three species of wild ruminants infected with resistant (MHco4) strain of *H. contortus*. Day (D) 0 (day of infection), D16, D37, D58, D77 (days post infection), D77 (day of treatment), D99 (D21 after treatment).

age, sex, pregnancy and season (Küker *et al.*, 2015). Vengušt *et al.* (2006) and Casas-Díaz *et al.* (2008) also reported that the method of capture and fixation of wild ruminants can greatly affect the blood parameter values. Vengušt *et al.* (2006) in fallow deer recorded higher values in total erythrocyte count, haemoglobin and haematocrit in subjects immobilized by chemical methods compared to animals immobilized by physical methods. The aim of this study was to describe changes in total number of erythrocytes and leukocytes, haematocrit and haemoglobin and leukogram values (differential cell blood counts) in wild ruminants

(mouflons, fallow deer, roe deer) experimentally infected with the model parasite *H. contortus*.

Material and Methods

Three species of wild ruminants – six European mouflons (*Ovis musimon*), six fallow deer (*Dama dama*) and six roe deer (*Capreolus capreolus*) with an average age of 1.5 years were experimentally infected with the model parasite *H. contortus*. Each animal was housed individually, fed with meadow hay and a commercial

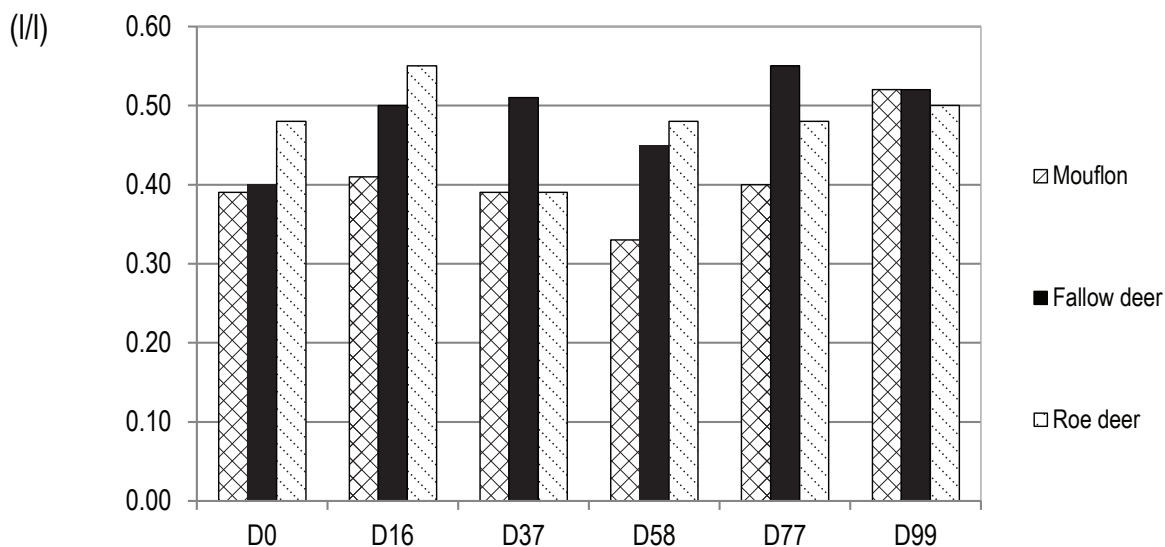


Fig. 2 Results of haematocrit values (l/l) in three species of wild ruminants infected with resistant (MHco4) strain of *H. contortus*. Day (D) 0 (day of infection), D16, D37, D58, D77 (days post infection), D77 (day of treatment), D99 (D21 after treatment).

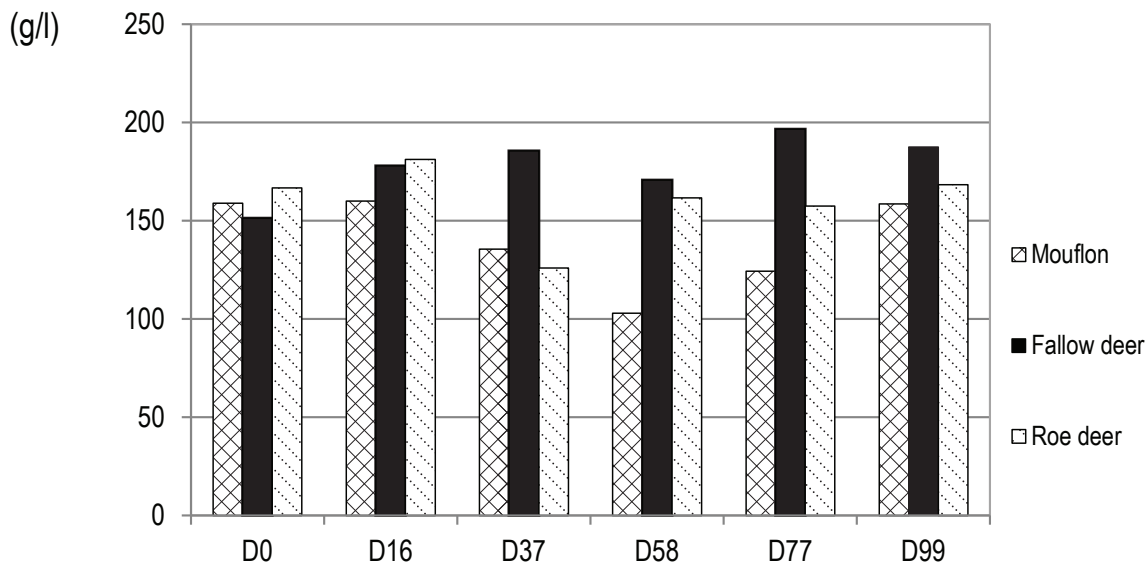


Fig. 3 Results of haemoglobin values (g/l) in three species of wild ruminants infected with resistant (MHco4) strain of *H. contortus*. Day (D) 0 (day of infection), D16, D37, D58, D77 (days post infection), D77 (day of treatment), D99 (D21 after treatment).

concentrate with free access to water, mineral and salt licks. All animals were treated *per os* with albendazole (10 mg / kg body weight, ALDIFAL 2.5 % susp. a.u.v., MEVAK, Slovakia) prior to the experiment for the purpose of utilising parasite free animals. Faecal samples from all experimental animals were examined and negative coprological findings were confirmed before the experiment. After a week, each animal was infected *per os* with 8000 L3 larvae of isolate of *H. contortus* (MHco4). MHco4 is a multi-resistant (ivermectin, BZ, rafoxanide, closantel) field isolate from South

Africa (Van Wyk & Malan, 1988). The dynamics of haematological parameter values in all animals were monitored for 99 days. Blood samplings were performed on day (D) 0, day of infection, D16, D37, D58 and D77 days post infection (p.i.). On D77 p.i., levamisole was orally administered to all experimental animals with Ripercol Drench (Elanco, Germany) at a dose of 2 ml / 10 kg body weight. Blood was again collected on D99 p.i. of the experiment (D21 after treatment). Each animal was immobilized prior to the blood collection by applying Hellabrun mixture (125 mg xylazine

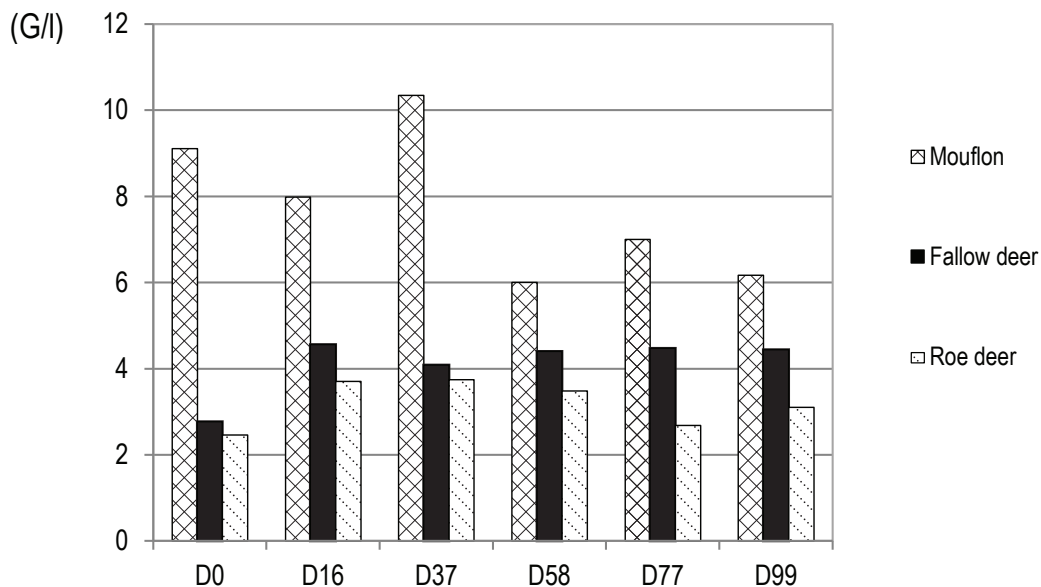


Fig. 4 Results of the total leucocyte count (G/l) in three species of wild ruminants infected with resistant (MHco4) strain of *H. contortus*. Day (D) 0 (day of infection), D16, D37, D58, D77 (days post infection), D77 (day of treatment), D99 (D21 after treatment).

Table 1. Leucogram results - granulocytic line in three species of wild ruminants infected with resistant (MHco4) strain of *H. contortus* (%).

Day of blood collection		Mouflon (<i>Ovis musimon</i>)	Roe deer (<i>Dama dama</i>)	Fallow deer (<i>Capreolus capreolus</i>)
Average ± SD				
Neutrophilic granulocytes (%)	D0 - infection	33.80 ± 1.51	45.60 ± 0.63	93.20 ± 0.35
	D16	21.80 ± 5.58	34.13 ± 1.59	85.33 ± 1.11
	D37	51.17 ± 2.01	33.20 ± 2.46	83.90 ± 0.53
	D58	50.00 ± 2.16	36.60 ± 0.88	86.10 ± 0.77
	D77 + treatment	54.67 ± 5.43	32.46 ± 6.64	88.20 ± 1.15
	D99 - D21 post treatment	41.00 ± 4.32	39.20 ± 0.65	87.13 ± 0.12
	Eosinophilic granulocytes (%)	D0 - infection	1.80 ± 0.43	3.16 ± 0.12
D16		2.10 ± 0.16	3.13 ± 0.26	7.26 ± 0.60
D37		1.67 ± 0.47	2.46 ± 0.36	8.30 ± 0.43
D58		2.33 ± 0.47	2.33 ± 0.47	8.63 ± 0.52
D77 + treatment		2.00 ± 0.0	1.97 ± 0.04	9.00 ± 1.48
D99 - D21 post treatment		3.24 ± 1.43	1.96 ± 0.04	8.36 ± 0.57
Basophilic granulocytes (%)	D0 - infection	0.93 ± 0.73	4.80 ± 0.24	1.36 ± 0.33
	D16	0.63 ± 0.44	3.40 ± 0.29	4.63 ± 0.52
	D37	0.33 ± 0.47	4.60 ± 0.08	4.10 ± 1.42
	D58	0.33 ± 0.47	2.63 ± 0.44	2.56 ± 0.70
	D77 + treatment	0.67 ± 0.47	4.90 ± 0.08	1.36 ± 0.46
	D99 - D21 post treatment	2.10 ± 0.78	4.33 ± 0.26	2.16 ± 0.52

hydrochloride 100 mg ketamine hydrochloride / 1 ml) at the following doses: mouflon 1.5 – 2.0 ml, fallow deer 2.0 – 2.5 ml and roe deer 0.2 – 0.3 ml. Blood was collected from the jugular vein into an ethylene-diamine-tetraacetic acid anticoagulant tube (EDTA) and processed on an IDEXX ProCyt DxTH (IDEXX Laboratory, Cymedica). The mean and ± statistical deviations were calculated using the Excel 2010 statistical program (Microsoft Int.).

Individual counts of eggs per gram (EPG) for each experimental animal were determined by a modified McMaster technique with a sensitivity of 50 EPG (Coles *et al.*, 1992; 2004). Three grams of faeces from each animal were mixed with 42 ml of water and passed through a sieve. The filtrate was centrifuged at 605 g for 2 min. The sediment was mixed with a sugar solution with specific gravity 1.28 and centrifuged under the same conditions. One millilitre of the mixture was then transferred to McMaster chambers. Tukey-Kramer test (one-way ANOVA) was used to evaluate the haematological parameters. A significance level of less than 5 % (P<0.05) was considered statistically significant.

Ethical Approval and/or Informed Consent

Animal use and study design were approved by the Ethics Committee of the Institute of Parasitology of the Slovak Academy of

Sciences in accordance with the national legislation in Slovakia – Animal Welfare Act No. 3/2009.

Results

Mouflons

In the parameters of the red blood count (total erythrocytes, haematocrit and haemoglobin values), mild anaemia was noted on D37, D58 and D77 p.i. (Figs. 1, 2, 3), with the most pronounced decrease in these parameters on D58 p.i. Mild anaemia was caused due to erythrocyte loss during *H. contortus* infection. Considering the day of infection, leukocytopenia with neutrophilia and lymphopenia was detected in the total leukocyte count (Fig. 4). The most pronounced leukopenia was determined on D58 and D77 p.i. and neutrophilia and lymphopenia on D37 p.i. (Table 1, 2, Fig. 4). Changes in these values persisted until the end of the experiment. Values recorded in total leukocytes, neutrophil granulocytes and lymphocytes indicate a high level of parasitic infection. Simultaneously, a low level of posthemorrhagic anaemia was confirmed with the presence of acute local mucosal inflammation. Parasitic infection may be accompanied by eosinophilia. Changes in the values of eosinophil granulocytes were caused by *H. contortus* infection. Similarly, in our results eosinophilia was observed throughout the

Table 2. Leucogram results - Agranulocytic line in mouflons (*Ovis musimon*), roe deer (*Capreolus capreolus*) and fallow deer (*Dama dama*) during the experimental infection with resistant (MHco4) strain of *H. contortus* (%).

Day of blood collection		Mouflon (<i>Ovis musimon</i>)	Roe deer (<i>Dama dama</i>)	Fallow deer (<i>Capreolus capreolus</i>)
Average ± SD				
Lymphocytes (%)	D0 - infection	61.53 ± 1.26	39.80 ± 0.53	2.06 ± 1.06
	D16	71.90 ± 4.30	53.33 ± 2.51	2.56 ± 1.14
	D37	42.70 ± 2.65	54.03 ± 2.08	3.70 ± 1.10
	D58	45.33 ± 2.35	54.36 ± 0.73	2.70 ± 0.74
	D77 + treatment	40.33 ± 4.02	56.63 ± 6.50	1.43 ± 0.49
	D99 - D21post treatment	50.53 ± 2.60	50.43 ± 0.75	2.33 ± 1.13
	Monocytes (%)	D0 - infection	1.60 ± 0.50	6.63 ± 0.89
D16		3.57 ± 1.30	6.00 ± 0.81	0±0
D37		3.47 ± 0.44	5.70 ± 0.53	0±0
D58		2.00 ± 0.81	4.06 ± 0.17	0±0
D77 + treatment		2.66 ± 1.24	4.03 ± 0.12	0±0
D99 - D21post treatment		2.77 ± 0.20	4.06 ± 0.16	0±0

experiment. Monocytosis was recorded throughout the experiment with maximal value on D16 p.i. (Table 2), what indicated an acute inflammatory process due to parasitic infection.

Fallow deer

The increase in red blood counts was determined on all sampling days, with a maximum on D77 p.i. (Figs. 1, 2, 3). Leukocytosis with neutropenia and lymphocytosis was recorded in on all sampling days (Table 1, 2, Fig. 4). The eosinophil granulocyte values decreased throughout the experiment. In fallow deer group, basophilia was not detected (Table 1). Monocyte levels decreased on D58 p.i. (Table 2).

Following the treatment with levamisole on D99 p.i., red blood cell maximal values were recorded in all experimental species what could be related to the immunostimulatory effect of levami-

sole (Figs. 1, 2, 3). The changes recorded in the dynamics of the haematological parameters of the red and white blood line during parasitic infection with resistant strain (MHCo4) of *H. contortus* were statistically insignificant in all experimental species of wild ruminants. Changes recorded in red and white blood counts were associated with the rate of parasitic infection, expressed as eggs per gram of faeces – EPG (Table 3).

Roe deer

Mild anaemia was recorded on D37, D58 and D77 p.i. with the most pronounced decrease on D37 p.i. in comparison with the day of infection (Figs. 1, 2, 3). Leukocytosis with neutropenia and lymphocytosis was determined in total leukocyte count on all sampling days (Table 1, 2, Fig. 4). Changes in the mentioned parameters in roe deer could be caused by a lower intensity of parasitic infec-

Table 3. Mean egg count±standard deviation for the three species of wild ruminants – European mouflon (*Ovis musimon*), roe deer (*Capreolus capreolus*) and fallow deer (*Dama dama*) during the experimental infection with resistant (MHco4) strain of *H. contortus*.

	EPG D16	EPG D37	EPG D58	EPG D77	EPG D99
Mouflon n=3	0.0 ± 0.0	12 250 ± 105.48	25 400 ± 432.00	19 850 ± 550.00	0 ± 0
Fallow deer n=3	100 ± 60.28	150 ± 55.20	150 ± 55.54	0 ± 0	0 ± 0
Roe deer n=3	0.0 ± 0.0	50 ± 47.25	150 ± 57.35	0 ± 0	0 ± 0

EPG, eggs per gramme, number of *H. contortus* eggs in 1 gramme of faeces

tion without an acute abomasal mucosal inflammation (Table 3). Eosinophilia was noted in the roe deer throughout the experiment with a maximum value on D77 p.i. in comparison with the day of infection (Table 1). It can be assumed that the recorded basophilia followed by eosinophilia may have resulted from an allergic reaction to the immune mobilizing pharmaceutical metabolites.

Discussion

It is well known that many species of gastrointestinal nematodes are common to domestic and wild ruminants due to their frequent common grazing on pastures. Little is known about the haematological profile of wild ruminants infected with *H. contortus*, one of the most pathogenic hematophagous gastrointestinal nematodes occurring in domestic and wild ruminants. Ferté *et al.* (2000) summarised and compared the prevalence of *H. contortus* in three species of wild ruminants (red, roe, and fallow deer) from surveys carried out across Europe. A total of 36 surveys in 17 European countries recorded the incidence of *H. contortus* in roe deer, with prevalence ranging from 0.3 to 85%. *H. contortus* was found in red deer in nine countries, with prevalence between 5 and 25%, and in fallow deer in four countries (4–7%) (Ferté *et al.*, 2000). Cerutti *et al.* (2010) confirmed the low host specificity of *H. contortus* by finding it in common populations of roe deer, chamois, alpine ibex, and domestic goats and sheep in various alpine areas. Mašek *et al.* (2009) noted the differences in blood analyses under physiological conditions between mouflon and sheep, Ciberej *et al.* (2007) described haematological profile of mouflons; however no data compares changes in blood parameters between three different species of wild ruminants during infection with hematophagous parasites.

The first sign of the *H. contortus* infection is a decrease in values in red blood cell count. The red and white blood count may interpret differences related to nutritional status, disease condition and environmental impact. The progression and prognosis of the disease depend on several factors: parasitic infection intensity, age, breeds and host health. The main pathogenic mechanism of *H. contortus* parasite lies in a direct damage to the gastric mucosa and subsequent blood sucking (Angulo-Cubillan *et al.*, 2007). Haemorrhagic anaemia is a result of a strong infection characterized by pale gingiva and conjunctival sac. There is also a decrease in total erythrocyte count, hypoalbuminemia and total protein in haematological parameters (Alvarez *et al.*, 2000). Lavin *et al.* (1997) described similar changes in red blood counts in wild Spanish goats with present *H. contortus* in the abomasum. Macrocytic hypochromic anaemia, anisocytosis, poikilocytosis and occasionally the Howell-Jolly body were diagnosed in the erythrocytes. The authors assumed that anaemia was caused by acute bleeding. Kolodziej-Sobocińska *et al.* (2016) reported similar changes in European bison infected with *Ashworthius siedmi* (*Trichostrongylidae*). Erythrocyte loss can be balanced by increased reticulocyte production, but if losses are too high anaemia occurs. Our

results indicated that mild anaemia occurred only in mouflons and roe deer. The level of decline in red blood cell parameters correlated with increasing intensity of parasitic infection expressed in EPG values. After the deworming, the red blood cell values were adjusted, confirming that irreversible changes did not occur in the early therapy of the disease in the blood and the organism was able to regenerate relatively quickly. Post-haemorrhagic anaemia in experimental wild ruminants was reversible as erythrocyte reserves in the bone marrow and spleen were sufficient to compensate for their loss in peripheral blood. Higher values of red blood cell parameters were noted in fallow deer, probably due to a lower sensitivity to infection with *H. contortus*. It can be assumed that the indicated parasitic infection rate in fallow deer was not high enough and long-term to cause a decrease in these values, respectively. Parasitic infection of animals in poor nutritional and condition status might also be of higher intensity because parasites would encounter less opposition to their survival. It is generally acknowledged that discrepancy in susceptibility is one reason why parasites tend to be concentrated in individual host (Beldomenico & Begon, 2009). Peinado *et al.* (1999) confirmed that the physiological values of total erythrocytes, haematocrit and haemoglobin are higher in young fallow deer. Similarly, Vengušt *et al.* (2006) reported that the total erythrocyte count, haemoglobin and haematocrit are also affected by the trapping method. Haematological values in fallow deer immobilized by chemical methods were higher than in fallow deer immobilized by physical methods. The effect of catecholamine release is expected to cause spleen contraction and subsequent release of erythrocytes into peripheral blood. The results of haematological parameters may also be influenced by season, age, gender and pregnancy (Küker *et al.*, 2015). In mouflons, leukopenia was recorded with neutrophilia and lymphopenia, eosinophilia and monocytosis, indicative of intense haemonchosis accompanied by post-haemorrhagic anaemia and suspected local acute mucosa inflammation in abomasum. Kraft & Dürr (2005) described pathological neutrophils with a regenerative left shift even during posthemorrhagic anaemia. In the fallow deer and roe deer, leukocytosis with neutropenia and lymphocytosis was recorded. Haematological parameters for roe deer and fallow deer did not indicate an acute inflammation associated with low parasitic infection intensity. Distinct eosinophilia accompanied by basophilia was recorded in the roe deer after D16 p.i. It can be assumed that an allergic reaction of the organism to the interaction of the parasitic infection and the immobilizing agent used has occurred. In comparison to the study of Peinado *et al.* (1999) our eosinophil granulocyte baseline values in roe deer group were increased. Eosinophilia can be caused by the chemicals used to immobilize experimentally infected wild ruminants. Similar changes in the white blood count in wild ruminants which can result from an organism's response to local or total inflammation as a result of parasitic and allergic reactions were reported by Ciberej *et al.* (2007).

Conclusion

Our results indicated that changes in both red and white blood counts observed in infected wild ruminants were temporary. The degree of damage caused by *H. contortus* was dependent on the number of parasites present and wild ruminant's species sensitivity. Changes in the red blood cell indicated a development of haemorrhagic anaemia. Changes in the white blood count could be attributed to the intensity of infection and the animal's response to pathogenesis of *H. contortus* and its subsequent local inflammatory response with the presence of haemorrhages. These changes would be more pronounced in case of a severe parasitic infection, poor body condition or in immunosuppressed hosts.

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Conflict of Interest

Authors state no conflict of interest.

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