BIORHYTMIC VARIATIONS OF HAEMATOLOGIC PARAMETERS IN DOGS

Olga-Alina RADA[1], Adelina PROTEASA[2], Mihaela OSTAN[1]

[1]Banat's University of Agricultural Sciences and Veterinary Medicine Timişoara, Faculty of Agriculture, Romania
[2]Banat's University of Agricultural Sciences and Veterinary Medicine Timişoara, Faculty of Veterinary Medicine, Romania
radaolga2005@gmail.com

Abstract: *Continuous stress conditions on animal farms and the decreasing efficacy of antibiotics emphasise the necessity of finding new solutions for the prevention and treatment of infectious diseases. Chronobiology can be an alternative solution given the fact that living organisms have a non-specific and specific temporal and functional defence structure that varies in a circadian and seasonal way. The authors assessed the circadian variations of hemoleucogram in dogs in this prophylaxis and therapeutic context. Experiments were made on eight common breed dogs that were sampled blood to produce haematologic parameters, the blood samples were analysed using an automatic haematologic analyser ADVIA 2120 Autoslide. Discrete and mean values in the studied parameters were compared to literature reference values and data were processed statistically using the non-parametric test Mann-Whitney U. Results pointed out the fact that haematologic parameters measured at three different times in the nychtemeron ranged within physiological limits for this species. Haemoglobin content was statistically significantly higher in the morning than in the evening ($p<0.05$) and higher at noon than in the evening ($p<0.05$). The mean erythrocyte volume was statistically significantly higher at noon than in the morning ($p<0.05$) and the mean haemoglobin concentration was statistically significantly higher in the morning than at noon ($p<0.001$) or in the evening ($p<0.001$). The mean number of thrombocytes and leukocytes was within physiological limits without statistically significant variations in the nychtemeron. The share of neutrophils, lymphocytes and eosinophils did not vary significantly statistically. The share of basophile granulocytes was within physiological variation limits; in the nychtemeron, the values were significantly higher in the morning than at noon or in the evening ($p<0.05$).*

Key words: *chronobiology, biorhythm, haematologic parameters, dogs, therapy*

INTRODUCTION

In the context of continuous stress on animal farms and of decreasing efficacy of antibiotics (the rate of genetic adaptation in pathogen germs is quicker than the discovery and production of new antibiotics), we need to find new solutions for the prevention and treatment of infectious diseases.

Chronobiology might be an alternative solution since living organisms have a temporal and functional structure of unspecific and specific defence that varies circadianly and seasonally (1).

The support of defence in living organisms are leukocytes: the circadian variations of their number and their share – proven in both cattle and rabbits (2, 3) – can become important and effective in specific prevention and antibiotic therapy of some infectious diseases.

MATERIAL AND METHODS

The trial was carried out on eight dogs of common breed aged 2.5-9 and weighing 12-30 kg. The dogs were kept in individual cages and fed on standard granulated food.

Prior to the sampling proper, we secured the dogs with muzzles and we disinfected the election place with sanitary alcohol. For the haematological examination, the blood samples

were harvested from the lateral saphenous vein in vacutainers with anticoagulant (EDTA). Each blood sample was labelled and carried quickly to the analysis laboratory. Blood samples were analysed using an automated ADVIA 2120i Autoslide haematology analyser.

The discrete and mean values in the parameters analysed were compared to the reference values in literature (4).

Data were processed statistically using the Mann-Whitney nonparametric test.

RESULTS AND DISCUSSIONS

The haematological exam

The mean values and the reference values of the haematological parameters are shown in Table 1, a and b, below. Overall, all the values of haematological parameters we measured are within physiological limits.

Table 1 a

The mean values and the reference values of the haematological parameters in dogs

Values	Red blood cells (mil./mm^3)			Haemoglobin (g/100ml)			Hematocrit (%)		
	M	**N**	**E**	**M**	**N**	**E**	**M**	**N**	**E**
Mean	7720000 ±	7723750 ±	7606250 ±	17,78 ±	17,69 ±	17,05 ±	49,38 ±	51,05 ±	49,65 ±
Reference	6,15-8,70[1]			14,1-20[1]			43,3-59,3[1]		

Table 1 b

The mean values and the reference values of the haematological parameters in dogs

Values	MCV (fL)			MCH (pg)			MCHC (g/dL)			RDW (%)		
	M	**N**	**E**	**M**	**N**	**E**	**M**	**N**	**E**	**M**	**N**	**E**
Mean	63,96 ±1,51	66,14 ±1,88	65,30 ±1,87	23,04 ±0,57	22,93 ±0,67	22,43 ±0,63	36,00 ±0,12	34,68 ±0,40	34,36 ±0,26	12,89 ±0,40	13,06 ±0,39	13,00 ±0,25
Reference	63,0-77,10[1]			21,1-24,8[1]			29,9-35,6[1]			11,9-14,9[1]		

MCV - mean erythrocyte volume; MCH - mean concentration in haemoglobin; MCHC - mean concentration in haemoglobin; RDW - index of erythrocyte distribution.

M- in the morning; N – at noon; E – in the evening:

[1] - The reference values: H. Tvedten, Small Animal Clinical Diagnosis by Laboratory Methods, 2004 (4);

Nychtemeral variations of red blood cells and the hematocrit, as well as the mean concentration in haemoglobin (HEM/MCH) and the index of erythrocyte distribution (IDR/RDW), do not differ statistically significantly between blood sampling intervals. In exchange, there were statistically significant differences in the mean erythrocyte volume (VEM/MCV) and the mean concentration in haemoglobin (CHEM/MCHC). Thus, the values of VEM/MCV at noon were statistically significantly higher ($p<0.05$) than in the morning. CHEM/MCHC was significantly higher in the morning than at noon ($p<0.001$) and in the evening ($p<0.001$). These variations are under the incidence of the content in haemoglobin which has significantly higher values in the morning than in the evening ($p<0.05$) and at noon than in the evening ($p<0.05$). There are no statistically significant differences between the values of haemoglobin in the morning and at noon (Figure 1).

The values of the mean individual number of thrombocytes are shown in Table 2. We can see that the mean number of thrombocytes is within physiological limits and that, since the

differences between blood samplings are low, there are no statistically significant differences ($p>0.05$) (Figure 2).

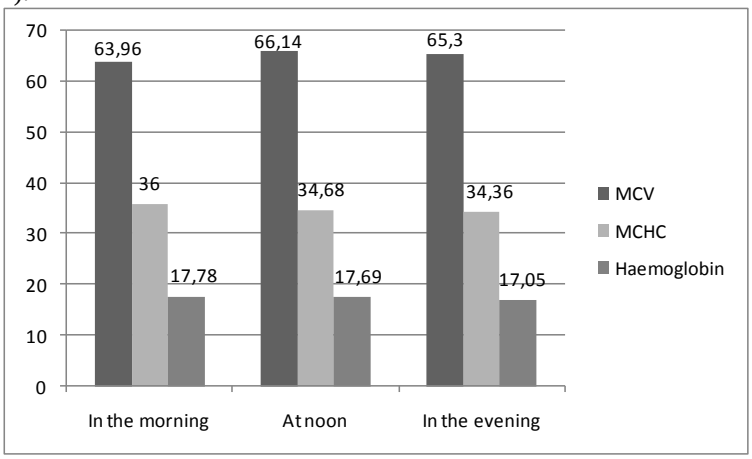

Fig. 1. Nychtemeral variations of the mean erythrocyte volume (MCV), the mean concentration in haemoglobin (MCHC) and the content in haemoglobin in dogs

Table 2

The values of the mean individual number of thrombocytes in dogs

Values	**Trombocite (thou/mm^3)**		
	In the morning	At noon	In the evening
Mean	297 750± 54562,55	320 375± 57730,75	298875,00± 52256,07
Reference	166-575		

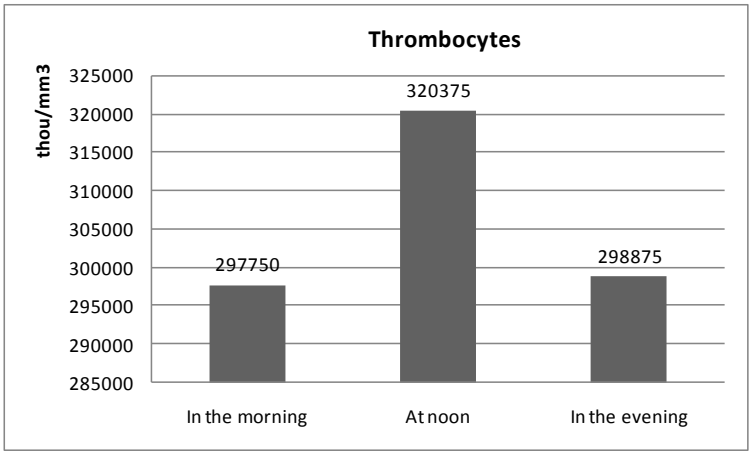

Fig. 2. Nychtemeral variations of the mean individual number of thrombocytes in dogs

The leukocytes

The mean comparative values of the number of leukocytes and their share in the leukocytary formula measured at intervals of six hours in nychtemeron are shown in Table 3 a and b.

As for the total number of leukocytes, we could see that the mean values ate within physiological limits of variation, closer to their upper limit. From a statistic point of view, there is no statistically significant difference ($p>0.05$) between the values obtained at different time intervals (Figure 3).

Table 3a

The mean comparative values of the number of leukocytes and their share in the leukocytary formula in dogs

Values	Leukocytes (thou/mm^3)			Neutrophils (%)			Lymphocytes (%)		
	M	N	E	M	N	E	M	N	E
Mean	12 678,75 ±2171,71	12 162,50 ±1751,85	12 447,50 ±2926,93	51,57 ±6,73	53,60 ±6,18	54,04 ±8,02	27,39 ±3,62	24,96 ±3,01	24,12 ±4,73
Reference	6,02-16,02[1]			63,52-85,68[1] 60-75[2]			14,06-20,14[1] 12-30[2]		

Table 3b

The mean comparative values of the number of leukocytes and their share in the leukocytary formula in dogs

Values	Monocyte (%)			Eosinophils (%)			Basophils (%)		
	M	N	E	M	N	E	M	N	E
Mean	4,15± 0,58	3,90± 0,95	4,93± 1,62	16,30± 6,54	17,18± 5,93	16,43± 7,32	0,57± 0,21	0,36± 0,12	0,47± 0,37
Reference	0-4,98[1] 3-10[2]			0-8,78[1] 2-10[2]			0-0[1] 0-2[2]		

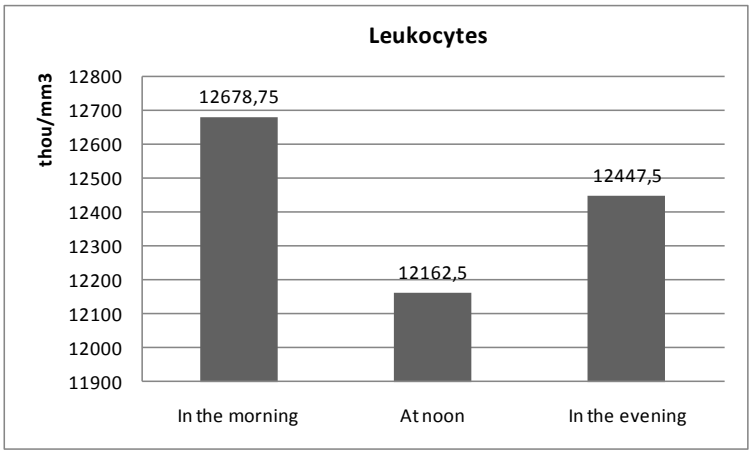

Fig. 3. Nychtemeral variations of the mean comparative values of the number of leukocytes

As for the leukocytary formula, the share of neutrophils at the three sampling intervals is below the lower limit of reference values, which can suggest neutropenia if all the dogs were clinically healthy. Neutropenia is usually associated with leucopoenia, which is not the case here, since leukocytes are towards the upper limit of the reference values. Neutropenia is associated with the presence of immature neutrophils and has a clinical value when the share of immature forms is above 10% of the number of neutrophils. Neutropenia is also frequently associated with Gram-negative bacterial infections in the diseases of the red marrow of the bones and anaemia, and thrombocytopenia is associated with intoxications and myelotoxicity caused by the cancer chemotherapy.

In all these cases, neutrophils go below 2,000 cells/µl; in our case, the number of neutrophils was about 9,000/µl, so that we can ignore all the situations in which they could be integrated within a pathological case. If we compare blood sampling times, there were no statistically significant differences per nychtemeron ($p>0.05$).

The share of lymphocytes was within physiological variation limits, closer to the upper limit. Though there were no statistically significant differences between the values measured at six-hour intervals, the highest differences were in the morning and in the evening, close to the statistically significant limit ($p=0.092$).

In the previous trials that suggest vaccinating animals when there is the largest number of lymphocytes for better antibody production in dogs, we suggest, given that the largest number of lymphocytes in our dogs was in the morning, to vaccinate the animals as early in the morning as possible.

Monocytes share 3.90-4.93% of the white elements and were within physiological variation (0-3.98%/3-10%); they are the circulating precursors of foxed macrophages, daily variations being below the statistical significance threshold ($p>0.05$).

Eosinophils recorded higher values, almost twice the upper value of the reference values (0-8.78%/2-10%), ranging between 16.30% and 17.18%. This suggests an allergy state very likely caused by intestinal parasites. Nychtemeral differences of eosinophils, in exchange, did not record any statistically significant differences.

Basophils were within physiological variation limits (0-2%), with values between 0.35% and 0.57%. The maximum value was in the morning (0.57%), followed by a statistically significant decrease at noon (after six hours) ($p<0.05$). between the blood samplings in the morning and in the evening and between the blood samplings at noon and in the evening, there were no statistically significant differences ($p>0.05$) (Figure 4).

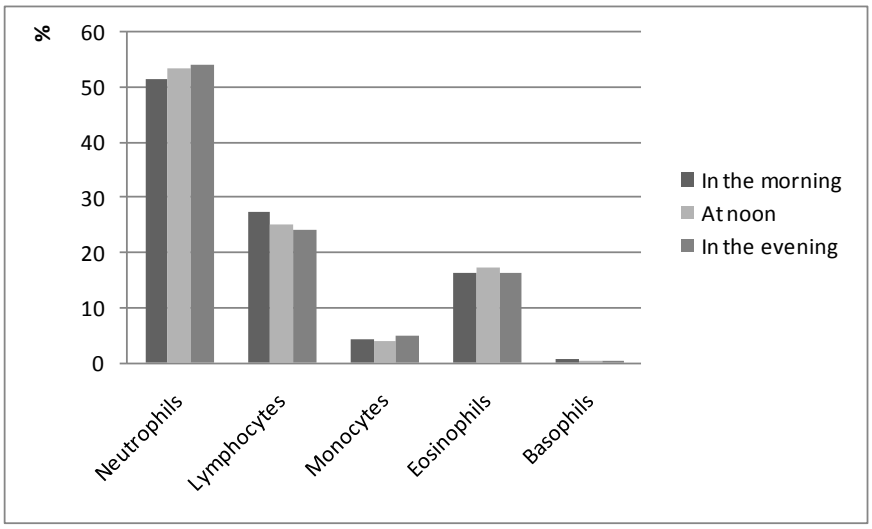

Fig. 4. Nychtemeral variations of leukocytes share in the leukocytary formula in dogs

CONCLUSIONS

1. Mean erythrocyte parameters measured in eight common breed dogs aged 2.5-9 were within physiological limits at the three blood sampling times during a nychtemeral interval (six-hour intervals).

2. There were statistically significant differences in haemoglobin, in mean erythrocyte volume and in mean haemoglobin concentration:

- blood haemoglobin content was statistically significantly higher in the morning than in the evening ($p<0.05$) and at noon than in the evening ($p<0.05$). there were no statistically significant differences between the haemoglobin values in the morning and at noon ($p>0.05$);
- mean erythrocyte volume (VEM/MCV) was statistically significantly higher at noon than in the morning ($p<0.05$);
- mean concentration in haemoglobin (CHEM/MCHC) was statistically significantly higher in the morning than at noon ($p<0.001$) and in the evening ($p<0.001$), respectively.

3. The mean number of thrombocytes was within physiological limits, with no statistically significant variations per nychtemeron.

4. The number of leukocytes was within physiological variation limits with no significant differences per nychtemeron.

5. The share of neutrophils was below the reference limit without varying significantly per nychtemeron.

6. The share of lymphocytes in the leukocytary formula was closer to the upper physiological variation reference limit, but it did not vary statistically significantly per nychtemeron; the mean values recorded in the morning were the highest, closer to the statistically significant threshold.

7. The share of eosinophils was about twice as much as the upper limit of the reference values, suggesting intestinal parasitism; per nychtemeron, there was no statistically significant variation.

BIBLIOGRAPHY

1. AMMED IJAZ, GOHAR, A., AHMED, N., AHMED, M., Haematological profile in cyclic and non-cyclic and endometric cross breed cattle, International Journal of Agriculture and Biology, 1560-8530, 05-3-332-334, 2003.
2. GHEORGHE, L., Corelaţia echilibrului fiziologic al organismului cu bioritmul profilului immunologic la taurine, teză de doctorat, USAMV-FMV, Iaşi, 2011.
3. MANOLESCU, N., ALEXANDRA, N., AVRAM, N., BARZĂ, H., COMIŞEL, V., Tratat de hematologie animală, vol. I, II, Ed. Fundaţiei România de mâine, Bucureşti, 1999.
4. TVEDTEN, H., Small Animal Clinical Diagnosis by Laboratory Methods, Fourth Edition, 2004.