



INVESTIGATION OF SOME HAEMATOLOGICAL AND BLOOD BIOCHEMICAL PARAMETERS IN CATTLE SPONTANEOUSLY INFECTED WITH BOVINE LEUKOSIS VIRUS

Sandev Nikolay¹, Zapryanova Dimitrinka², Stoycheva Ivanka³,
Rusenova Nikolina¹, Mircheva Teodora²

¹*Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria*

²*Department of Pharmacology, Physiology of Animals and Physiologic Chemistry, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria*

³*Department of Economics, Faculty of Economics, Stara Zagora, Bulgaria*

Received 28 June 2013; Received in revised form 30 July 2013; Accepted 26 August 2013

ABSTRACT

The aim of the present study was to follow out the alterations in some haematological and blood biochemical parameters in cattle spontaneously infected with enzootic bovine leukosis virus with regard to the *in vivo* differentiation of bovine leukosis stages. The experiment included 76 cows at various ages and body weight. Serological leukosis tests were done by agar-gel immunodiffusion test with a commercial kit of Synbiotics (France), containing standardised gp 51 antigen and positive serum approved by the EU. On the basis of haematological results, the cows were divided into three groups: first group – EBL-seropositive with normal haemogramme; second group – EBL seropositive with altered haemogramme and third group – controls. In cows from the first and the second group, a statistically significantly increased blood cell counts was established compared to healthy controls. The total WBC were increased in the second group (leukocytosis) up to $33.21 \times 10^9/l$ vs reference range of $5-10 \times 10^9/l$ as well as lymphocyte percentages (lymphocytosis) – 81.89% (reference 40–63%). A reduction in the proportion of neutrophils to 12.78% (relative neutropenia) vs the reference range of 22–49% and monocytes (monocytopenia) to 1.78% (reference range 2–6%) was observed. A statistically significant reduction in Ca concentrations (4.41 mg/dl) and higher inorganic phosphate levels (5.28 mg/dl) were established in cows from the second group. Also, ASAT activity was considerably lower – 47.03 U/l, while alkaline phosphatase increased slightly within the reference range up to 167.68 U/l and 165.81 U/l in groups one and two, respectively. The present haematological and whole blood/serum biochemical results in cows spontaneously infected with EBL virus could be used as prognostic markers of the course of the disease, to distinguish the stages of infection with regard to alive diagnostics.

Key words: enzootic bovine leukosis, cows, haematological and blood biochemical assays

INTRODUCTION

Enzootic bovine leukosis (EBL) is a chronic viral infection manifested with hyperplastic proliferative events in haemopoietic organs accompanied by quantitative and qualitative changes in peripheral blood leukocytes. The disease, after a prolonged

incubation period, undergoes three stages of development: stage I – virus carriership and antibody production; II – preclinical (haematological) related to changes in leukocyte classes (leukocytosis and lymphocytosis) and III – neoplastic, characterized with neoplastic growth in internal organs. Apart from the haematological changes, altered blood enzyme profile (elevated or reduced levels) and blood biochemistry parameters of diseased animals are also observed. A number of authors (1-4) reported changes in alkaline phosphatase (ALP) activity in some animal species. Spatar, F. V., (5) demonstrated a marked increase in ALP activity and inorganic phosphate in cattle with leukosis,

Corresponding author: Prof. Sandev Nikolay, PhD

E-mail address: n_sandev@abv.bg

Present address: Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria tel: +359 42 699 602;

Copyright: © 2013 Sandev N. This is an open-access article published under the terms of the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Competing Interests: The authors have declared that no competing interests exist.

whereas Hisamutdinov, F. F. (6) observed reduced ALP levels only in the tumour stage of EBL. In sheep experimentally infected with EBL virus and spontaneously infected cattle with altered blood cell counts, increased blood serum LDH activity was reported in sheep, but reduced LDH levels in cattle (7, 8). In cattle with tumours of internal organs, Antonov, S., et al. (9) reported marked relative and absolute decrease in albumins and very high blood serum globulins concentrations.

The aim of the present study was to follow out the alterations in some haematological and blood biochemical parameters in cattle spontaneously infected with enzootic bovine leukosis virus with regard to the *in vivo* differentiation of bovine leukosis stages.

MATERIALS AND METHODS

Haematological parameters were investigated in 30 cows of different breeds, 3 to 14 years of age, weighing 350 to 500 kg, divided into three groups: group I – seropositive for leukosis with negative haemogramme (11 cows); group II – seropositive for leukosis with altered haemogramme (9 cows) and group III – controls (10 cows).

For blood biochemistry investigations, the sample of animals was enlarged. The total number of observed animals was 46 with distribution per groups as followed: group I – seropositive for leukosis with negative haemogramme (21 cows); group II – seropositive for leukosis with altered haemogramme (15 cows) and group III – controls (10 cows). The total number of cows in the studied farm was 150.

Serological tests for leukosis were performed by agar gel immunodiffusion commercial kit (Synbiotics, France) containing a standardized glycoprotein antigen gp 51 and positive serum, approved by the EU.

Blood samples were assayed on automated haematological analyzer BC-2008 Vet for red blood cell counts (RBC), total white blood cell counts (WBC), mean corpuscular volume (MCV), haematocrit (HCT) and haemoglobin (HGB) contents. Differential white blood cell counts were determined by routine methods.

The blood concentrations of calcium, inorganic phosphate, triglycerides and cholesterol were determined on an automated analyzer BS-3000 P (Sinnowa LTD Nanjing, China) with commercial kits (Gisse Diagnostics, Italy).

Transaminases (ASAT, ALAT) and total protein were assayed with commercial kits of Human, Germany. The activity of both enzymes was determined by kinetic methods recommended by the International Federation of Clinical Chemistry at 37°C. Alkaline phosphatase activity was assayed by a method recommended by the German Society of Clinical Chemistry, also at 37°C with a kit of Chema Diagnostica, Italy. Gamma glutamyltransferase activity was determined by a kit of Hospitex Diagnostics, Italy.

Statistical processing of data was done by the paired Student's t-test at a level of significance 0.05 and degrees of freedom $df = n_1 + n_2 - 2$.

RESULTS

The results from the haematological investigations are presented in Table 1.

Table 1. Haematological parameters in healthy cows and cows spontaneously infected with bovine leukosis virus

| Group | Number | RBC ($\times 10^{12}/l$) | Ht (%) | MCV μm^3 | Hb (g/l) | WBC ($\times 10^9/l$) | WBC classes (%) | | | |
|-------|--------|-------------------------------|------------|------------------|-------------|----------------------------|-----------------|------------|------------|------------|
| | | | | | | | E_0 | Sg | L | M_0 |
| I | 11 | 5.54 | 26.45 | 48.52 | 99.45 | 9.84 | 3.09 | 36.64 | 56.09 | 3.00 |
| n_1 | | ± 0.23 | ± 0.92 | ± 2.49 | ± 3.22 | ± 0.58 | ± 0.28 | ± 0.77 | ± 0.58 | ± 0.38 |
| II | 9 | 6.34 | 27.37 | 43.42 | 105.33 | 33.21 | 1.78 | 12.78 | 81.89 | 1.78 |
| n_2 | | ± 0.16 | ± 0.59 | ± 1.31 | ± 2.41 | ± 3.46 | ± 0.32 | ± 0.86 | ± 1.24 | ± 0.22 |
| III | 10 | 5.95 | 26.60 | 41.65 | 99.80 | 9.38 | 2.46 | 32.27 | 60.37 | 3.50 |
| n_3 | | ± 0.29 | ± 1.27 | ± 1.48 | ± 5.42 | ± 0.86 | ± 0.35 | ± 2.14 | ± 2.71 | ± 0.45 |

RBC – red blood cell counts; Ht – haematocrit; MCV – mean corpuscular volume; Hb – haemoglobin; WBC – total white blood cell counts; WBC classes – differential blood cell counts; E_0 – eosinophils; Sg – segmented neutrophils; L – lymphocytes; M_0 – monocytes

No changes were observed in the cows from group III. In animals from the first and the second group, blood cell counts were higher as compared to healthy cows. The total white blood cell counts were increased in group II (leukocytosis): $33.2 \times 10^9/l$ (reference values $5-10 \times 10^9/l$). Lymphocytosis

– 81.89% was also observed (reference values 40-63%). Neutrophil counts were lower (relative neutropenia – 12.78% vs reference values of 22-49%); monocytopenia was also present (1.78% vs reference values of 2-6%). Blood serum biochemical parameters are presented in Table 2.

Table 2. Blood biochemical parameters in healthy cows and cows spontaneously infected with bovine leukosis virus

| Group | Number | P (mg/dl) | Ca (mg/dl) | ASAT (U/L) | ALAT (U/L) | ALP (U/L) | GAMMA GT (U/L) | TP (g/L) | TC (mmol/L) | TG (mmol/L) |
|-----------------------|--------|--------------|---------------|----------------|----------------|------------------|-------------------|----------------|----------------|----------------|
| I n ₁ | 21 | 3.32 ±0.30 | 5.90 ±0.42 | 42.05 ±3.73 | 37.46 ±4.05 | 167.68 ±14.35 | 26.56 ±3.03 | 74.56 ±2.30 | 2.82 ±0.14 | 0.70 ±0.09 |
| II n ₂ | 15 | 5.28 ±0.27 | 4.41 ±0.55 | 47.03 ±3.83 | 34.29 ±4.52 | 165.81 ±11.26 | 24.47 ±2.47 | 72.37 ±2.01 | 3.27 ±0.21 | 0.23 ±0.05 |
| III n ₃ | 10 | 3.24 ±0.32 | 9.78 ±0.21 | 84.24 ±9.93 | 32.22 ±1.43 | 125.06 ±9.99 | 24.95 ±0.76 | 69.80 ±1.85 | 2.73 ±0.16 | 0.25 ±0.01 |

P - inorganic phosphate; Ca - calcium; ASAT - aspartate aminotransferase; ALAT - alanine aminotransferase; ALP - alkaline phosphatase; GAMMA GT - gamma glutamyltransferase; TP - total protein; TC - total cholesterol; TG - triglycerides

Serum calcium was statistically significant lower (4.41 mg/dl) and inorganic phosphate – significantly higher (5.28 mg/dl) in group II. Blood enzymes showed statistically significantly lower activity of ASAT (47.03 U/L) and a slight increase in ALP in cows from group I and II (167.68 U/L and 165.81 U/L, respectively).

DISCUSSION

The results of this study showed that cows from group II were in the second stage of leukosis infection, also called preleukemic stage (10,11). This statement is supported by increased total leukocyte counts and a proportionally increased lymphocyte percentage. A very specific finding for lymphoid leukosis is the increase of the relative, and then of the absolute number of peripheral blood lymphocytes. The marked monocytopenia is also typical, which according to Simonyan, (10) is usually observed in cattle in an advanced (leukemia) stage of infection. The reduction in monocytes and the relative neutropenia, are probably due to spontaneous remissions, and the further course of leukosis was

accompanied by stabilization of the blood picture. This period could last from 1 to more than 2 years (12). The decreased blood calcium and increased inorganic phosphate concentrations corresponded to data provided by Spatar and Tsimbal (5) indicating a pathological process. The development of hypocalcaemia is important for leukosis genesis, as it is related to altered permeability of cell membranes which is regulated by calcium. The increased serum inorganic phosphate in diseased animals is related to extensive degradation of cells at various stages of maturity and release of phosphate compounds. In our study, no change in total protein concentrations was observed in group II contrary to hypoproteinaemia reported by Kuzin and Zakrepina (13) in cows with altered white blood cell counts.

Blood analysis of enzymes showed reduction in ASAT and a slightly increased ALP activity in cows from group I and II. These changes depend on the stage of bovine leukosis, and in the terminal stage of EBL, ALP tended to decrease but ASAT activity – to increase (6). There were no statistically significant changes in the other studied blood biochemical indices.

The results from the haematological and blood biochemical investigation of cows spontaneously infected with enzootic bovine leukosis virus and healthy cows could be used as diagnostic guidelines for the spread of leukosis and are important in differentiating the stages of infection. They could be also used as prognostic factors for the development of bovine leukosis with regard to *in vivo* diagnostics.

REFERENCES

1. Sarvaiya, N.P., Metha, V.M., Patel, D.M., Jaiswal, R.S., Nigam, R. (1991). The effect of short term showering on blood serum alkaline phosphatase and serum total proteins in Surti Buffalo Heifers. *Ind. J. Anim. Res.*, 25, 50-52.
2. Center, S.A., Slater, M.R., Manwarren, T., Prymak K. (1992). Diagnostic efficacy of serum alkaline phosphatase and glutamittransferase in dogs with histologically confirmed hepatobilliary disease, 270 cases (1980-1990). *J. Amer. Vet. Med. Assos.*, 201, 1258-1264.
3. Hank, A.M., Hoffman, W.E., Sanecki, R.K., Schaeffer, D.J., Dorner J.E. (1993). Quantative determination of equine alkaline phosphatase isoenzymes in foal and adult serum. *J. Vet. Internal. Med.*, 7, 20-24.
4. Guergueva, T.M. (1997). Comparison between three methods of alkaline phosphatase determination in plasma samples with different degree of hemolysis in cattle sheep and pigs. *Bulgarian Journal of Agricultural Science*, 3, 217-224.
5. Spatar, F. V., Tsimbal, G.L. (1978). Blood biochemical parameters in bovine leukosis. *Animal Husbandry, Shtinitsa, Kishinev*, 118-123.
6. Hisamutdinov, F. F. (1979). On changes of blood serum activity of alkaline phsophatase, aspartate aminotransferase in bovine leukosis. *Theoretical and practical veterinary medicine, Leukosis, Tartu*, 2, 123-126.
7. Sandev, N. (1988). Investigations on some epizootological and immunological features of enzootic bovine leukosis. Doctoral thesis, Stara Zagora (In Bulgarian)
8. Sandev, N., Binev, R., Katsarova, R., Hvarchilkov, V. (1999). Investigations on some haematological and blood biochemical parameters in bovine leukosis. *Proceedings of the Union of Scientists - Rousse*, 4, 28-31.
9. Antonov, S., Lalov, H. (1978). Investigations on changes in serum proteins in cattle with leukosis. *Veterinary Science (Sofia)*, 6, 42-45.
10. Simonyan, G. A. (1985). Lymphoid bovine leucosis. *Veterinaria*, 3, 35-38.
11. Nahmanson, V. M. (1986). *Bovine Leukosis*. Rosselhozizdat, Moscow, 124-126.
12. Lemesh, V.M., Drogun, A.G., Yakubov, V.N., Luchko, V. (1985). *Bovine Leukosis*. Uradzhaj, Minsk, 96-125.
13. Kuzin, A. I., Zakrepina, E.N. (1997). Effect of bovine leukosis on milk productivity and quality. *Veterinaria*, 2, 19-21.