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Analysis Of Hemo-Biochemical Status Of Cows Infected With Retroviruses.

Ekaterina S Krasnikova[1], Aleksandr V Krasnikov[1]*, Vyacheslav V Annikov[1], Andrey S Rykhlov[1], Albert K Galiullin[2], and Azat M Alimov[2].

[1]Saratov State Agrarian University named after N.I. Vavilov, Teatralnaya Square 1, Saratov city, 410012, Russia
[2]Kazan State Academy of Veterinary Medicine by N.E. Bauman, Sybirsky Tract Street 35, Kazan, 420029, Russia

ABSTRACT

Retroviral infections of the cattle (viral immunodeficiency (BIV) and enzootic bovine leukosis (BLV)) are wide-spread around the world and are often recorded in the form of co-infection. The study of blood values in these abnormalities is of great diagnostic and prognostic significance. The purpose of the study is a comparative analysis of morphological and biochemical blood values of intact cattle latently infected with retroviruses, as well as cows with specific immunological (IDR) and hemobiochemical (CBC, BBT) manifestation of BLV infection. It has been established that blood values of BLV-carriers and BIV-infected animals with mono-infection are characterized by minor shifts in morphological and biochemical blood values. With retroviral co-infection the blood is characterised by a sharp decrease in the number of blood corpuscles, the increase in ESR and the density of lymphocytes and new forms of leukocytes, moderate basophilia, a sharp decline in protein level and activity of alkaline phosphatase, a significant increase in bilirubin, BUN and creatinine, increase of transaminases, which is an evidence of changes in metabolic and reactive properties of the body.
Keywords: cattle, leukosis, immunodeficiency, hemo-biochemical status.

Corresponding author

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INTRODUCTION

During the practice the veterinarian has to meet not only with the acute manifestation of the disease, but with its asymptomatic, chronic course. Not manifesting as specific, striking clinical performance, the disease can disrupt body homeostasis, gradually reducing breeding and productive efficiency of animals [1,2]. The fact that in this situation other animals become infected is not less important.

In the number of cases it is possible that a single animal is a carrier for multiple infections, the so-called mixed infection or co-infection. Agents can enter the body both simultaneously and sequentially. The presence of several infectious agents may change the clinical performance of the disease, complicate the course and diagnosis of diseases [3,4].

However, another urgent issue is anthropozoonotic infections potentially dangerous to humans. The danger may come from both the animal being the source of infection, and products taken from it. Raw materials obtained from the diseased and infected animals may be of poor quality due to lower nutritional value and the content of toxic substances [5,6].

Blood plays a vital role in the fight of the immune system against infections and inflammation. The presence of such kinds of diseases leads to a sharp increase of antibody levels, the number and appearance of which help to judge the presence or absence of this or that agent. Modern research methods allow to determine the functioning of the immune system and if there are any silent infections in the body at the molecular level. Both serological and molecular genetic methods of research have their specificities in the diagnosis of various infections. For example, in some viral infections the infectious agent integrates with the genome of the infected body before the immune system has time to fully react to it [7,8]. In some cases, it was noted that the virus, after getting into the body, is quick to inactivate and eliminate, but the immune "footprint" remains for several months [9]. That is why the carriage of a virus does not always correlate with the diagnostic titer of antibodies. Such biological peculiarities belong to the agents of viral immunodeficiency (*BIV*) and enzootic bovine leukosis (*BLV*) of the cattle [10-12].

However, the presence of the virus, regardless of its form, causes homeostasis change. Blood is often called the "mirror" of the body, as being presented in the organs and tissues, it binds them together and reflects all processes occurring in it. The ratio of blood components provides an insight into what processes occur in the body. Thus, hemato-biochemical status gives an opportunity to judge the course of infection and the extent of damage of individual organs and systems and the body as a whole [13, 14].

The research in this area is highly relevant in the study of enzootic bovine leukosis of the cattle. Leukosis causes a huge economic damage both to the productive and breeding livestock all around the world. This is mainly associated with the culling of the diseased animals and elimination of virus carriers from breeding [15]. It is established that in some cases, *BLV*- infection occurs in combination with another retroviral infection – viral immunodeficiency of the cattle. The researchers state that both agents have an immunosupression impact, affecting immunocompetent cells: *BLV* – mostly B-lymphocytes, and *BIV* – T-lymphocytes [16]. However, even in the infected body the clinical signs of the disease do not always develop. Approximately 30% of the cattle infected with leukosis virus, is characterised by a constant lymphocytosis, while B-cell lymphosarcoma, which manifests itself in a visual increase in lymph nodes and spleen, develops in only 1-5% of the animals [17]. In viral immunodeficiency in the cattle it is quite difficult to differentiate the specific clinical features. However, in the blood of more than 50% of the animals it is possible to find out lymphopenia [18]. Accordingly, the study of the blood with these abnormalities will have both diagnostic and prognostic value.

In this regard the purpose of the study is a comparative analysis of some morphological and biochemical blood values of intact cattle latently infected with retroviruses, as well as animals with specific immunological and hemobiochemical manifestations of *BLV* infection.

MATERIAL AND METHODS

Material for the study included 32 samples of peripheral blood obtained from cows of Kazakh white-headed breed from the affected with enzootic bovine leukosis cattle from Ozernoe, LLC of the Saratov region.

All cows were aged 5-7 years old, were in identical conditions of feeding and maintenance, had a satisfactory clinical condition.

DNA isolation and cleaning from the samples was carried out using a set of DNA-sob-V (InterLabService, Russia) according to the supplied set of instructions. The presence of DNA of *BLV* and *BIV* proviruses was checked by the method of classical polymerase chain reaction (PCR) using a set "Leukosis" (InterLabService, Russia) and by an original technique using a set PCR-Mix and a buffer for applying (RPC "Litech", Russia) and with the addition of primers for the gag BIV gene (synthesis of Sintol, CJSC, Russia). Oligonucleotide sequences were synthesized on the basis of research by Kolotvin V.V. and Velikhov A.F. (2006) Primers: gag 1: 5'-GTCTTCCCACATCCGTAACATCTCCT-3' и gag 2: 5'-CCCCAGGTCCCATCAACATTCATCAG-3). The amplification conditions were adapted for the thermocycler T100 BioRad (USA): initial incubation at 95°C for 2 min, denaturation at 95°C for 20 sec., annealing at 58°C for 20 sec, elongation at 72°C for 40 sec. – 35 cycles, terminal incubation at 72ºC for 2 min. The detection of amplification products was carried out using horizontal electrophoresis in 2% agarose gel with addition of 0.5 μg/l of ethidium bromide under standard conditions with the application of the set "EF" (InterLabService, Russia) and photographic recording of the results on the BioRad equipment.

The study of the morphological blood composition was carried out on the automatic hematology analyzer PCE-VET (USA). Biochemical studies of blood serum were carried out on the semi-automatic biochemical analyzer BioChemSA (USA).

The information about the serologic status of animals was derived on the basis of data from the Federal State Budgetary Institution "Saratov Interregional Veterinarian Laboratory" according to the results of the study of animals by immunodiffusion (IDR).

RESULTS AND DISCUSSION

According to the results of serologic (IDR), molecular genetic (PCR) and hematologic studies the animals were divided into 5 groups (Fig.1).

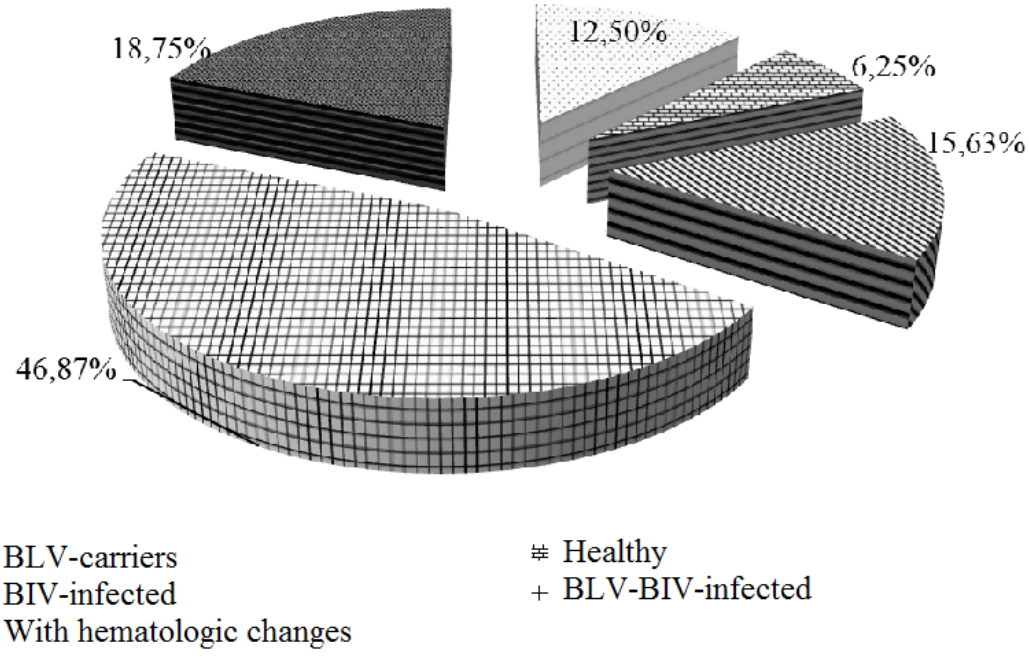

Figure 1: The structure of the herd in the study.

The group of nominally healthy (I) included animals the blood of which did not contain any antibodies to *BLV*, *BLV* and *BIV* provirus, as well as there were no changes in the number of lymphocytes. In the group of

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BLV-carriers (II) there were cows the serum of which had only *BLV* provirus. In the group of cows with hematologic changes (III) there were animals the serum of which contained antibodies to *BLV* and provirus in the background of lymphocytosis. Among *BIV*-infected animals (IV) there were cows having only *BIV* provirus in their blood. The last group (V) included cows coinfected with both retroviruses, while all animals of this group were marked with hematologic changes of this or that character.

The results of hematologic studies of I – V cattle groups are presented in Tables 1 - 3.

Table 1: Morphological composition of the blood of the tested animals (M,m,n=32)

Value	Nominally healthy (n=2)	BLV-carriers (n=4)	Patients with leukosis (n=6)	BIV-carriers (n=5)	BLV-BIV infection (n=15)
Erythrocytes, 1012/l	6.2±0.2	4.7±0.4$^{\#*}$	3.7±0.3$^{\#*}$	5.1±0.4$^{\#*}$	2.7±0.3$^{\#*}$
Hemoglobin, g/l	125.0±5.0	94.0±4.0*	83.0±5.0$^{\#*}$	98.0±6.0*	55.0±2.0$^{\#*}$
Hematocrit, %	55.0±5.0	40.0±4.0$^{\#*}$	35.0±3.0$^{\#*}$	44.0±3.0$^{\#*}$	29.0±2.0$^{\#*}$
Leukocytes, 10^9/l	9.3±0.,3	11.6±0.7$^{\#*}$	15.6±1.9$^{\#*}$	6.8±0.4$^{\#*}$	3.1±0.2$^{\#*}$
Thrombocytes, 10^9/l	390.0±15.0	290.0±10.0$^{\#*}$	230.0±10.0$^{\#*}$	160.0±8.0$^{\#*}$	74.0±6.0$^{\#*}$
ESR, mm/hour	3.0±1.0	4.0±2.0$^{\#*}$	6.0±2.0$^{\#*}$	7.0±1.0$^{\#*}$	8.0±2.0$^{\#*}$

Notes: * - statistically significant differences between the control and the experimental groups, # - statistically significant differences between the experimental groups,

Table 2: Blood leukogram of the tested animals (M,m,n=32)

Value	Nominally healthy (n=2)	BLV-carriers (n=4)	Patients with leukosis (n=6)	BIV-carriers (n=5)	BLV-BIV infection (n=15)
Basophils, %	0.0±0.0	2.0±1.0*	1.3±0.3$^{\#*}$	2.0±1.0*	2.0±1.0*
Eosinophils, %	3.0±1.0	2.8±0.2	1.4±0.2$^{\#*}$	5.2±1.1$^{\#*}$	2.9±0.3
Monocytes, %	4.0±1.0	2.8±0.2$^{\#*}$	2.4±0.2$^{\#*}$	6.5±1.8$^{\#*}$	3.6±0.2$^{\#}$
Myelocytes, %	0.0±0.0	0.6±0.4$^{\#*}$	3.8±0.8$^{\#*}$	0.0±0.0$^{\#*}$	4.3±0.9$^{\#*}$
New, %	0.0±0.0	0.8±0.2$^{\#*}$	3.2±0.6$^{\#*}$	1.8±0.8$^{\#*}$	3.3±0.6$^{\#*}$
Stab, %	4.0±1.0	4,5±0,5*	3.5±0.5$^{\#*}$	8.4±2.4$^{\#*}$	3.7±0.3*
Segmented, %	33.0±2.0	32.2±3.2$^{\#}$	11.6±2.2$^{\#*}$	47.8±3.5$^{\#*}$	10.9±0.5$^{\#*}$
Lymphocytes, %	56.0±2.0	54.3±3.6$^{\#}$	72.8±4.6*	28.3±3.2$^{\#*}$	69.3±2.5*

Notes: * - statistically significant differences between the control and the experimental groups, # - statistically significant differences between the experimental groups

The data from the Tables 1 and 2 show that in the group of nominally healthy animals the morphological blood values, including the readings in leucogram, were within the physiological norm. In the group of BLV-carriers the morphological blood composition was characterized by the reduction of quantity of erythrocytes by 17.7% and hemoglobin by 21.6% compared to the control group, a slight leukocytosis with a neutrophilic nucleus shift to the left and ESR increasing by 1 mm/hour, as well as moderate basophilia. Animals, having in their blood both antibodies to BLV and provirus in the background of lymphocytosis, had morphological changes of the blood of a clearly pronounced nature and characterized by a 1.5 decrease in hemoglobin concentration and 1.7 decrease in the erythrocyte concentration in the background of ESR twofold increase and thrombocytopenia (41 %). The leucogram, with pronounced leukocytosis - 67.7 % compared to the control group, showed the substantial fluctuations consisting in the increase of lymphocytes content by 30 % and the increase in the number of new forms to 3.2 % of the total number of neutrophils. Among BIV-infected animals the morphological blood values had a clear trend and were characterized by the number of erythrocytes and hemoglobin at the lower limit of the physiological norm with 2.3 increase of ESR. The leucogram recorded the pronounced lymphocytopenia (twofold) and thrombocytopenia (2.4 times) in the background of leukocytopenia (by 26.8 %) with neutrophilic nucleus shift to the left. The group of cows coinfected with the two retroviruses, changes in the morphological composition of the blood were significant and showed sharp decrease in the number of hemoglobin and erythrocytes (2.3 times), thrombocytes (5.3-

times) with 2.7 increase of ESR. The leucogram noted the increase of lymphocytes density by 23.8 % and sevenfold increase of new forms of leukocytes with a moderate basophilia.

Table 3 - Biochemical blood values of the tested animals (M,m,n=32)

Value	Nominally healthy (n=2)	BLV-carriers (n=4)	Patients with leukosis (n=6)	BIV-carriers (n=5)	BLV-BIV infection (n=15)
Conjugated bilirubin, µmol/l	1.5±0.5	3.2±0.6*	5.3±0.8#*	3.1±0.4*	8.4±0.6#*
Total bilirubin, µmol/l	5.6±0.9	7.6±1.4#*	15.6±1.9#*	6.8±0.8#*	18.5±0.7#*
AAT, Unit/l	29.4±2.2	30.2±1.8#	47.4±2.6*	48.2±2.2*	97.3±2.8#*
ALT, Unit/l	44.3±2.5	48.1±2.7#*	66.1±3.1#*	91.4±3.9#*	153.2±3.6#*
Total protein, g/l	78.0±5.0	82.0±4.0#*	98.5±4.6#*	63.7±5.2#*	31.2±1.2#*
Alkaline phosphatase, Unit/l	57.3±2.3	82.8±3.8#*	230.0±15.0#*	120.0±10.0#*	45.2±1.7#*
Creatinine, µmol/l	65.1±2.9	73.3±2.6#*	124.4±5.1*	124.4±5.3*	136.4±6.2#*
BUN, mmol/l	5.8±0.6	6.5±0.5#*	9.3±0.7#*	7.5±0.8#*	18.3±0.9#*
Albumins, g/l	37.0±4.0	31.1±3.2#*	20.2±3.1*	24.1±3.3#*	21.4±1.9*

Notes: * - statistically significant differences between the control and the experimental groups, # - statistically significant differences between the experimental groups,

As the data from Table 3 show, in the group of nominally healthy animals blood biochemical values were within the physiological norm. BLV-carriers had the blood biochemical parameters characterized by increased level of bile pigments: 1.3 and 2.1 times increase of conjugated and unconjugated bilirubin respectively, and 1.4 times increase of alkaline phosphatase compared with the control group. In the group of the cows having both antibodies to BLV and provirus in their blood in the background of leukocytosis, the biochemical study showed hypoalbuminemia (by 45.4 %) in the background of the increase of total protein level by 26.3%, a significant increase in the amount of bilirubin (conjugated and unconjugated) (2.8 and 3.5 times respectively), and a 4 times increase in alkaline phosphatase activity, as well as the upper limit of normal activity of AAT and ALT. Biochemical changes in the blood of BIV-infected animals were characterized by a decrease in total protein level by 19.2 % and albumin by 35.1 %, content of bilirubin and AAT in the upper limits of physiological norm, 2.1 times increase of alkaline phosphatase activity, increase AAT and ALT (1.6 and 2 times respectively) and higher creatinine concentration (1.9 times). Biochemical blood values of animals coinfected with retroviruses were characterized by a sharp decrease in the protein level (2.5 times) and the activity of alkaline phosphatase (4 times), with a significant increase in the content of total and conjugated bilirubin (3.3 and 5.6 times respectively), as well as the increase in transaminases activity (more than 3 times), high BUN content (3.2 times) and high creatinine content (2.1 times).

In our opinion, basophils responsible for producing histamine will stimulate the development of allergic reactions in BLV-carriers and if there is retroviral co-infection of the cattle, thus reducing the diagnostic value of allergic tests. Cytopeniae indicate a decrease in the protective and trophic properties of the blood, and reducing the number of hemoglobin that is a hypoxia in all organs and tissues. In this case increased ESR can act as a marker of the development of the related inflammatory infections and kidney and liver damages and endocrine disorders. Leukocytosis with a neutrophilic nucleus shift to the left indicates the formation of acute inflammatory processes in the body.

We have identified biochemical changes in the form of increased creatinine level which in this case may indicate an impairment of filtering ability of the kidneys in consequence of the death of the nephrons, and an increase of transaminase activity may indicate the inflammatory degenerative changes in the liver, including due to impairment of detoxification function of the kidneys. The increase in bilirubin concentration in this case may act as a marker of hepatitis and hemolytic anemia, which is showed by the increased activity of alkaline phosphatase as well. The decrease in total protein allows us to assume the development of nephrotic pathologies – protein excretion by the kidneys, and, at the same time, it indicates pathologies of the liver (relative decrease in the albumin fraction).

Our findings are comparable with the results of the Russian and foreign researchers. M.C. Frie et al. (2018) report that approximately 30% of BLV-infected cattle have the developed persistent lymphocytosis. S.

Bhatia et al. (2013) state a decrease in CD4/CD8 ratio, and an increased lymphocyte proliferation already in 2-6 weeks after infecting calves with BIV. According to the data of S. Carpenter et al. (1992) in the blood of the calves artificially infected with BIV there was neutropenia found in 5-15 days which was aggravated in 4 weeks after infection. Research of I.S. Ponomareva (2012) confirms our data that the hematological parameters of cows in conditions of infectious process are characterized by leukocytosis, anemia and thrombocytopenia. Our data correlate with the results of the researches of F. G. Gizatullina (2015) and I. S. Ponomareva (2012), which testify that the blood of the cattle with BLV-infection and the cows with hematological changes shows significant changes in biochemical values: hyperproteinemia with the decrease in globulin and albumin concentration, increased level of alkaline phosphatase, creatinine and ALT.

CONCLUSION

Summarizing the obtained data, we can conclude that the blood values of BLV-carriers are characterized by a slight leukocytosis with a shift of neutrophil nucleus to the left, moderate basophilia, a small decrease in the concentration of erythrocytes and haemoglobin and a slight increase in the level of bile pigments and alkaline phosphatase. Among BLV-infected animals with a pronounced humoral response the blood is characterised by a sharp decrease in the concentraion of hemoglobin and erythrocytes, ESR increase, minor thrombocytopenia, lymphocytosis with a predominance of immature white blood cells, hypoalbuminemia in the background of the increase in total protein level, a significant increase in bilirubin concentration and akaline phosphatase activity. BIV-infected animals show a moderate ESR increase, lymphopenia, thrombocytopenia, a slight shift of neutrophil nucleus to the left, a small decrease in total protein and albumin level, increased activity of alkaline phosphatase and ALT, high concentration of creatinine. It was made clear that with retroviral co-infection the blood is characterised by a sharp decrease in the number of blood corpuscles, the increase in ESR and the density of lymphocytes and new forms of leukocytes, moderate basophilia, a sharp decline in protein level and activity of alkaline phosphatase, a significant increase in bilirubin, BUN and creatinine, increase of transaminases.

Thus, the results of our research show that viral leukosis and immunodeficiency of the cattle, in the form of both monoinfection and co-infection, are accompanied by significant and diverse changes in morphological and biochemical blood values, which indicates a change in metabolic and reactive properties of the body.

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