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Features of humoral immunity in cows infected with the leukaemia virus

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ABSTRACT

The article presents the results of studying the dynamics of the formation of antibodies and immune complexes, reveals the prospects for improving the early diagnosis of cattle leukaemia. Studies were conducted for 6 months on an experimental group of animals consisting of 12 cows. The titers of free and bound antibodies in blood serum and milk were determined by enzyme-linked immunosorbent assay. The results of studies showing that changes in titers of anti-leukaemia antibodies in the blood serum of cows naturally infected with BLV (bovine leukaemia virus) are significantly different from experimental infection data are adduced. In cows infected with BLV, there is no definite relationship between antibody titers in milk and in blood serum. With sufficiently high titers of serum antibodies, antibody titers in milk can be minimal for the same cows; conversely, with low titers of serum antibodies, there can be high antibody titers in milk. In the titers of antibodies free and bound in the immune complexes in blood serum with the development of the disease, a certain dependence is traced. With a decrease in titers of free antibodies, in most cases, an increase in titers of "bound" antibodies is observed, i.e., the degree of formation of circulating immune complexes (CECs) increases. There is no clearly defined dependence in the dynamics of changes in titers free and bound in immune complexes of antibodies in milk samples. They can remain at the same level for a long time, both at low and rather high levels.

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INTRODUCTION

A characteristic feature of the BLV infection in cattle is the lifelong persistence of the virus and virus-specific antibodies in a sick animal. Therefore, serological methods for detecting specific serum antibodies are the most practical, efficient, and widely used in the diagnostics of cattle leukemia.

The BLV has a depressing effect on the body due to genetic parasitism in B-lymphocytes, damage to other immunocompetent cells, organs of the immune system (spleen, lymph nodes, etc.) and chronic stress exposure. Against the background of immune depression, resistance to infectious, invasive, and non-infectious pathologies decreases. Moreover, the BLV and antibodies thereto persist in the blood for life.

It is known that in chronic infections caused by viruses of the Retroviridae family, antigen-antibody immune complexes appear in biological fluids of

the body, which have a certain value in the pathogenesis of these infections. The immune complexes in the blood serum of humans and animals can be formed from any classes or subclasses of immunoglobulins. According to (Yakobson *et al.*, 1998), immune complexes in the serum of BLV-seropositive cows contain antigens of the virus and IgG and IgM. The ratio of antibodies in complexes of "bound antibodies" varies with the age of the animal and the stages of the disease. Methods for the quantitative determination of immune complexes have been developed. (Schetters *et al.*, 1990), adapted the ELISA method for measuring antigen-specific immune complexes (ASICs) containing retroviral antigens.

Serological research methods are the basis for the diagnosis of leukemia in cattle at the present stage. The most used and legal methods for diagnosing cattle leukemia are agar gel immunodiffusion (AGID) and enzyme immunoassay (ELISA) (Jaworski *et al.*, 2016). The basis of these methods is the interaction of specific precipitating antibodies with the BLV antigens, which can be detected 3-6 weeks after infection.

The aim of this work was to study the dynamics and characteristics of the formation of antibodies and immune complexes in the blood serum and milk of the BLV-infected cows.

MATERIALS AND METHODS

Samples of blood and milk were obtained from the BLV-infected cows. The detection of antibodies to the BLV in serum and milk samples was performed by the ELISA method using the commercial test systems. The reaction results were taken into account visually and spectrophotometrically. Circulating immune complexes (CICs) were isolated by precipitation in polyethylene glycol (PEG). At the same time, blood serum samples were mixed in a 1:1 ratio with a 7% solution of PEG-6000 in 0.1 M borate buffer (pH 8.8), mixed and incubated at + 4° C for 72 hours. The precipitate was precipitated by centrifugation at 5000g for 20 minutes and washed three times with a ten-fold volume of PEG in concentrations: 3.5%, 7%, and 10.5% in borate buffer. The isolated immune complexes were dissolved in normal saline, and their activity in ELISA was studied. The complexes were broken up with preliminary incubation of samples at 50° C for 2 hours.

To study PEG for free immunoglobulins, studies were carried out to determine the titer of anti-BLV antibodies in a pool of blood and milk serum before and after treatment with PEG-6000. As a result, it was found that after deposition of the CEC from the pool of blood and milk with a 3.5% PEG solution, the titers of anti-leukemia antibodies in ELISA did not change and were equal to the titers of antibodies in the initial sample.

RESULTS AND DISCUSSION

The experimental group of animals consisted of 12 cows. Of these, 2 (nos. 5 and 6) are hematologically ill, and the rest are positively responsive in AGID. Observations were carried out for 6 months. The titers of bound and free antibodies in the studied samples were determined by enzyme-linked immunosorbent assay every two months. The study results are presented in Table 1 and Table 2.

The data presented in the table show that changes in titers of anti-leukaemia antibodies in the blood serum of cows naturally infected with BLV are significantly different from experimental infection data are adduced. If the algorithm for changing antibody titers is three months in the case of experimental infection, then it turned out to be different with the natural infection of cows with the leukemia virus. During the study period, serum antibody titers in individual samples remained unchanged both at sufficiently high and relatively low levels. However, in general, changes in antibody titers in the studied samples occurred sinusoidally.

Mammerickx *et al.* (2010), created an experimental model of chronic calf leukemia infection and noted that there were no serious differences in cellular immunity in the development of cattle leukemia in the field and in laboratories. However, they and other authors noted that about 30% of ELISA-positive livestock are susceptible to lymphocytic leukemia (lymphocytosis), which is accompanied by suppression of immunity and various patterns of development of humoral immunity with disease progression (Ungar-Waron *et al.*, 1992, 1999; Konishi *et al.*, 2018). Antibody titers both in milk and in blood serum of hematologically ill animals significantly decrease as the disease develops. Similar results are given in the studies of some foreign researchers (Ruggiero and Bartlett, 2019).

Antibody titers in the milk of infected cows do not correlate with those in serum. With sufficiently high titers of serum antibodies, antibody titers in milk can be minimal (sample nos. 12, 10, 4) for the same cows; conversely, with low titers of serum antibodies, there are high antibody titers in milk (nos. 3,8). In addition, despite significant changes in serum antibody titers over the study periods, anti-leukemia antibody titers in milk may not change (nos. 1, 9).

Table 1: Dynamics of changes in titers of antibodies free and bound in the immune complexes against gp51 in blood serum.

Nos. of samples	ELISA antibody titers					
	study 1		study 2		study 3	
	free	bound	Free	Bound	Free	Bound
1	1:216	1:216	1:648	1:216	1:72	1:216
2	1:216	1:648	1:24	1:216	1:72	1:72
3	1:72	1:648	1:72	1:216	1:648	1:216
4	1:216	1:72	1:648	1:72	1:648	1:72
5	1:216	1:216	1:24	1:72	1:8	1:72
6	1:216	1:216	1:216	1:72	1:24	1:24
7	1:72	1:216	1:72	1:216	1:216	1:216
8	1:72	1:648	1:72	1:648	1:72	1:216
9	1:216	1:648	1:24	1:648	1:72	1:216
10	1:648	1:72	1:216	1:72	1:24	1:216
11	1:216	1:216	1:216	1:216	1:72	1:648
12	1:648	1:216	1:216	1:648	1:72	1:216

Table 2: Dynamics of changes in titers of antibodies free and bound in the immune complexes against gp51 in milk samples.

Nos. of samples	ELISA antibody titers					
	study 1		study 2		study 3	
	free	Bound	free	Bound	free	Bound
1	1:8	1:8	1:8	1:8	1:8	1:2
2	1:8	1:8	1:8	1:2	1:16	negative
3	1:16	1:2	1:16	1:2	1:16	1:8
4	1:2	1:8	1:8	1:8	1:8	1:8
5	1:2	negative	negative	1:2	negative	negative
6	1:8	1:2	1:2	1:4	negative	1:2
7	1:8	1:8	1:8	1:4	1:16	1:2
8	1:2	1:8	1:16	1:2	1:8	1:8
9	1:8	1:16	1:8	1:8	1:8	1:16
10	1:8	1:2	1:2	1:2	1:2	1:2
11	1:2	1:16	1:16	1:8	1:16	1:8
12	1:2	1:8	1:8	1:2	1:8	1:8

Comparing the titers of free and "bound" antibodies in blood serum (Table 1), we can say that they change with the development of the disease, and a certain relationship is traced between them. With a decrease in titers of free antibodies, in most cases, an increase in titers of "bound" antibodies is observed, i.e., the degree of formation of circulating immune complexes (CECs) increases. Typical examples of such changes are samples No. 1, 3, 9, 10, 11.

In the dynamics of changes in titers of antibodies free and bound in the immune complexes against gp51 in milk samples, there is no clearly defined dependence. Titers of free and bound milk antibodies can remain at the same level for a long time, both at low and rather high levels. There are a lot of reports in the scientific literature on the results of studies to determine the relationship between the level of antibodies to cattle leukemia virus and proviral load (PVL) in milk and blood samples. There is a significant positive correlation between PVL and serum antibody titers, a negative correlation between milk PVL and antibody titers in milk, as well as between blood and milk antibody titers (Kono *et al.*, 1982; Khudhair *et al.*, 2016).

Most researchers note that the determination of CEC is of great importance in monitoring the epizootic

situation of leukemia and can serve as an indicator of infection of animals with the virus. The identification and study of the composition of immune complexes contribute to the explanation of the pathogenetic aspects of cattle leukemia.

CONCLUSIONS

It is generally accepted that ELISA as compared with AGID is a more sensitive method, especially in the early stages of antibody formation, when the level of antibodies is lower than the threshold determined in AGID. However, this advantage quickly passes, the titers grow quickly and 2-3 weeks after reach the same level of effectiveness. Both methods have their strengths and weaknesses and are fairly well comparable. However, our data indicate that the ratio between free and bound antibodies in the blood serum is constantly changing in the process of the development of the infectious process. Therefore, one-time serological tests in the diagnosis of cattle leukemia may not be effective enough. A study of the dynamics of the formation of immune complexes reveals the prospects for improving the early diagnosis of animal leukemia. Serological methods for the diagnosis of cattle leukemia should also be aimed at detecting antibodies bound in the composition of the CEC in biological samples, taking into account the ratio between free and bound antibodies in serum, constantly changing for 2-4 months.

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