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INSUFFICIENTLY STUDIED MINOR VIRAL INFECTIONS IN LIVESTOCK OF UKRAINE

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Summary. For the first time in Ukraine the presence of genetic material of bovine immunodeficiency virus and bovine foamy virus in cattle on Ukrainian farms was detected by scientists of the Laboratory of Leukemia Study and the Laboratory of Molecular Diagnostics of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'. The associative nature of animal infection with leukemia, immunodeficiency and spumavirus pathogens is recorded. In the future, it is planned to study the properties of pathogens, adaptation to homologous cell cultures and accumulation of viral material in order to develop domestic means of serological diagnosis of immunodeficiency and spumavirus infection

Keywords: epizootic situation, leukemia, immunodeficiency, spumavirus, PCR

Introduction. Among the minor viral diseases of cattle, leukemia, bovine immunodeficiency and bovine spumavirus infection are the most notable. What these diseases have in common is that the pathogens, BLV (Bovine Leukemia Virus), BIV (Bovine Immunodeficiency Virus) and BFV (Bovine Foamy Virus), cause an immunosuppressive state in the body of infected animals, especially during the clinical course of the disease, which makes it impossible to obtain reliable efficacy of treatment and prevention measures, and causes a decrease in the volume and quality of livestock products, loss of valuable gene pool and often death of animals. In addition, pathogens belonging to the retrovirus family also pose a potentially dangerous medical and social threat, as they are structurally similar to the causative agents of AIDS and human T-cell leukemia.

Bovine leukemia, one of the most common slow infectious diseases of cattle, has been well studied in terms of its prevalence in the world livestock industry through the introduction of serological and molecular-genetic research methods. With the introduction of legislation in most European countries, the disease has been eradicated, although there are still some regions with a limited number of infected animals without clinical manifestations of the disease. There is still a significant presence of leukemia in the livestock industry in Canada and the United States—in both North American countries, bovine leukemia occurs not only at the level of seroconversion, but also at the level of clinical manifestation of the disease.

Animals infected with bovine immunodeficiency virus are recorded in many countries of the world, and often associated infection with both infectious immunodeficiency virus and bovine leukemia is recorded. It is worth noting that the serological testing of cattle for immunodeficiency in different countries, based on the materials of individual scientific publications, revealed a significant prevalence of the disease.

Thus, seropositivity rates differ across countries. In the United States, the seropositivity rate is at 4%, whereas in the Netherlands, it is at 1.4%, in Canada, 5.5%, in Germany, 6.6%, and in France, 4%. Immunodeficiency has been determined in several countries, including the UK, Sweden, Costa Rica, Venezuela, New Zealand, and Australia, based on laboratory test results. The rate between seropositive and healthy cattle typically range from 1–7%, although in some herds with a chronic course of the disease (epizootic stationarity), the infection rate can reach as high as 50%. Of the 64% of animals with lymphosarcoma, lymphadenopathy, and other disorders, 74% were infected with the immunodeficiency pathogen (Kolotvin, 2007; Krasnikova, 2011; Supotnitskiy, 2009; Meas et al., 2002).

According to individual authors' materials, infectious bovine immunodeficiency has been reported in Japan, France, Canada, Iran, Argentina, Germany, the Netherlands, Italy, Brazil, Turkey, Cambodia, Pakistan, and Australia with infection rates ranging from 1 to 50% or more (Meas et al., 2002; Romen et al., 2007; Murray et al., 2006).

The scientific literature indicates that BFV seropositivity among cattle in certain livestock farms in developed countries ranges from 30% to 45%, and the resulting infection is prevalent worldwide. Some instances report the occurrence of a two- or even three-variant course, signifying persistent leukemia pathogens, immunodeficiency, and spumavirus infection. The diseases cause significant harm to livestock production, impacting both animal resistance and the volume and quality of products (Romen et al., 2007; Murray et al., 2006; Orr, O'Reilly and Scholl, 2003).

The information reports in the world scientific literature necessitated conducting scientific research on

the epizootic condition of livestock in Ukraine with regards to minor infections. The Laboratory of Leukemia Study of the NSC 'IECVM' initiated such studies within Ukraine.

The purpose of this research is to examine the incidence of minor viral infections in cattle, namely leukemia, immunodeficiency, and spumavirus infection, within the Ukrainian livestock industry.

Materials and methods. We analyzed 10–15 stabilized blood samples obtained from cows in individual farms located in northern and central regions of Ukraine. On these farms, anti-leukemia health improvement measures were taken during the active phase (farms No. 6 in Kharkiv, No. 1 in Kirovohrad, No. 2 in Poltava, and No. 4 in Cherkasy regions) and at the final stages of recovery.

Animals infected with leukemia virus were detected through serological methods, including RID and ELISA, as well as molecular-genetic methods such as PCR. The serological examination of bovine serum for leukemia was carried out on the basis of the Laboratory of Leukemia Study, using the 'Kit of dry components for the serological diagnosis of bovine leukemia in the immunodiffusion reaction' (RID) (manufactured by LLC 'SRE Veterinary Medicine', Kharkiv) and the 'Kit for the detection of antibodies to bovine leukemia virus by ELISA' (manufactured by VMRD, USA).

The total DNA extraction from the biological material samples was performed through the use of the sorbent method (Boom et al., 1990).

The PCR assay was performed using the Thermo Scientific DreamTaq Green PCR Master Mix (2X) reagent kit according to the manufacturer's instructions.

Detection of BFV proviral DNA was performed by standard PCR using primers Int 3 (forward primer, 5`-TCCCGCCTAAAGCTGATAGA-3`) and Int 4 (reverse primer, 5`-CAAACCTGAAATGGCTTGGT-3`), the target of which was the 241-base pair (bp) region of the *pol* gene of the virus (Materniak et al., 2013); detection of BIV proviral DNA was performed using Pol + (forward primer, 5`-GATTTTAGGGAATTAAATAA-3`) and Pol – (reverse primer, 5`-ACCCATCCTTGTGGTAGAACT-3`) primers, the target of which was the 235-bp region of the *pol* gene of the virus (Moody et al., 2002); detection of BIV proviral DNA was performed using BLV-env-3 (forward primer, 5`-CCACAAGGGCGGCGCCGGTTT-3`) and BLV-env-4 (reverse primer, 5`-GCGAGGCCGGTC CAGAGCTGG-3`) primers, the target of which was the 444-bp region of the *env* gene of the virus (Fechner et al., 1996).

The amplification results were visualized through horizontal electrophoresis on a 1.5% agarose gel utilizing 10 μL of the amplification product. Electrophoresis was carried out for 40 min at an electric field intensity of 12 V/cm. The molecular weight marker with a resolution of 100 bp (GeneRuler 100 bp DNA Ladder, Thermo Scientific) was used to determine the amplicon length. The results of the amplification procedure were recorded utilizing the Image Lab 5.2.1 gel imaging software and a BioRad Universal Hood II transilluminator.

Ninety blood samples were collected from cows at seven milk-producing farms in Ukraine's five different regions that were recovering from bovine leukemia. The samples underwent molecular genetic testing to detect genetic material of BIV, BFV, and BLV.

Results and discussion. An epizootic survey was conducted on several livestock farms in central and eastern Ukraine, with implementation of anti-leukemia health measures in conjunction with the Laboratory of Leukemia Study of the NSC 'IECVM'. Specifically, two farms in the central region — Kirovohrad (No. 1) and Poltava (No. 2) — underwent examination of their cattle.

In the first case, the epidemiological survey was based on detecting individual cows infected with leukemia virus in a herd that was previously leukemia-free. In the second case, a high prevalence of leukemia virus infection was found in the herd. In addition to serological tests for leukemia in animals over 6 months old, samples of stabilized blood were collected for molecular genetic studies to identify the genetic material of leukemia pathogens, infectious immunodeficiency, and spumavirus infections in cattle. 10 samples were collected from cows of farm No. 1, and 15 samples from animals of the same category were collected from the herd of farm No. 2.

Similar work was undertaken on farms in Sumy (No. 3), Cherkasy (No. 4), and No. 5, No. 6, and No. 7 in Kharkiv region. Each of these farms implements anti-leukemia health measures which have proven largely efficacious throughout the year. Regular serological tests are performed on livestock beginning at six months of age, followed by the removal of seropositive individuals. The level of seropositivity was reduced from 12% to 0.2%. In some cases, the health improvement measures resulted in the stabilization of the epizootic situation. For instance, despite several serological tests throughout the year, the level of infection among cattle at farm No. 6 in Kharkiv Region remained at 7-8% without declining. It is noteworthy that farm No. 4 in Cherkasy Region falls within the central-western zone. The cattle on this farm exhibit a 13% infection rate of leukemia virus among cows and receive anti-leukemia health measures. Here, along with repeated serological screenings for leukemia in cattle starting at 6 months of age and subsequent isolation of leukemia-compromised animals, 15 stabilized blood samples were obtained to conduct a molecular-genetic research program aimed at identifying the genetic material of leukemia, bovine immunodeficiency, and spumavirus infection in cattle.

The qualitative characteristics of these studies on the isolation of BIV, BFV, and BLV DNA are shown in Table 1.

Table 1 — Results of molecular-genetic study of cattle blood samples

Region	Farm	n	Genetic material detected		
			BLV	BFV	BIV
Kirovohrad	No. 1	10	1	2	1
Poltava	No. 2	15	4	1	—
Sumy	No. 3	10	—	1	1
Cherkasy	No. 4	15	2	1	—
Kharkiv	No. 5	15	—	—	1
	No. 6	15	3	2	—
	No. 7	10	—	1	—

Table 1 shows that the leukemia virus was detected in three cases out of 15 samples of citrated blood taken from the herd of farm No. 6 in the Kharkiv Region, which could be attributed to the fact that the samples were taken from animals of a herd positive for bovine leukemia, where the above disease is in an active stage. In other instances, concerning farms in different regions, the isolation of the leukemia pathogen was sporadic, as anti-leukemia health measures were in their final stages.

Regarding the detection of immunodeficiency and spumavirus infections in cattle, it is important to note that two farms, No. 1 and No. 3, had animals with associative infections. In three other cases, animals from farms No. 2, No. 4, and No. 6 were infected with a combination of leukemia and spumavirus pathogens. In one instance, farm No. 1 had animals infected with a combination of leukemia, bovine immunodeficiency, and spumavirus infections. Thus, the persistence of pathogens causing minor cattle infections such as leukemia, bovine immunodeficiency, and spumavirus infection is observed among the animals on seven farms in five regions of Central and Eastern Ukraine.

Conclusions. 1. The retroviruses responsible for slow infections in cattle, which are leukemia, immunodeficiency, and spumavirus (BLV, BIV, BFV) belong to the Retroviridae family. They have a wide distribution in livestock farms globally.

2. Selective studies of blood samples collected from cattle at risk of leukemia farms in the Kharkiv, Sumy, Kirovohrad, Poltava, and Cherkasy regions demonstrated the presence of BIV and BFV genetic material by utilizing molecular-genetic methods. Additionally, some cases reveal that the pathogenesis of animal infection can be attributed to the association of leukemia pathogens, immunodeficiency, and spumavirus infection in cattle.

3. The above is aimed at a detailed study of the epizootic state of livestock in Ukraine regarding minor viral infections of cattle with the aim of identifying potential areas for improvement in the national program for the regulation of animal products and consumer protection.

Prospects for further use of the obtained results. Indication of the genetic material of immunodeficiency and spumavirus pathogens, study of their properties, adaptation to homologous cell cultures, accumulation of viral material for the development of a domestic serological diagnostic tool.

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