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SUSCEPTIBILITY OF RABBITS, AS HETEROLOGOUS ANIMALS, TO BOVINE LEUKEMIA VIRUS

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Summary. Given the ability of the bovine leukemia virus (BLV) to overcome the interspecies barrier under experimental conditions — leading to the development of an infectious process in pigs, monkeys, rats, capybaras, and other animal species — the study of the susceptibility of various animal species to the pathogen and the determination of their potential role in the epizootic process is relevant and requires further research. Therefore, the investigation of the possible use of laboratory animals, particularly rabbits, for studying the infectious process in leukemia is of scientific interest and may contribute fundamental knowledge about the ability of BLV to cross the species barrier. The possibility of infecting rabbits was studied by subcutaneous inoculation of stabilized blood, followed by assessment of hematological, serological, and molecular-genetic indicators in animals from both the experimental and control groups at distant time points after inoculation. Every 15 days, hematological parameters (ESR, hemoglobin level, and leukocyte differential count) were examined in both groups. Seroconversion in the infected animals was determined using the agar gel immunodiffusion test. At the same time, the presence of the virus's genetic material was detected by polymerase chain reaction (PCR) using a specific primer pair. Analysis of hematological results from the experimental and control rabbit groups at later stages after infection revealed that 60 days after inoculation, there was an increase in leukocyte count due to a rise in band neutrophils and lymphocytes. Most hematological parameters (hemoglobin, neutrophils, basophils, ESR) in the experimental group returned to baseline levels, except for lymphocyte count. Seroconversion in the experimental group animals was observed starting from day 60 post-infection, with peak levels recorded between days 105–120. The presence of the leukemia virus in the animals during this period was confirmed by molecular-genetic studies, which correlated with the hematological findings, particularly the development of lymphocytosis starting on day 60, which is characteristic of the infectious process typical of BLV infection. Thus, the study experimentally confirmed the ability of the bovine leukemia virus to cross the species barrier and induce an infectious process in heterologous animal species, namely rabbits

Keywords: infectious process, hematology, lymphocytosis, seroconversion, molecular-genetic research

Introduction. Among viral cattle diseases, leukemia is considered the most significant neoplastic disease, caused by Bovine leukemia virus (BLV). The pathogen belongs to the Retroviridae family, *Deltaretrovirus* genus, which also includes human T-cell leukemia viruses (HTLV-1, HTLV-2, HTLV-3), simian T-lymphotropic viruses (STLV), and bovine leukemia viruses (BLV) (Hossain, Tan and Satou, 2025). The disease is classified as a slow or minor infection due to its long incubation period and chronic manifestation. A recent comparative analysis of HTLV-1/BLV provirus integration sites in host genomes from primary tumors and asymptomatic infection stages was conducted using high-throughput sequencing, mapping, and RNAseq. This research demonstrated that HTLV/BLV proviruses integrate near cancer driver genes, contributing to malignancy development via polyclonal expansion of infected cells (Forlani et al., 2021).

In cattle, the disease manifests in two stages after the incubation period: the seroconversion stage and the clinical-hematological stage. The seroconversion stage has no pronounced clinical symptoms, as the virus remains in a latent state; thus, infected animals are classified as carriers. After a prolonged latent infection lasting 3 to 8 years, approximately 30% of BLV-infected cattle develop persistent lymphocytosis, and fewer than 5% eventually develop malignant B-cell lymphoma (Marawan et al., 2021; Lv et al., 2024). The clinical-hematological stage is characterized not only by significant lymphocytosis but also by immunosuppression, along with a decline in productivity and product quality. BLV infects the epithelial cells of the mammary gland in dairy cows, reducing their antimicrobial capacity. BLV-encoded microRNAs (BLV-miRNAs) can modify host genes and promote viral replication. According to recent data (Lian et al., 2023), BLV-miR-B1-5p suppresses the expression of the *mucin 1* (MUC1) gene in bovine mammary epithelial cells, significantly enhancing the adhesion of *Staphylococcus aureus* — one of the most common mastitis pathogens.

According to Buehring, Choi and Jensen (2001), immunoblotting of sera from 257 people who had contact with BLV-infected animals or consumed unsterilized/raw milk and untreated beef products containing proviral BLV DNA fragments revealed antibodies to the BLV capsid antigen in 74% of cases. Although the presence of BLV genomic fragments and the p24 protein in bovine and human mammary tissue and cell cultures is not directly linked to disease manifestation, this demonstrates the virus's tropism to these tissues. Furthermore, research indicates a significantly higher BLV detection rate in breast cancer patients compared to healthy control groups (Lv et al., 2024). The *tax* gene in BLV is responsible for oncogenic

activity, suppressing DNA excision repair mechanisms and causing oxidative cellular damage. This may be associated with various cancers, including breast and lung cancer. A literature review confirms that BLV infection is statistically associated with breast cancer (Saeedi-Moghaddam, Mohammaditabar and Mozhgani, 2024; Khatami et al., 2020). This raises significant socio-biological concerns regarding public health and safety, as milk, dairy products, and beef, are key sources of human nutrition, and consuming them raw may be a route of BLV transmission to humans.

Transmission of BLV to susceptible cattle occurs via blood, as well as through all bodily secretions and excretions containing infected lymphocytes. Two transmission pathways are distinguished: vertical (transplacental) and horizontal, including iatrogenic transmission (via human involvement). The alimentary route concerns calf infections during suckling, with an increased risk when milk is contaminated with blood lymphocytes, especially in mastitis-afflicted carrier cows. Other horizontal transmission cases involve mass veterinary procedures using inadequately disinfected tools, and surgical or obstetric interventions performed without proper aseptic precautions. Biting flies, such as the stable fly (*Stomoxys calcitrans*), pose a significant risk for horizontal spread, transferring the virus from infected to healthy animals. The critical role of biting flies in BLV epidemiology was highlighted in epidemiological studies conducted in the USA and Japan, where stringent fly control eliminated new BLV cases in beef cattle herds (Marawan et al., 2021).

Studies have shown that BLV can infect human mammary and lung cell lines, as well as HeLa cell cultures. Literature also confirms that various cell lines derived from primates (chimpanzees, rhesus macaques), dogs, pigs, sheep, goats, and bats can be infected with BLV through inoculation with cell-free viral preparations. In all cases, viral replication was observed (Graves and Ferrer, 1976; Bai et al., 2020).

Under natural conditions, BLV is transmitted among domestic cattle (*Bos taurus*), zebu (*Bos indicus*), and water buffalo (*Bos bubalis*). Besides cattle, BLV can infect sheep and goats, causing leukemia and lymphoma. Sheep experimentally infected with BLV are considered the best model for studying leukemia/lymphoma, as the disease develops in about 20 months (Forlani et al., 2021). The ability of BLV to cross species barriers has been confirmed experimentally. Successful infections have been established in pigs, monkeys, rats (Buehring, Choi and Jensen, 2001), capybaras, and rabbits (De Oliveira et al., 2016). When rabbits and rats were inoculated with material derived from FLK cells, about 30% of animals became seropositive to BLV and developed symptoms of lymphoid leukemia, including immunosuppression, increased lymphocyte and lymphoblast counts, and preneoplastic lymphoid cell clusters in the liver, lungs, kidneys, and lymph nodes. BLV genetic material was detected in sick animals by PCR, confirming the virus's role as the etiological agent of the observed lymphoid leukemia (Dimitrov et al., 2012). Thus, the investigation of the use of laboratory animals — particularly rabbits — for studying the infectious process of leukemia is of scientific interest and may provide fundamental insights into the ability of BLV to overcome interspecies barriers (Dimitrov et al., 2013).

The **research objective** is to determine the feasibility of using laboratory animals, particularly rabbits, for studying the infectious process of leukemia.

Materials and methods. The study was conducted on 10 rabbits weighing 2–2.5 kg. The animals were divided into two groups — experimental and control. The rabbits in the experimental group (n = 5) were subcutaneously inoculated with 1.0 cm^3 of EDTA-stabilized blood from cattle infected with the bovine leukemia virus (BLV). The animals in the control group received an equivalent volume of phosphate-buffered saline.

Experiments on rabbits were conducted following the recommendations of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and under Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017). Under the current procedure, the research program was reviewed and approved by the Bioethics Committee of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'.

To study the effect of inoculating biological material from a BLV-infected bovine donor on the rabbits, serum and EDTA-stabilized blood samples were collected from animals in both the experimental and control groups every 15 days. At the same time, clinical observation of the rabbits was conducted.

Hematological examinations of EDTA-stabilized blood were performed using light microscopy by counting the cellular elements of the leukocyte fraction (lymphocytes, basophils, band and segmented neutrophils, atypical and immature forms of the mentioned cells) and determining their proportions. Blood smears were stained using the Romanowsky-Giemsa method (Wittekind and Gehring, 1985). Additionally, at each stage, the erythrocyte sedimentation rate (ESR) and hemoglobin levels were measured (Dudchenko et al., 2019).

Serological testing for the presence of specific antibodies in the blood serum of experimental and control rabbits to BLV was conducted using the agar gel immunodiffusion (AGID) test, employing a 'Set of liquid stabilized components for serological diagnosis of bovine leukemia by immunodiffusion (AGID)' produced by LLC 'Scientific Research Enterprise 'Veterinary Medicine' (Kharkiv, Ukraine).

Detection of proviral BLV DNA was carried out using a pair of BLV-env 3–4 primers according to WOAH recommendations (Fechner et al., 1996), flanking a 444 bp fragment of the *env* gene of BLV. Reverse transcription and DNA synthesis were performed using

MMLV reverse transcriptase following the manufacturer's guidelines. Amplification was carried out using a Biometra thermocycler (USA). Visualization of PCR results was performed by horizontal gel electrophoresis in 1.5% agarose gel.

Results and discussion. Immediately before the inoculation of blood from BLV-infected cattle, baseline hematological parameters were determined in rabbits of both the experimental and control groups. It was established that hemoglobin levels, the number of erythrocytes and leukocytes, as well as the ratio of leukocyte fraction elements, were within normal ranges: hemoglobin — 116.0 ± 14.0 g/l, erythrocytes — 6.8 ± 0.4×10^6/ml, leukocytes — 5.8 ± 2.2×10^3/ml, erythrocyte sedimentation rate (ESR) — 2.0 ± 1.2 mm/h. It should be noted that the ESR in animals of the experimental group increased significantly by day 30 after experimental infection of the rabbits, with a trend toward decreasing values starting from day 45 after the inoculation of the biological material and continuing until the end of the experiment. From day 60 onward, the ESR returned to within the normal range. Between days 30 and 45 post-infection, concentration was increased by 20–25 units in rabbits of the experimental group compared to the control group, where no changes in hematological parameters were recorded. By day 60 of the study, the hemoglobin concentrations of the experimental and control animals were nearly equal and remained stable until the end of the observation period.

Analysis of the leukocyte formula results in both groups at later stages after infection showed that, by day 60 post-inoculation of the biological material, there was an increase in the number of leukocyte fraction cells (up to 9.1 ± 0.7×10^9/l), due to elevated numbers of band neutrophils and lymphocytes (Table 1).

Table 1 — Dynamics of leukocyte fraction cell concentration at later stages after rabbit infection

Observation period, days	Leukocyte fraction cells, ×10^9/l							
	Segmented neutrophils		Band neutrophils		Basophiles		Lymphocytes	
	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control
15	29.4 ± 2.6	25.4 ± 2.6	4.5 ± 1.2	5.6 ± 1.2	1.4 ± 0.5	1.3 ± 0.2	48.3 ± 4.0	47.6 ± 4.0
30	26.6 ± 3.3	23.4 ± 3.3	3.6 ± 1.4	3.8 ± 1.5	0.8 ± 0.4	2.2 ± 0.3	59.1 ± 3.0	52.2 ± 3.0
45	19.4 ± 2.6	27.1 ± 1.9	4.5 ± 1.1	4.1 ± 0.7	0.5 ± 0.3	3.2 ± 0.6	67.3 ± 4.0	49.4 ± 5.0
60	18.7 ± 2.2	24.6 ± 2.4	5.8 ± 1.4	4.3 ± 1.1	0.8 ± 0.4	2.6 ± 0.5	85.2 ± 5.0	51.3 ± 4.0
75	21.2 ± 3.5	26.4 ± 3.7	3.8 ± 1.5	3.2 ± 0.2	0.9 ± 0.4	1.8 ± 0.3	80.6 ± 3.0	47.6 ± 3.0
90	23.7 ± 2.3	30.3 ± 2.1	3.3 ± 1.2	4.4 ± 1.2	1.6 ± 0.2	1.6 ± 0.2	81.8 ± 4.0	49.1 ± 5.0
105	21.9 ± 3.9	32.5 ± 2.9	3.9 ± 0.7	5.5 ± 1.3	0.8 ± 0.3	1.8 ± 0.3	88.4 ± 3.0	50.6 ± 2.0
120	20.8 ± 2.7	33.4 ± 2.7	4.1 ± 1.1	5.9 ± 1.3	1.2 ± 0.4	1.2 ± 0.1	67.2 ± 5.0	49.4 ± 6.0
135	20.8 ± 2.7	32.4 ± 2.7	4.0 ± 1.1	5.7 ± 1.3	1.1 ± 0.4	1.3 ± 0.1	68.2 ± 5.0	49.4 ± 6.0

As shown in Table 1, the number of lymphocytes began to increase as early as 30 days after inoculation of the biological material, reaching a peak value of 88.4 ± 3.0×10^9/l, and gradually decreased by day 135 (the end of the observation period) to 68.2 ± 5.0×10^9/l. It should be noted that by day 30 after the experimental infection of rabbits, most hematological parameters in the blood of the experimental group (leukocytes, neutrophils, basophils, ESR) returned to their baseline levels. This indicates that the presence of BLV in the rabbits' bodies does not significantly affect their hematological parameters. However, a notable shift in the leukocyte fraction composition — toward a marked increase (up to 80–88%) in the proportion of lymphocytes — suggests the development of an immunosuppressive state in the experimental animals. Additionally, during this period, the erythrocyte sedimentation rate (ESR) increased to 3–4 mm/hour.

Serological and molecular genetic analyses revealed that the genetic material of BLV was detected in two rabbits from the experimental group as early as 15 days after the start of the experiment. Later, at 30–45 days, BLV genetic material was detected in four experimental animals, and it continued to be present throughout the 120-day observation period. On day 135, a negative result was obtained in one of the four previously infected rabbits, in which BLV genetic material had previously been identified in blood samples. The results of serological and molecular-genetic tests of biological material (serum and stabilized blood) from animals in the experimental and control groups are presented in Table 2.

Table 2 — Results of serological and molecular-genetic studies of biological material from rabbits

Group	Method	Observation period after infection, days								
		15	30	45	60	75	90	105	120	135
Experimental n = 5	AGID	0/5	0/5	0/5	1/5	2/5	3/5	3/5	4/5	3/5
	PCR	2/5	3/5	4/5	4/5	4/5	4/5	4/5	4/5	3/5
Control n = 5	AGID	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	PCR	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

As shown in Table 2, the presence of antibodies to BLV was detected in one experimental animal 60 days after the start of the study. Subsequently, antibodies to

bovine leukemia virus (BLV) were identified in two animals (on day 75), three animals (on days 90–105), and four animals (on day 120). These findings correlate with the hematological results, particularly the development of lymphocytosis beginning on day 60 of observation, which is characteristic of the typical infectious process associated with bovine leukemia.

Thus, it has been experimentally proven that the bovine leukemia virus is capable of crossing the species barrier and inducing an infectious process in heterologous animal species, specifically in rabbits. This opens the possibility of using rabbits as a model for studying the leukemia virus *in vivo*. Furthermore, considering the susceptibility of other animal species to BLV and the virus's potential to overcome interspecies barriers (even under artificial conditions), their possible role in the epizootic process of leukemia should also be considered.

Conclusions. 1. Inoculating rabbits with biological material containing the bovine leukemia virus (BLV) results in the persistence of the pathogen in 60% of experimental animals, as confirmed by molecular-genetic and serological studies.

2. BLV persistence does not cause significant hematological changes in rabbits. However, it leads to a redistribution of leukocyte subpopulations toward marked lymphocytosis. These changes correlate with serological findings, indicating the development of an infectious process and an immunosuppressive state in the rabbits.

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