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To cite this article: A A Shabeykin *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **315** 042036

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# Risk assessment for interspecies transmission of enzootic bovine leukemia

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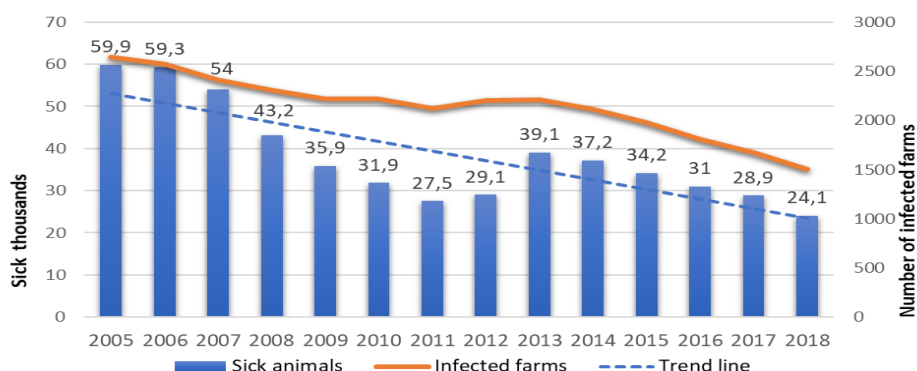
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**Abstract.** Conducted three series of infecting the rabbits with bovine leukemia virus (BLV) using milk and blood from cows having enzootic leukemia with different doses of infecting material and different mode of administration showed a high degree of repeatability of the experiment. The obtained results testify to the ability of BLV to successfully overcome the interspecific barrier when ingested by an alimentary pathway with milk, as well as by the direct injection of infected lymphocytes into the bloodstream.

## 1. Introduction

Enzootic bovine leukosis is a chronic lymphoproliferative disorder that causes a persistent increase in the number of circulating B-lymphocytes. In infected animals, B-cell lymphosarcoma evolves in 1–5% of cases [1].

In the Russian Federation, cattle leukemia is the most common infectious disease of farm animals. According to the Department of Veterinary Medicine of the Ministry of Agriculture of the Russian Federation, in 2018, cattle leukemia accounted for 67.1% of all reported cases of infectious diseases of cattle.



**Figure 1.** Incidence of bovine enzootic leukemia in Russian Federation in 2005-2018.

The graph shows that with a general trend of declining in the incidence, significant fluctuations in annual rates are possible, which is evident in the situation of 2011-2013, when the increase in the incidence over two years was 42.2% and it took five years to further level the situation. This allows



predicting the persistence of consistently high epizootic tension in the Russian Federation for at least the next decade.

Epizootic ill-being of enzootic bovine leukosis is recorded worldwide. The disease is most intensely registered in the Americas, Australia, Africa and Asia. According to the US Department of Agriculture's Animal and Plant Health Inspection Service (APHIS), 89.0% of dairy farms and 38% of US beef farms have seropositive animals. Moreover, in 74.8% of dairy farms, the estimated prevalence of bovine leukemia virus inside herds is 25% or higher.

According to the classification of the International Committee on the Taxonomy of Viruses (ICTV), the bovine leukemia virus (BLV) belongs to the Retroviridae, the genus Deltaretrovirus, which, in addition to BLV, combines Primate T-lymphotropic virus 1, Primate T-lymphotropic virus 2 and Primate T-lymphotropic virus 3.

Like all agents of retroviruses, BLV uses a reverse transcription mechanism to copy viral RNA into complementary DNA, which is then inserted into host chromosomal DNA as a provirus.

According to the mode of transmission, retroviruses are subdivided into exogenous retroviruses and endogenous retroviruses. While exogenous variants of viruses are transmitted between their hosts horizontally, endogenous viruses arise as a result of infection of primary germ cells and then transmit vertically from parents to their offspring [2].

In the course of millions of years of evolution, endogenous retroviruses of each animal species gradually accumulated in their genome. When considered the most fully studied human genome, then the proportion of endogenous retroviruses in its composition is approx. 8% [3].

All types of viruses belonging to the genus Deltaretrovirus are considered exogenous, with horizontal transmission, but recent studies have shown the presence of an endogenous deltaretrovirus in the genome of long-fingered bats, which confirms the fundamental possibility of the existence of endogenous forms [4].

The main and practically the only reservoir for the bovine leukemia virus is domestic cattle. In the wild, BVL is also detected in some members of the Bovinae subfamily [1].

The problem of cattle leukemia is directly related to the safety of human health. Despite the fact that cases of natural human infection with enzootic bovine leukosis have not been identified, the probability of recombination between it and HTLV is not excluded, which may lead to oncogenic properties of the virus that are dangerous to humans.

The virus is able to multiply in cattle cells, also in cells of a number of other mammals, including humans. According to its genetic structure, the virus contains all the genes inherent in the HTLV family, including the TAX gene, which has transactivating, transforming functions, and is an oncogene. BLV can be a real risk for the development of cancer in humans.

At the same time, one of the most important aspects of studying the biological properties of enzootic bovine leukosis is the potential ability of the virus to infect xenogenic species of animals, including humans, who eat livestock products and closely contact cattle in the course of economic activity.

For a long time it was believed that cattle leukemia is not infectious and dangerous for humans [5]. However, the majority of all human studies were based on the assumption that the lymphoid tissue is the most prevalent site of localization of the BVL virus, and only blood samples were subjected to research. Expansion of the search area to other types of human tissues led to fundamentally different results. The study of archival samples of mammary gland tissues obtained from 239 donors showed that DNA of provirus of bovine leukemia occurs in the epithelium of the mammary gland in women in 29% of cases. However, in the case of the incidence of breast cancer, the incidence of BLV DNA was significantly higher and was 59% [6].

The high incidence of BVL in human tissue samples indicates the need for further research on the mechanisms of transmission of the virus and its biology.

The most likely route of penetration of BVL into the human body is alimentary. The possibility of crossing the interspecific barrier for BVL in experiments has been proven for rabbits, sheep, and goats [1]. Therefore, the purpose of the experiment was to check the effectiveness of infection of xenogenic animals in conditions as close as possible to natural ones, including alimentary.

## 2. Materials and research methods

Three series of studies on infection the rabbits with vaccinated material were carried out. Milk and blood from cows received from enzootic bovine leukosis infected animals served as infective material. In the milk of donor cows, no lymphocyte material was detected. A total of 53 intact California rabbit breeds at the age of 3 months were infected. In each series of experiments, rabbits were divided into three experimental and one control group. Rabbits of the first group received milk from cow infected with leukemia; the second group of milk from leukemia infected cows with the addition of 5 ml of whole blood of the same cow. Rabbits were injected *per os* milk 50 ml twice a day for 30 days. Rabbits of the third experimental group were injected with 1 ml of blood from cow infected with leukemia intravenously once. The rabbits of the control group received milk from a healthy cow. In each series of the experiment, the number of lymphocytes obtained by the blood of a cow differed and varied from  $2.0 \times 10^9$  to  $6.0 \times 10^9$  ( $2.7 \times 10^9$  -  $8.1 \times 10^9$  copies of provirus), when administered *per os* and from  $1.9 \times 10^7$  to  $2.9 \times 10^7$  lymphocytes, when administered intravenously. Rabbits were kept in vivarium in individual cages on a standard feeding ration.

Control studies were subjected to blood, which was obtained from the marginal vein of the ear of rabbits during the first month of the experiment weekly, then with an interval of 1 month. Observations in the first experiment were carried out for 270 days, in the second– 300 days and in the third –380 days [7].

Serological studies were performed by ELIZA-test using diagnostic test systems in blocking variant for the detection of antibodies to glycoprotein gp51 leukemia (IDEXX. USA), as well as in the diffuse precipitation reaction, using a commercial set for the diagnosis of bovine leukemia (Kursk Biofactory – “Bioc”, Russia) [21].

For identification of provirus DNA fragments, PCR was used in various variants (including the development of VIEW) [8].

## 3. Research results

In a series of three experiments on rabbits, a process was modeled that was closest to the natural conditions, when infected with enzootic bovine leukosis cows were the source of infection. The virus can be transmitted with milk by the alimentary route; with milk and blood in the alimentary way when drinking milk from a cow suffering from mastitis; only with blood, which is possible when transmitted by iatrogenic route.

The use of different doses and ways of infection, the change of donors of infectious material, did not affect the repeatability of the experiment. By the end of each series of experiments, seroconversion and BVL DNA provirus sequences were detected in 50% of rabbits. As a result of oral administration of milk and milk with blood infected with enzootic bovine leukosis, in 25–50% of cases, rabbits showed all signs of infection. When administered intravenously to rabbits, blood obtained from a cow infected with the infected with enzootic bovine leukosis, signs of infection were observed in 100% of cases.

In assessing the emerging biological risks, two facts are most important. Firstly, the milk given to the rabbits of the first group did not contain lymph/leukocytes. Secondly, the intravenous administration of blood to the rabbits of the third group was carried out once. These facts indicate the breadth of options for effective transitions of BVL to a new host. All studied methods of virus transmission are possible only in anthropogenic conditions. Long-term milk drinking and parenteral transfer of a relatively large volume of blood are possible only with the participation of man and do not occur in the wild.

The domestication of cattle and the subsequent use of milk in human food and for feeding other animal species by evolutionary standards for the development of viruses occurred relatively recently. A high percentage of the prevalence of the BVL virus in the epithelium of the mammary gland of women [6] indicates the process of further evolution of the virus in new conditions for it. The BVL strategy, as a typical agent of retroviruses, aims to spread in the population of its host primarily without the rapid development of clinical signs of disease leading to the death of animals. This strategy is partially provided by the possibility of a temporary or permanent absence of circulation of viral particles in the host's blood. When injected into the human body, the main strategy of BVL seems to be to avoid contact

with the immune system of the new host, when the virus creates its DNA copy in the epithelial tissue, without seroconversion. Originally several thousand years ago, the trans-species BVL transition with milk was supposed to represent a dead-end “spillover effect”, but the current data on the occurrence of BVL provirus in the mammary gland epithelium in 29% of women is difficult to explain by using raw milk and meat products and can be said about the risks arising from the formation of endogenous forms in humans.

#### 4. Conclusion

In the anthropogenic environment, new vectors of contacts of infectious agents with other species of animals and humans are created, providing conditions for the emergence of emergent infectious diseases [9], including those mediated with BVL. Registered high prevalence of leukemia among cattle in the Russian Federation and the nature of the dynamics of changes in the incidence of animals in recent years enable to predict the persistence of the unfavorable epizootic situation even in the long term [10]. Given that non-pasteurized milk from small private farms goes on sale to the markets, this creates high levels of the risk of the BVL trans-feed transition in the present and in the future, including high risks of human infection with a potential cancer virus.

#### Acknowledgements

The work was carried out within the framework of the state assignment under the program of fundamental scientific research of the state academies of sciences on the subject No. 0578-2018-0003 and No. 0578-2014-0025

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