Part 1. Veterinary medicine

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STUDY OF THE SPREAD OF MINOR VIRAL CATTLE INFECTIONS (LEUKEMIA, IMMUNODEFICIENCY, AND SPUMAVIRUS INFECTION) USING POLYMERASE CHAIN REACTION

Biloivan O. V., Didyk T. B., Yurko P. S., Korneikova O. B., Paliy A. P., Gorbatenko S. K., Bryl N. F.

National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: silverscreen91@gmail.com

Summary. The aim of the study was to investigate the prevalence of minor cattle infections (leukemia, bovine immunodeficiency and spumavirus infection) using the polymerase chain reaction (PCR). Blood samples were collected from cows in conditionally leukemia-free farms in ten regions of Ukraine to determine the presence of these infections. The samples were examined via classical PCR to detect the genetic material of the specific fragment of the *ENV* gene of the leukemia virus using *BLV-env-3/BLV-env-4* primers recommended by the OIE. To identify the proviral DNA of bovine foamy virus (BFV), primers *Int 1-Int 2* and *Int 3-Int 4* were used, and for the detection of bovine immunodeficiency virus (BIV) proviral DNA, a pair of primers RT_+(–) flanking the conservative domain of reverse transcriptase and a pair of primers flanking the *pol* gene of the BIV were selected. The situation concerning leukemia is most severe in Sumy and Kharkiv regions. A significant percentage of animals carrying the foamy virus was observed in farms in Kirovohrad, Kherson, Donetsk, and Kharkiv regions. Moreover, genetic material of the immunodeficiency virus was found in samples from Kirovohrad, Donetsk, and Kherson regions. These results indicate a significant prevalence of minor infections among cattle in Ukraine due to a lack of awareness among livestock workers, highlighting the necessity for comprehensive sanitary and preventive measures

Keywords: foamy virus, DNA, Ukraine

Introduction. Among the infectious diseases of cattle, particular attention is given to the viruses known as slow or minor infections. These include leukemia caused by the bovine leukemia virus (BLV), spumavirus infection caused by the bovine foamy virus (BFV), and bovine immunodeficiency caused by the bovine immunodeficiency virus (BIV). All these agents belong to the retrovirus family and share antigenic relatedness. One of the main features of these diseases is the lack of distinct clinical signs, a latent course and a prolonged incubation period. They are not notably lethal; however, especially when they have an associated course, they can cause significant losses in cattle farming. They exhibit immunosuppressive effects in the infected animals, significantly reducing the effectiveness of preventive and curative measures, as well as the productivity levels and quality of animal products. Furthermore, these infections lead to a loss of genetic diversity within the livestock (Constable et al., 2017; Scobie et al., 2001; Straub and Levy, 1999; Meas et al., 2002).

Leukemia in cattle is a well-studied disease concerning the specifics of infectious and epizootic processes, as well as diagnostic directions and eradication methods based on legislative acts adopted in countries with developed livestock industries. Examples include European countries where, through legislative programs, the disease has been eradicated, except for isolated cases reported in certain countries where the disease occurs sporadically without clinical manifestation. This applies to livestock farms in Ukraine as well.

Regarding the other minor viral infections mentioned earlier, it is worth noting the lack of information available in both OIE materials and scientific literature. It is known that spumavirus infection and bovine immunodeficiency have a wide distribution in livestock in various countries around the world. According to some authors, bovine immunodeficiency in cattle has been reported in Japan, France, Canada, USA, Iran, Argentina, Germany, the Netherlands, Italy, Brazil, and other countries. The infection rate ranges from 3% to 50% or even higher. In some livestock farms in developed countries, 35% to 45% of cattle are seropositive for the spumavirus infection, and the difficulties caused by this pathogen are widespread worldwide (Meas et al., 2002; Romen et al., 2007; Murray et al., 2006; Orr, O'Reilly and Scholl, 2003; Krasnikova and Larionova, 2014).

It is worth noting the complete lack of information regarding the presence and spread of the spumavirus infection and bovine immunodeficiency in domestic animal husbandry. In recent years, monitoring studies on the prevalence of these minor infections in livestock farms in the central and eastern regions of Ukraine have

been conducted by scientists from the Laboratory for the Study of Leucosis and the Laboratory of Molecular Diagnostics at the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (NSC 'IECVM') exclusively using molecular-genetic methods due to the absence of domestic serological diagnostic tools (Gorbatenko et al., 2023a, 2023b). Even with limited sample sizes, the presence of genetic material of leukemia, spumavirus and immunodeficiency viruses among the livestock of several farms has been identified.

The **purpose of this report** is to emphasize the spread of these diseases not only in livestock worldwide but also within the livestock farms of Ukraine. This accentuates the necessity to deepen research aimed at studying the properties of immunodeficiency and foamy viruses. It is also important to develop technology for accumulating their viral mass and to design and implement domestic serological diagnostic tools for these less-studied minor viral diseases in cattle. This approach will help establish the real epizootic situation, introduce large-scale anti-epizootic measures, ultimately leading to the improvement of animal health and the quality of livestock products.

Materials and methods. The blood samples were collected from apparently healthy cows in various farms from different regions like Kirovohrad (n = 10), Poltava (n = 15), Sumy (n = 25), Cherkasy (n = 15), Kharkiv (n = 40), Chernihiv (n = 10), Donetsk (n = 10), Kherson (n = 10), Mykolaiv (n = 10), and Zaporizhzhia (n = 10) regions (Table 1). Blood samples were collected with a 3% EDTA solution and sent for analysis on the same day as collection. The total DNA extraction was performed using spin columns and reagents from the IndiSpin QIAcube HT Pathogen Kit (Germany). The samples were analyzed using classical PCR with DreamTaq Green PCR Master Mix (ThermoFisher, USA) to detect the genetic material of the specific *ENV* gene fragment of the leukemia virus, utilizing the *BLV-env-3/BLV-env-4* primers recommended by the OIE (2018) and described by Fechner et al. (1996). The amplification was carried out following this thermal profile: 2 min of denaturation at 94 °C; 30 cycles of 30 s at 95 °C, 30 s at 58 °C, and 60 s at 72 °C; followed by a final elongation step of 4 min at 72 °C. Samples showing a band corresponding to the 444 bp amplification product were considered positive. For the positive control sample, DNA isolated from the virus-containing fluid of the FLK-BLV culture was used.

The detection of proviral DNA of bovine foamy virus (BFV) was conducted using two pairs of primers: *Int 1-Int 2* (outer pair, amplification product length 430 bp) and *Int 3-Int 4* (inner pair, amplicon length 221 bp). A 'nested' version of the PCR was selected for the detection of proviral DNA of BFV following the recommendations of the authors (Materniak-Kornas et al., 2013).

For the detection of bovine immunodeficiency virus (BIV) proviral DNA, a pair of primers was used: RT_+(–), which flank a conservative domain of the reverse transcriptase (PCR amplicon length 495 bp), and another pair, BIV_Pol_+(–), which flank the *pol* gene of BIV (PCR amplicon length 235 bp). The amplification was performed using standard PCR protocols following the recommendations of the authors (Moody et al., 2002).

Results. Based on the results presented in Table 1, it can be seen that the situation concerning bovine leukemia is most critical in Sumy (33.3% positive from the total number of samples) and Kharkiv (32.5%) regions. Regarding other minor viral infections in cattle, a significant percentage of animals were identified as carriers of the foamy virus in several regions, which is concerning. For instance, the highest percentages of positively reacting animals were found in Kirovohrad (70%), Kherson (60%), Donetsk (50%), and Kharkiv (30%) regions, based on the obtained results. However, the situation concerning the circulation of the immunodeficiency virus among livestock appears less complex. Nonetheless, 40% of the examined samples from Kirovohrad, Donetsk, and Kherson regions exhibited genetic material of the mentioned pathogen.

Table 1 — The results of the molecular-genetic examination of blood samples from cattle in conditionally healthy herds regarding bovine leukemia virus (BLV) in various regions of Ukraine aimed to detect the presence of minor viral infections genetic material

Region	Farm	Number (n)	Presence of genetic material		
			BLV	BFV	BIV
Kirovohrad	1	10	1	7	4
Poltava	2	15	4	2	–
Sumy	3	15	5	10	1
Cherkasy	4	15	1	2	1
Kharkiv	5	15	1	2	–
	6	15	3	2	–
	7	10	9	8	–
Chernihiv	8	10	2	2	1
Donetsk	9	10	2	5	4
Kherson	10	10	2	6	4
Mykolaiv	11	10	1	1	–
Zaporizhzhia	12	10	2	2	–

The obtained results were interpreted based on the presence of characteristic bands of specific lengths observed after horizontal gel electrophoresis of the amplification products, specifically: 444 bp for the proviral DNA of the leukemia virus, 221 bp for the genetic material of the foamy virus, and 235 bp for the immunodeficiency virus (Figs 1, 2).

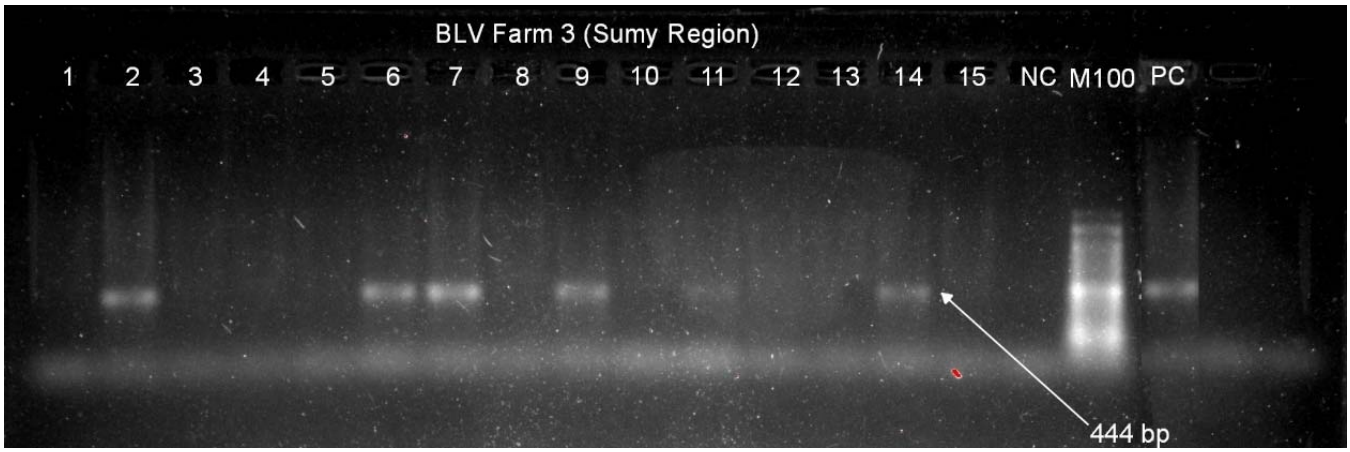

Figure 1. Gel-electrophoresis results of the amplification products for the presence of proviral DNA of BLV using samples obtained from one of the farms in Sumy Region are depicted. In the illustration, PC stands for the positive control sample, while NC represents the negative control sample.

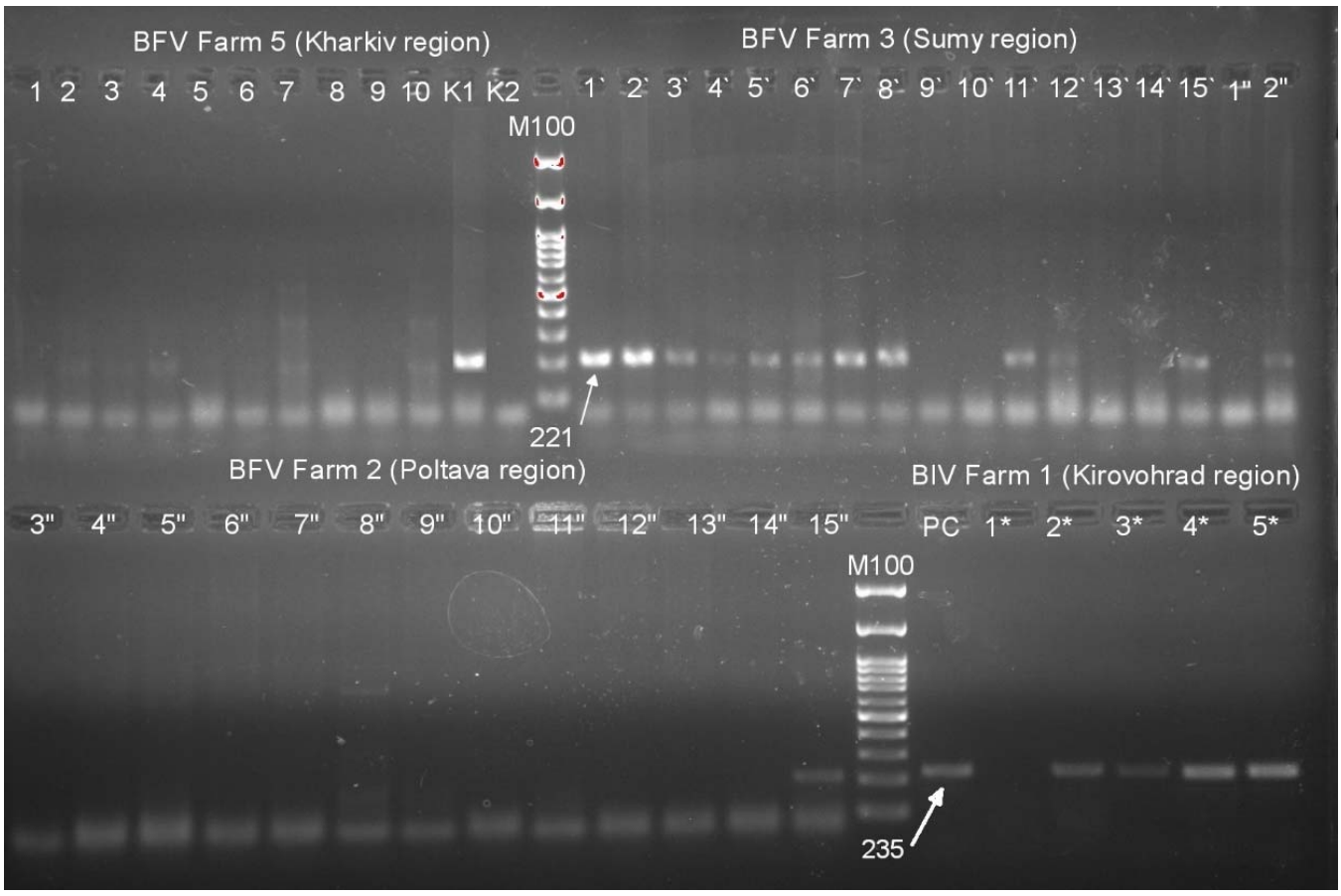

Figure 2. Gel-electrophoresis results of amplification products for the presence of proviral DNA of BFV and BIV using samples from specific farms in Kharkiv, Sumy, Poltava, and Kirovohrad regions. K1 represents the positive control sample for BFV DNA, PC — the positive control sample for BIV DNA, and K2 — the negative control sample.

Conclusions. The obtained results indicate a significant prevalence of agents causing minor infections among livestock in Ukraine, attributed to the lack of awareness among animal husbandry personnel. This underscores the necessity for comprehensive health-boosting measures against epizootic diseases. Given the above, it is essential to emphasize the need for an in-depth study of agents causing foamy virus infections and immunodeficiency, along with the development of methods for accumulating their viral mass.

It is worth noting that although molecular-genetic methods enable relatively rapid detection of pathogens in specific farms, they only allow for a limited sample of animals to be studied. To conduct comprehensive

screening of the entire livestock and track immunity dynamics and stages, the application of classical serological diagnostic methods remains pertinent. Therefore, the development and implementation of domestic serological diagnostic tools for lesser-known minor viral infections in cattle to assess the actual epizootic status in Ukrainian animal husbandry and the implementation of large-scale anti-epizootic measures remain essential tasks in domestic veterinary science.

References

Constable, P. D., Hinchcliff, K. W., Done, S. H. and Grünberg, W (2017) 'Enzootic bovine leukosis (Bovine lymphosarcoma)', in *Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats*. 11th ed. Elsevier, pp. 785–794. doi: 10.1016/B978-0-7020-5246-0.00011-5.

Fechner, H., Kurg, A., Geue, L., Blankenstein, P., Mewes, G., Ebner, D. and Beier, D. (1996) 'Evaluation of polymerase chain reaction (PCR) application in diagnosis of bovine leukaemia virus (BLV) infection in naturally infected cattle', *Journal of Veterinary Medicine, Series B*, 43(1–10), pp. 621–630. doi: 10.1111/j.1439-0450.1996.tb00361.x.

Gorbatenko, S. K., Kornieikova, O. B., Rudova, N. H., Dunaiev, Yu. K., Stegniy, B. T. and Kornieikov, O. M. (2023a) 'Insufficiently studied minor viral infections in livestock of Ukraine', *Journal for Veterinary Medicine, Biotechnology and Biosafety*, 9(1–2), pp. 8–11. doi: 10.36016/JVMBBS-2023-9-1-2-2.

Gorbatenko, S. K., Rudova, N. G., Kornieikova, O. B., Stegniy, M. Yu., Kovalenko, L. V., Kuznetsova, O. V. and Miahkykh, N. V. (2023b) 'Study the distribution and biological characteristics of field isolates of Bovine immunodeficiency and Spumavirus infection pathogens' [Vyvchennia rozpovsiudzhennia ta biolohichnykh vlastyvostei polovykh izoliativ zbudnykiv imunodefitsytu i spumavirusnoi infektsii velykoi rohatoi khudoby], *Veterinary Medicine [Veterynarna medytsyna]*, 109, pp. 61–66. doi: 10.36016/VM-2023-109-11. [in Ukrainian].

Krasnikova, E. S. and Larionova, O. S. (2014) 'Biosafety of the animal origin products from cattle infected with bovine leukemia virus or bovine immunodeficiency virus' [K voprosu o biologicheskoy bezopasnosti produktsii, poluchennoy ot zhivotnykh, infitsirovannykh virusami enzooticheskogo leykoza i immunodefitsita KRS], *Vestnik veterinarii*, 2, pp, 85–88. [in Russian].

Materniak-Kornas, M., Osiński, Z., Rudzki, M. and Kuźmak, J. (2017) 'Development of a recombinant protein-based ELISA for detection of antibodies against bovine foamy virus', *Journal of Veterinary Research*, 61(3), pp. 247–252. doi: 10.1515/jvetres-2017-0034.

Meas, S., Usui, T., Ohashi, K., Sugimoto, C. and Onuma, M. (2002) 'Vertical transmission of bovine leukemia virus and bovine immunodeficiency virus in dairy cattle herds', *Veterinary Microbiology*, 84(3), pp. 275–282. doi: 10.1016/S0378-1135(01)00458-8.

Moody, C. A., Pharr, G. T., Murphey, J., Hughlett, M. B., Weaver, C. C., Nelson, P. D. and Coats, K. S. (2002) 'Confirmation of vertical transmission of bovine immunodeficiency virus in naturally infected dairy cattle using the polymerase chain reaction', *Journal of Veterinary Diagnostic Investigation*, 14(2), pp. 113–119. doi: 10.1177/104063870201400204.

Murray, S. M., Picker, L. J., Axthelm, M. K. and Linial, M. L. (2006) 'Expanded tissue targets for foamy virus replication with simian immunodeficiency virus-induced immunosuppression', *Journal of Virology*, 80(2), pp. 663–670. doi: 10.1128/JVI.80.2.663-670.2006.

OIE (World Organisation for Animal Health). (2018) 'Chapter 3.4.9. Enzootic Bovine Leukosis', in *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. 12th ed. [version adopted in 2018]. Paris: WOAH. Available at: https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.04.09_EBL.pdf.

Orr, K. A., O'Reilly, K. L. and Scholl, D. T. (2003) 'Estimation of sensitivity and specificity of two diagnostics tests for bovine immunodeficiency virus using Bayesian techniques', *Preventive Veterinary Medicine*, 61(2), pp. 79–89. doi: 10.1016/j.prevetmed.2003.08.001.

Romen, F., Backes, P., Materniak, M., Sting, R., Vahlenkamp, T. W., Riebe, R., Pawlita, M., Kuzmak, J. and Löchelt, M. (2007) 'Serological detection systems for identification of cows shedding bovine foamy virus via milk', *Virology*, 364(1), pp. 123–131. doi: 10.1016/j.virol.2007.03.009.

Scobie, L., Jarrett, O., Venables, C., Sayers, A. R. and Weightman, S. (2001) 'Prevalence of bovine immunodeficiency virus infection in cattle in Great Britain', *Veterinary Record*, 149(15), pp. 459–460. doi: 10.1136/vr.149.15.459.

Straub, O. and Lévy, D. (1999) 'Bovine immunodeficiency virus and analogies with human immunodeficiency virus', *Leukemia*, 13(S1), pp. S106–S109. doi: 10.1038/sj.leu.2401324.