Part 2. Biotechnology

UDC 602.3:619:616.98:578.82/83.085.23:636.22/.28 **DOI 10.36016/JVMBBS-2020-6-2-3**

STUDY OF BIOLOGICAL PROPERTIES OF FIELD ISOLATES OF CATTLE MINOR INFECTIONS AGENTS ON HOMOLOGICAL CELL CULTURES

Gorbatenko S. K. [1], Solodiankin O. S. [1], Lymanska O. Yu. [1], Rudova N. G. [1], Kulyk I. O. [1], Kornieikov O. M. [1], Stegniy M. Yu. [1], Kuznetsova O. V. [1], Dunaeva O. V. [2], Gorbatenko V. P. [3]

[1] National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: st.gorbatenko@gmail.com
[2] Kharkiv National Medical University, Kharkiv, Ukraine
[3] Kharkiv State Zooveterinary Academy, Kharkiv, Ukraine

Summary. Biological properties of field isolates of bovine immunodeficiency virus and bovine foamy virus on homological cell cultures (fetal bovine lung and bovine coronary artery endothelial cells) were investigated. Pathogens of bovine slow infections, namely bovine immunodeficiency virus and bovine foamy virus, are able to integrate into cell cultures of homologous to cattle type, which is confirmed by the results of PCR. There has been determined the presence of genetic material of pathogens of bovine immunodeficiency (BIV) and spumavirus infection (BFV) in the cultivation of lymphocytes of field isolates in the culture of bovine coronary artery endothelial cells (BCAEC) at the level of 5th passage, and in the cell culture of fetal bovine lung (FBL) — at the level of 10th passage. In the process of integration of pathogens of immunodeficiency and spumavirus infection of cattle in continuous cell cultures FBL and BCAEC, morphological changes in the state of the monolayer by the principle of syncytiation and vacuolation are observed

Keywords: bovine immunodeficiency virus, bovine foamy virus, cell cultures, PCR, cattle

Introduction. The profitability of animal husbandry in countries with developed livestock industries is hampered by many chronic viral infectious diseases, the causative agents of which, due to immunosuppressive effects, reduce the effectiveness of specific prevention, productivity, and quality of livestock products, general resistance of livestock. These include leukemia, immunodeficiency, spumavirus infection of cattle. The causative agents of the above diseases, namely bovine leukemia virus (BLV), bovine immunodeficiency virus (BIV), and bovine foamy virus (BFV) are genetically and antigenically related retroviruses, which, affecting cattle, cause a slow course of the disease (Mousavi et al., 2014).

In Ukraine, the infectious process, the dynamics of the epizootic process in bovine leukemia, the peculiarities of the disease in livestock farms, the issue of eradication of the disease at the legislative level have been studied. However, the question of the spread and mechanism of the infectious process in slow infections of cattle caused by BIV and BFV, in Ukraine has not been studied (Bhatia, Patil and Sood, 2013). Minor infections, in particular immunodeficiency and spumavirus infection, are widespread in animal husbandry worldwide (Kolotvin, 2007; Materniak-Kornas et al., 2017; Mousavi et al., 2014; Rodrigues et al., 2019; Santos et al., 2019; Meas et al., 2003).

It is known that the causative agents of the above slow infections may not manifest themselves in the infected organism for many years, causing, in most cases, associated, two- or three-variant course of the disease and cause significant damage to livestock both in terms of animal resistance and product quantity and quality (Krasnikova, 2011; Supotnitskiy, 2009; Fedorov and Verkhovsky, 1996; Bhatia, Patil and Sood, 2013; Suarez et al., 1993).

Diseases caused by them are accompanied by non-plastic pathologies and are widespread among vertebrates. Losses from each of the diseases, and especially in the associated course, are due to the death and culling of animals, insufficient quantity and reduced quality of livestock products, loss of gene pool, the cost of anti-epizootic measures (Bhatia, Patil and Sood, 2013; Romen et al., 2007; Pinto-Santini, Stenbak and Linial, 2017).

In connection with the above program of scientific research provides for monitoring analysis of the epizootic state of animal husbandry in Ukraine for slow (minor) infectious diseases, primarily immunodeficiency and spumavirus infection, study the biological properties of pathogens in case of detection so that after the accumulation of biological mass BIV and BFV to design domestic means of retrospective diagnostics of the latter.

The aim of the study was to investigate of biological properties of field isolates of bovine immunodeficiency virus and bovine foamy virus on homological cell cultures (fetal bovine lung and bovine coronary artery endothelial cells).

Materials and methods. Donor animals infected with BIV and BFV were selected. Molecular genetic studies have confirmed the presence of BIV and BFV in lymphocytes, so we used a suspension of leukocyte fraction of animal blood (Hachiya et al., 2018; Materniak et al., 2013). Donor blood, collected in sterile vials with anticoagulant in the amount of 50 cm^3, was processed to isolate lymphocytes. To do this, the blood sample was diluted two times with buffered saline with the addition of penicillin 100 IU/cm^3, pH 7.2, then 8 cm^3 each was layered on a solution of triombrast (sodium amidotrizoate) with a density of 1.075, pre-poured into centrifuge tubes of 2 cm^3.

After centrifugation at 1,000 rpm for 40 min we took a layer of lymphocytes in a separate tube, washed once with buffered saline at 1,000 rpm for 10 min and one time with Eagle's medium by the above mode. The volume of the lymphocyte suspension was adjusted to 10 cm^3 with Eagle's medium to count the number of living cells with the addition of 0.1% trypan blue solution.

The concentration of live lymphocytes was adjusted to 1–3×10^6 cells/cm^3 in culture medium, which consists of 90% Eagle's medium, 10% native serum and 100 IU/cm^3 of penicillin.

To stimulate the production of the virus, short-term cultivation of lymphocytes that were isolated from the donor's blood was previously performed. For this purpose, the lymphocyte suspension was incubated for 48 h at a temperature of 37 ± 0.5°C, after that the number of living cells in the suspension of short-term cultured lymphocytes was determined.

The resulting suspension was tested for sterility by seeding on bacteriological growth media (nutrient agar and nutrient broth, enriched with glucose; Sabouraud and thioglycollate media) to exclude the presence of bacterial and fungal contamination.

Checking for the presence of BIV and BFV in lymphocytes after cultivation and the degree of their accumulation was assessed by polymerase chain reaction (PCR).

Two types of continuous monolayer cell cultures were used for infection, namely fetal bovine lung (FBL) and bovine coronary artery endothelial cells (BCAEC). Cell cultures for infection were taken at the level of 24–48 h of growth with 50–75% fulfillment of the monolayer. Reseeding of continuous cell cultures was performed in a nutrient medium consisting of 45% Eagle's medium, 45% of medium 199, 10% of bovine native serum, penicillin 100 IU/cm^3 and gentamicin 40 mg/cm^3.

A monolayer of FBL and BCAEC cell cultures with lymphocytes was incubated for 72 h at a conventional temperature 37 ± 0.5°C, after which it was washed twice with Hanks' solution, followed by re-replacement of the growth medium. Reseeding of cultures was carried out by the completion of the monolayer, on average every 3–4 days with a growth medium, the composition of which is indicated above.

Containers with uninfected monolayer of FBL and BCAEC cell cultures were used as controls. Each passage was monitored daily visually and using light microscopy. At the level of each third and fifth passages, samples were subjected to molecular genetic testing (PCR) to detect the genetic material of pathogens.

Primer systems Int 1-Int 2 (outer pair, length of amplified product is 430 bp) and Int 3-Int 4 (inner pair, length of amplified product 221 bp) were used to detect BFV proviral DNA by 'nested' version of PCR according to the recommendations of the developers (Materniak et al., 2013).

To detect BIV proviral DNA, a pair of primers RT_+(–) flanking the conserved reverse transcriptase domain (PCR product length 495 bp) and a pair of primers BIV_Pol_+(–) flanking the *pol* gene of bovine immunodeficiency virus (PCR product length is 235 bp) were used. Amplification was performed by standard PCR according to the recommendations of the developers (Moody et al., 2002).

Results and discussion. Microscopic studies of the continuous cultures after infection showed that the addition of short-term cultured lymphocytes did not cause destructive changes in the morphology of cells of both lines.

The cells of the monolayer were located densely, with clearly defined borders, a small number of vacuoles was observed in the cytoplasm, the nuclei had a typical oval shape.

After 1st passage, lymphocytes were still partially found, but after 2nd passages, lymphocytes were not detected during microscopy.

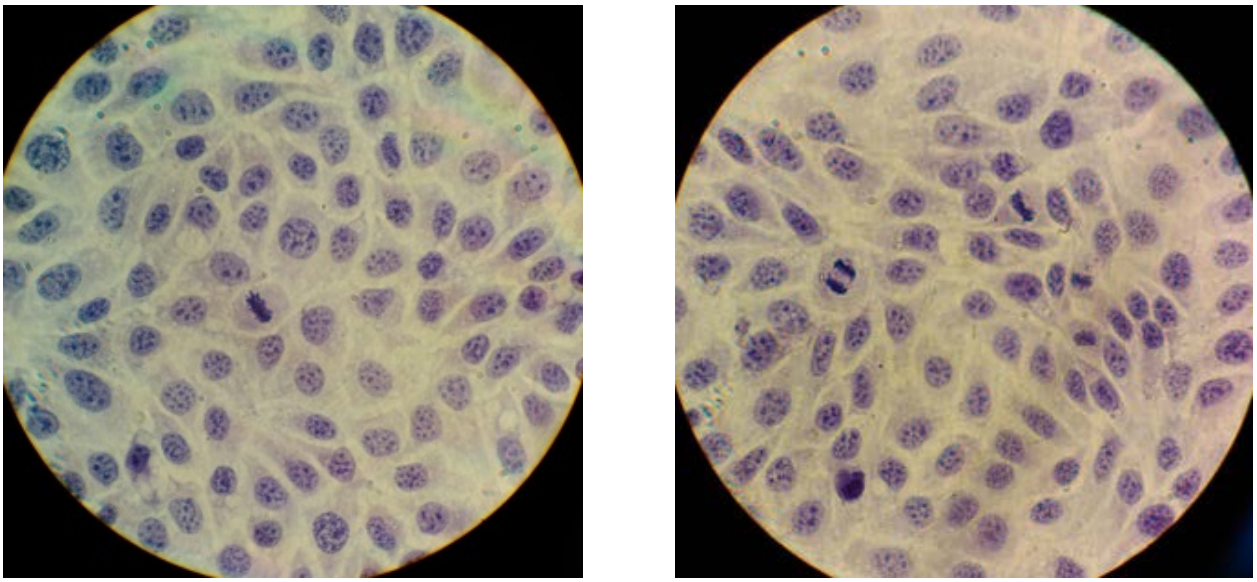
Observations of the state of the monolayer of cell cultures (FBL+BIV) and (FBL+BFV), at the level of 1st, 2nd, and 3rd passages established satisfactory filling of the monolayer, morphologically the cells of the experimental culture were similar to control, PCR at the level of 3rd passages showed the presence of BIV and BFV genetic material in monolayer cells.

At 4th–6th passages in the experimental cell culture morphological destruction of cells with signs of symplast formation was observed — in the culture there were enlarged cells with two or three nuclei, monolayer cells were removed from the glass with difficulty using trypsin-EDTA solution.

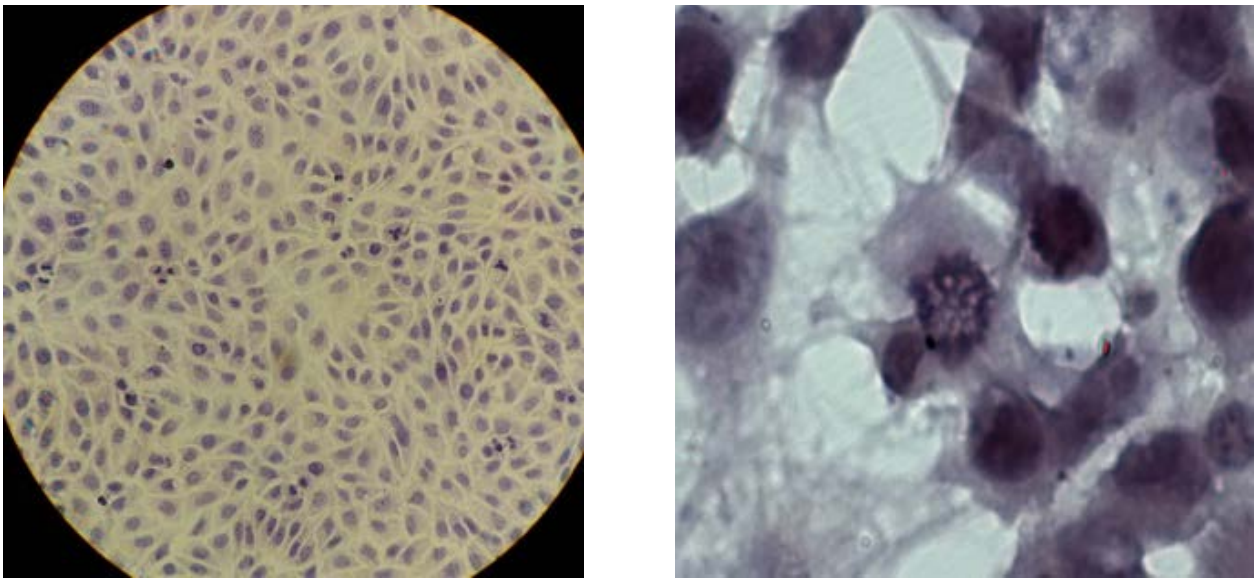
At the level of 7th–8th passages, the picture of the state of the monolayer remained similar, with a sharp increase in the number of dead cells in the culture fluid.

For a detailed study of the morphology of infected FBL cells, the cell suspension was seeded in test tubes with cover slides according to the conventional method, after the formation of a monolayer, culture cells were stained by Giemsa, and more intense staining was observed in the perinuclear zone.

Fig. 1 shows the changes in monolayer cells observed during the implementation of the research program.



Normal cell monolayer



Tripolar mitosis | Hollow metaphase

Figure 1. Changes in monolayer cells.

A total of 15 passages of culture (FBL+BIV) and (FBL+BFV) each were performed. By PCR genetic material of pathogens of immunodeficiency and spumavirus infection of cattle was still recorded at the level of 10th passage. Molecular-genetic study of cell culture in PCR at the level of 13th and 15th passages showed a negative result.

The gel picture (Fig. 2) shows the results of detection of genetic material of pathogens of immunodeficiency and spumavirus infection of cattle in the next passages of the culture of FBL cells.

A study on the possibility of integrating the field forms of pathogens of slow infections (BIV and BFV) in the continuous cell culture of BCAEC revealed a lower susceptibility of this culture cells to viruses of the Retroviridae family.

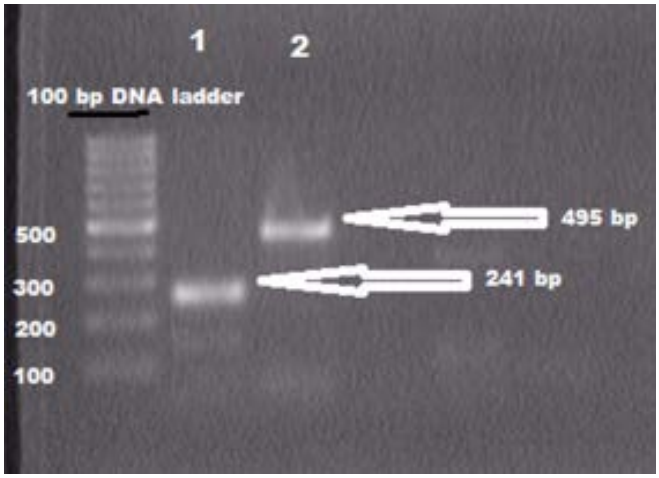


Figure 2. Gel picture of positive blood samples for BFV and BIV (100 bp DNA ladder — molecular weight marker, 1 — BFV positive sample, 2 — BIV positive sample).

According to the results of PCR at the level of 3rd passage, BIV and BFV genetic material in the cells of the monolayer of BCAEC culture was noted and already at this level of cultivation numerous vacuolation in infected monolayer cells (BCAEC+BFV) and destructive changes in the monolayer, which were expressed in its partial destruction with the formation of a numerical number of dead cells, were observed. At the level of 4th and 5th passages vacuolation and syncytial formation were observed in most (70–80%) of monolayer cells. At the level of 5th passage, PCR results showed the presence of genetic material of pathogens of retroviral infections, and material of 7th passage gave a negative result.

Conclusions. 1. Pathogens of bovine slow infections, namely bovine immunodeficiency virus and bovine foamy virus, are able to integrate into cell cultures of homologous to cattle type, which is confirmed by the results of PCR.

2. There has been determined the presence of genetic material of pathogens of bovine immunodeficiency (BIV) and spumavirus infection (BFV) in the cultivation of lymphocytes of field isolates in the culture of bovine coronary artery endothelial cells (BCAEC) at the level of 5th passage, and in the cell culture of fetal bovine lung (FBL) — at the level of 10th passage.

3. In the process of integration of pathogens of immunodeficiency and spumavirus infection of cattle in continuous cell cultures FBL and BCAEC, morphological changes in the state of the monolayer by the principle of syncytiation and vacuolation are observed.

References

Bhatia, S., Patil, S. S. and Sood, R. (2013) 'Bovine immunodeficiency virus: A lentiviral infection', *Indian Journal of Virology*, 24(3), pp. 332–341. doi: 10.1007/s13337-013-0165-9.

Fedorov, Yu. N. and Verkhovsky, O. A. (1996) 'Bovine immunodeficiency virus' [Virus immunodefitsita krupnogo rogatogo skota], *Veterinary* [*Veterinariya*], 10, pp. 17–19. [in Russian].

Hachiya, Y., Kimura, K., Oguma, K., Ono, M., Horikita, T. and Sentsui, H. (2018) 'Isolation of bovine foamy virus in Japan', *Journal of Veterinary Medical Science*, 80(10), pp. 1604–1609. doi: 10.1292/jvms.18-0121.

Kolotvin, V. V. (2007) *Bovine Immunodeficiency Virus: Indication of Infection and Prevalence in Farms of the Russian Federation [Virus immunodefitsita krupnogo rogatogo skota: indikatsiya infektsii i rasprostranennost' v khozyaystvakh Rossiyskoy Federatsii]*. The dissertation thesis for the scientific degree of the candidate of biological sciences. Moscow: All-Russian Scientific Research Institute of Experimental Veterinary Medicine named after Ya. R. Kovalenko. Available at: https://search.rsl.ru/ru/record/01003053744. [in Russian].

Krasnikova, E. S. (2011) 'Epizootic situation on Viral immunodeficiency of cattle in Saratov and the Saratov Region' [Epizooticheskaya situatsiya po virusnomu immunodefitsitu krupnogo rogatogo skota v gorode Saratove i Saratovskoy oblasti], *Vestnik Veterinarii*, 4, pp. 70–71. Available at: https://www.elibrary.ru/item.asp?id=17069902. [in Russian].

Materniak, M., Hechler, T., Lochelt, M. and Kuzmak, J. (2013) 'Similar patterns of infection with bovine foamy virus in experimentally inoculated calves and sheep', *Journal of Virology*, 87(6), pp. 3516–3525. doi: 10.1128/JVI.02447-12.

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., Yilmaz, Z., Usui, T., Torun, S., Yesilbag, K., Ohashi, K. and Onuma, M. (2003) 'Evidence of bovine immunodeficiency virus in cattle in Turkey', *The Japanese Journal of Veterinary Research*, 51(1), pp. 3–8. doi: 10.14943/jjvr.51.1.3.

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.

Mousavi, S., Haghparast, A., Mohammadi, G. and Tabatabaeizadeh, S.-E. (2014) 'Prevalence of bovine leukemia virus (BLV) infection in the northeast of Iran', *Veterinary Research Forum*, 5(2), pp. 135–139. PMID: 25568707.

Pinto-Santini, D. M., Stenbak, C. R. and Linial, M. L. (2017) 'Foamy virus zoonotic infections', *Retrovirology*, 14(1), p. 55. doi: 10.1186/s12977-017-0379-9.

Rodrigues, A. P. S., Fonseca Júnior, A. A., Lima, G. K., Bicalho, J. M., Leite, R. C. and Reis, J. K. P. (2019) 'Molecular detection of bovine immunodeficiency virus (BIV) in bovines from the state of Minas Gerais, Brazil', *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 71(2), pp. 711–714. doi: 10.1590/1678-4162-10495.

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.

Santos, A. F., Cavalcante, L. T. F., Muniz, C. P., Switzer, W. M. and Soares, M. A. (2019) 'Simian foamy viruses in Central and South America: A new world of discovery', *Viruses*, 11(10), p. 967. doi: 10.3390/v11100967.

Suarez, D. L., VanDerMaaten, M. J., Wood, C. and Whetstone, C. A. (1993) 'Isolation and characterization of new wild-type isolates of bovine lentivirus', *Journal of Virology*, 67(8), pp. 5051–5055. doi: 10.1128/JVI.67.8.5051-5055.1993.

Supotnitskiy, M. V. (2009) *Evolutionary Pathology. On the Question of the Place of HIV Infection and HIV/AIDS Pandemic Among Other Infectious, Epidemic and Pandemic Processes [Evolyutsionnaya patologiya. K voprosu o meste VICh-infektsii i VICh/SPID-pandemii sredi drugikh infektsionnykh, epidemicheskikh i pandemicheskikh protsessov]*. Moscow: Vuzovskaya kniga. ISBN 9785950203787. Available at: http://supotnitskiy.ru/book/supotnitskiy_evolucionnaya_patologia.pdf. [in Russian].