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PCR-SCREENING OF CLINICAL BLOOD SAMPLES THE PRESENCE OF BLV-, BIV-, BFV-INFECTED ANIMALS IN THE FARMS OF THE EASTERN REGION OF UKRAINE

O. Yu. Lymanska, I. O. Kulyk, O. B. Zlenko, N. H. Rudova, S. K. Horbatenko, A. P. Gerilovych

National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine", Molecular Diagnostics Laboratory, Pushkinska Str., 83, Kharkiv, 61023,

e-mail: antger2011@gmail.com

National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine", Kharkiv, Ukraine

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Retrovirus infections of cattle, including leukemia, are widespread in the whole world. In Ukraine, leukemia has been investigated for over 40 years, whereas minor retroviruses of cattle have not been studied yet. **Aim.** PCR-screening of samples of bovine peripheral blood, subject to diagnostic studies of leukemia, from the farms in the Eastern Ukraine (Kharkiv and Sumy regions). **Methods.** The genetic material of agents of retroviral infections in clinical samples was detected via conventional PCR and real-time PCR. **Results of Investigations.** The presence of BLV-, BIV-, BFV-infected animals among livestock was established. The number of animals, infected with leukemia, was 57–100 % from the total number of examined livestock on average. The presence of genetic material of BIV virus was either not detected at all, or detected in the amount of 62 % from the number of tested animals. At the same time, the presence of genetic material of BFV was in the range of 61.5–81 % from the total number of studied samples. A possible connection between the viruses of leukemia, immunodeficiency and foamy virus of cattle, circulating among the livestock in the farms of the Eastern region of Ukraine, was also established. **Conclusions**. The presence of genetic material of immunodeficiency virus in the leukemia-positive samples was insignificant – only 4 samples, whereas the presence of the foamy virus in the leukemia-positive samples was detected in 51 samples, which was 3 % and 42 % from the total number of investigated samples respectively. The absence of leukemia virus in the blood samples, containing the DNAs of two other minor retroviruses, was detected in one sample (0.8 %). The simultaneous presence of all three agents of retroviral infections was established in 36 samples (30 %).

Keywords: cattle, minor retroviruses, molecular diagnostics, polymerase chain reaction.

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At present, an urgent medical and veterinary problem worldwide is the control of incidence of retroviral infections among people and animals [1]. Retroviruses, endangering the epizootic safety of animal breeding, include bovine leukemia virus (BLV), bovine immunodeficiency virus (BIV), and bovine foamy virus (BFV). It is especially important to study the associated retroviral infections.

The presence of genetic and functional affinity between retroviruses of animals and the virus of human T-cell leukemia, common features of pathogenesis of the disease as well as the ability of overcoming interspecies barriers proves the urgency of studying these agents [2, 3].

Although the scientists, conducting serological studies as far as in 1975–1979, did not find any confirmation of the fact that bovine leukemia virus is of direct threat to humans, the discovery of the bovine leukemia virus DNA in human breast tissues in 2014 still may testify to the risk of infection and spreading of this virus in a human organism [4, 5].

On the contrary to bovine leukemia virus, which, although studied well enough, is still of enormous medical, social, and economic threat, minor retroviruses (BIV, BFV) are not studied in fine detail either globally or in Ukraine in particular, which gives background for potential related biorisks.

The serological examinations of cattle in different countries established a considerable incidence of bovine immunodeficiency virus (BIV) among livestock. For instance, BIV-seropositive animals constituted 4 % in the USA, 1.4 % – in the Netherlands, 5.5 % – in Canada, 6.6 % – in Germany, 4 % – in France. Immunodeficient animals were also registered in England, Sweden, Costa Rica, Venezuela, New Zealand, and Australia. The percentage ratio of seropositive animals to healthy cattle was 1–7 %. However, it amounted to 50 % in specific herds of animals with chronic diseases [6–10].

Brujeni G.N. *et. al.* studied a possible connection between the viruses of bovine immunodeficiency and leukemia. In many cases, the persistence of BIV virus in the organism of animals was accompanied with the presence of BLV, capable of causing lymphoid tumors and persistent lymphocytosis, while animals remained hematologically and clinically healthy. Out of 64 % of ID-seropositive animals with lymphosarcoma, lymphoadenopathy, and other disorders, 74 % were infected with BLV [11, 12].

Juliarena MA, Gutierrez SE, Ceriani C. demonstrated that the risk of BLV transmission from cattle without lymphocytosis may differ depending on the number of provirus copies. Thus, the animals with a higher number of copies (> 100 000) may be reservoirs of the infection, whereas the animal with a lower number of copies (< 100 copies) is not capable of transmitting the disease [13].

Rola-Łuszczak *et al.* conducted their work at creating real-time PCR for the identification of BLV and its use in the interpretation of the serological results. DNAs were isolated from the leukocytes of bovine peripheral blood, which resulted in inconclusive results of serological tests. Compared against traditional methods, real-time PCR detected 7.8 % more positive samples [14].

Our studies were aimed at conducting PCR-screening tests and establishing possible circulation of bovine immunodeficiency virus, bovine foamy virus and their association with bovine leukemia virus among livestock of the Eastern region of Ukraine.

MATERIALS AND METHODS

The samples of bovine peripheral blood, subject to diagnostic studies of leukemia in 2016, from the farms in the Eastern region of Ukraine (Kharkiv and Sumy regions) were selected. To prevent the clotting processes, blood was taken to a test-tube with EDTA.

The total DNA was isolated from the samples using the Ampliprime commercial kit (Central Scientific Research Institute of Epidemiology, the Ministry of Health of the Russian Federation, Moscow, Russia) according to the manufacturer's protocol.

The detection of BLV provirus DNA in the clinical samples was performed via polymerase chain reaction using basic PCR kits Gene PakTM (Isogen LLC, Russia) (Table 1).

The following protocol of amplification was used for pairs of primers BLV3 F/R:

1 step: denaturation – 95 °C – 2 min – 1 cycle;
2 step: denaturation – 95 °C – 30 sec,
annealing – 58 °C – 30 sec,
elongation – 72 °C – 30 sec; total – 40 cycles;
3 step: final elongation – 72 °C – 4 min – 1 cycle;

The length of the obtained amplicon was 440 base pairs (b.p.).

The detection for pairs of BIV RT primers was conducted according to the protocol:
1 step: denaturation – 94 °C – 10 min – 1 cycle;
2 step: denaturation – 94 °C – 30 sec,
annealing – 55 °C – 30 sec,
elongation – 72 °C – 2 min; total – 34 cycles;
3 step: final elongation – 72 °C – 5 min.

Table 1. Oligonucleotide sequences of primers for detection of retroviruses

Name of primer	Sequence	Source
BLV3 F/R	5′-GGTAGAGCGGACAAATGGAC-3′ 5′-TGACAGAGAGCGAGGAGAGTAAG-3′	2013, O.I.E.
BIV RT	5′-ATGCTAATGGATTTTAGGGA-3′ 5′-AACGCCATTTCTTGGGTGTG-3′	Cary A. Moody et al., 2002[15]
BFVInt3/4	5′-TCCCGCCTAAAGCTG ATAGA-3′ 5′-CAAACCTGAAATGGC TTGGT-3′	Materniak M, Sieradzki Z, Kuz´mak J., 2010 [16]

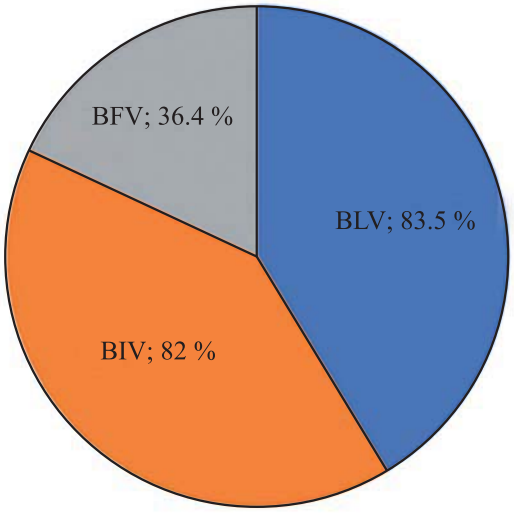

Fig. 1. The percentage ratio of BLV-, BIV-, BFV-positive samples

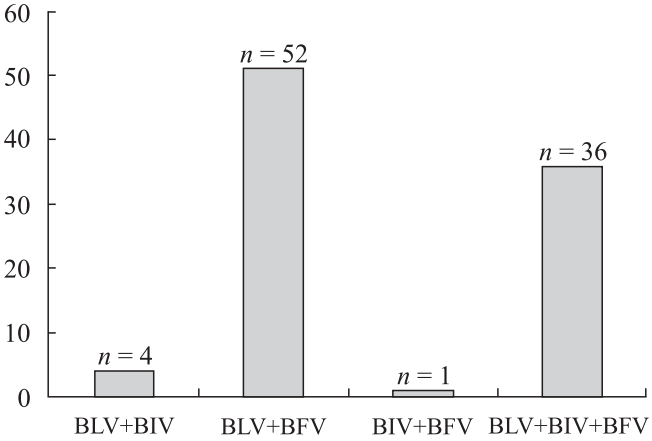

Fig. 2. Qualitative determination of retroviruses associations in clinical samples

The length of the amplicon was 495 b.p.

The detection of the BFV provirus DNA was conducted via real-time polymerase chain reaction using SybrGreen according to the following program.

1 step: denaturation – 95 °C – 15 min – 1 cycle;
2 step: denaturation – 94 °C – 30 sec,
annealing – 54 °C – 45 sec,
elongation – 72 °C – 1 min; total – 40 cycles;
3 step: final elongation – 72 °C – 5 min – 1 cycle.

The length of the amplicon was 241 b.p.

The analysis of amplification products of BLV3 F/R, BIV RT was conducted via electrophoresis in 1.5 % agarose gel, stained with ethidium bromide, under the voltage of U = 75 V, amperage I = 50 mA for 30–40 min and their further visualization in UV-light using the transilluminator.

The length of obtained amplicons was controlled using the molecular mass marker MassRules, Fermentas company.

RESULTS OF INVESTIGATIONS

PCR-screening was run on 121 samples of animal peripheral blood, subject to diagnostic examinations, from 4 farms in the Eastern region of Ukraine (Kharkiv and Sumy regions).

The study of bovine blood samples from the farms in the Eastern region of Ukraine established the presence of BLV-, BIV-, BFV-infected animals among the livestock.

Out of 121 blood samples, genetic material of leukemia virus was revealed in 101 samples, foamy virus – in 99 samples, and the genetic material of immunodeficiency virus – in 44 samples, which amounted to 83.5, 82, 36.4 % respectively (Fig. 1).

Here the number of infected samples was different for all 4 farms. The number of animals, infected with leukemia, was 57–100 % from the total number of examined livestock on average. The presence of genetic material of BIV virus was either not detected (0 % in the farms 1 and 2), or detected in the amount of 62 % from the number of tested animals. At the same time, the presence of genetic material of BFV was in the range of 61.5–81 % from the total number of studied samples (Table 2).

According to the data, obtained by some authors, BIV-seropositivity may increase among BLV-infected

Table 2. The incidence of detecting BLV-, BIV-, BFV-infected animals among bovine livestock of the Eastern region of Ukraine

Indices	Farm 1	Farm 2	Farm 3	Farm 4
Tested animals	10	14	60	37
Number of BLV-positive	10 (100 %)	8 (57 %)	53 (88 %)	30 (81 %)
Number of BIV-positive	0 (0 %)	0 (0 %)	37 (62 %)	7 (19 %)
Number of BFV-positive	7 (70 %)	13 (61.5 %)	49 (82 %)	30 (81 %)

cattle [3]. Based on the results of our studies, the presence of genetic material of immunodeficiency virus in the leukemia-positive samples was insignificant – only 4 samples, whereas the presence of the foamy virus in the leukemia-positive samples was detected in 51 samples, which was 3 % and 42 % from the total number of investigated samples respectively. The absence of leukemia virus in the blood samples, containing the DNAs of two other minor retroviruses, was detected in one sample (0.8 %).

The simultaneous presence of all three agents of retroviral infections was established in 36 samples, which constituted almost the third part of investigated samples (Fig. 2).

CONCLUSIONS

Therefore, the results of PCR-screening of bovine blood samples demonstrated the presence of BLV-, BIV, BFV-infected animals among the livestock. Possible connections between the viruses of leukemia, immunodeficiency and foamy virus of cattle, circulating among the livestock in the farms of the Eastern region of Ukraine, were established.

These are preliminary data, as the incidence range for minor retroviruses in Ukraine is yet to be determined. There are no data on the hematological status and clinical manifestations of diseases in these animals, there are no data on any previous investigations either. Therefore, to establish a potential role of minor retroviruses in bovine diseases, it is necessary to conduct large-scale and long-term (taking into account the incubation period) studies, the number of which is currently limited. From the standpoint of incidence of retroviral infections and ensuring food safety, it is urgent to obtain the information on the circulation of viruses in the population of susceptible animals.

PROSPECTS OF FURTHER STUDIES

Randomly selected samples can not reflect the whole picture of incidence of retroviral infection agents among bovine livestock. A great relevance of retroviruses for the etiology of immunodeficiencies of animals and humans, the lack of information about the incidence of minor retroviruses in Ukraine, as well as the presence of BLV-, BIV-, BFV-infected animals among the livestock highlight the urgency of this topic and require further large-scale studies.

ПЛР-скринінгові дослідження клінічних зразків крові на наявність BLV-, BIV-, BFV-інфікованих тварин в господарствах східного регіону України

О.Ю. Лиманська, І.О. Кулик, О.Б. Зленко, Н.Г. Рудова, С.К. Горбатенко, А.П. Герілович

e-mail: antger2011@gmail.com

Національний науковий центр "Інститут експериментальної і клінічної ветеринарної медицини", м. Харків, Україна

Pushkinska Str., 83, Kharkiv, 61023

Ретровірусні інфекції великої рогатої худоби (ВРХ), у тому числі й лейкоз, значно поширені у всьому світі. В Україні вивченням лейкозу займаються уже понад 40 років, у той час як мінорні ретровіруси ВРХ до цього часу не вивчалися. **Мета**. ПЛР-скринінг зразків периферійної крові від корів, що підлягали діагностичним дослідженням на лейкоз, із господарств Східного регіону України (Харківської та Сумської обл.). **Методи.** Детекцію генетичного матеріалу збудників ретровірусних інфекцій у клінічних зразках здійснювали шляхом класичної полімеразної ланцюгової реакції та полімеразної ланцюгової реакції у режимі реального часу. **Результати.** Показано наявність серед поголів'я ВРХ BLV-, BIV-, BFV-інфікованих тварин. Кількість тварин, інфікованих лейкозом, складала в середньому 57–100 % від числа досліджених. Наявність генетичного матеріалу вірусу BIV або не виявляли, або виявляли у кількості 62 % зразків від числа досліджених тварин. У той час наявність генетичного матеріалу пінистого вірусу була в межах 61.5–81 % від загальної кількості досліджених зразків. Також було встановлено можливий зв'язок між вірусами лейкозу, імунодефіциту та пінистого вірусу ВРХ, що циркулюють серед поголів'я худоби у господарствах Східного регіону України. **Висновки.** Присутність генетичного матеріалу вірусу імунодефіциту у зразках, позитивних щодо лейкозу, склала невелику кількість – лише 4 зразки, тоді як присутність пінистого вірусу у зразках, позитивних щодо лейкозу, було виявлено у 51 зразку, що склало 3 і 42 % відповідно від загальної кількості досліджених зразків. Відсутність у зразках крові вірусу лейкозу за наявності ДНК двох інших мінорних ретровірусів було виявлено в одному зразку (0.8 %). Наявність же одночасно всіх трьох збудників ретровірусних інфекцій було встановлено у 36 зразках (30 %).

Ключові слова: ВРХ, мінорні ретровіруси, молекулярна діагностика, полімеразна ланцюгова реакція.

ПЦР-скрининговые исследования клинических образцов крови на наличие BLV-, BIV-, BFV-инфицированных животных в хозяйствах восточного региона Украины

Ретровирусные инфекции крупного рогатого скота (КРС), в том числе и лейкоз, широко распространены во всем

мире. В Украине изучением лейкоза занимаются уже более 40 лет, в то время как минорные ретровирусы КРС до сих пор не изучались. **Цель.** ПЦР-скрининг образцов периферической крови от коров, подлежащих диагностическим исследованиям на лейкоз, из хозяйств Восточного региона Украины (Харьковской и Сумской обл.). **Методы.** Детекцию генетического материала возбудителей ретровирусных инфекций в клинических образцах осуществляли путем классической полимеразной цепной реакции и полимеразной цепной реакции в режиме реального времени. **Результаты**. Показано наличие среди поголовья КРС BLV-, BIV-, BFV-инфицированных животных. Количество животных, ин-фицированных лейкозом, составляло в среднем 57–100 % от числа исследованных. Наличие генетического материала вируса BIV или не устанавливали, или фиксировали в количестве 62 % образцов от числа исследованных животных. Наличие генетического материала пенистого вируса было в пределах 61.5–81 % от общего количества исследованных образцов. Также была установлена возможная связь между вирусами лейкоза, иммунодефицита и пенистого вируса КРС, циркулирующих среди поголовья скота в хозяйствах Восточного региона Украины. **Выводы.** Присутствие генетического материала вируса иммунодефицита в образцах, положительных по лейкозу, составило всего 4 образца, тогда как присутствие пенистого вируса в образцах, положительных по лейкозу, было обнаружено в 51 образце и составило 3 и 42 % соответственно от общего количества исследованных образцов. Отсутствие в образцах крови вируса лейкоза при наличии ДНК двух других минорных ретровирусов было обнаружено в одном образце (0.8 %). Наличие же сразу всех трех возбудителей ретровирусных инфекций было установлено в 36 образцах (30 %).

Ключевые слова: КРС, минорные ретровирусы, молекулярная диагностика, полимеразная цепная реакция.

REFERENCES

1. *Christou L.* The global burden of bacterial and zoonotic infections. *Clin Microbiol Infect.* 2011;**17**(3):326–30.
2. Detection of bovine immunodeficiency virus in blood and milk-derived leukocytes by use of polymerase chain reaction. Nash J.W. et al. Hanson L.A. *Am J Vet Res*. 1995;**56**(4):445–9.
3. *Fedorov YuN, Verkhovsky OA*. Bovine immunodeficiency virus: characteristics and role in pathology. *Veterinaria.* 2007;(1):18–22.
4. *The zoonotic* potential of bovine leukemia virus. Burridge MJ. *Vet Res Commun.* 1981;**5**:117–26.
5. *Burridge MJ, Shen HM, Jensen HM, Choi KY, Sun D, Nuovo G.* Bovine leukemia virus DNA in human breast tissue. Buehring GC. *Emerg Infect Dis.* 2014; **20**(5):772–82.
6. *Report* of the committee on bluetongue and bovine retrovirus. Black JW. *Proc U S Anim Health Assoc 93.* 1989:145–52.
7. *Horzinek M, Keldermans L, Stuurman T, Black J, Herrewegh A, Sillekens P, Koolen M.* Bovine Immunodeficiency virus: immunochemical characterization and serological survey. *J Gen Virol.* 1991;**72**:2923–8.
8. *McNab WB, Jacobs RM, Smith HE*. A serological survey for bovine immunodeficiency like virus in Ontario dairy cattle and association between test results production records and management practices. *Can J Vet Res.* 1994;**58**(1):36–41.
9. *Muluneh A.* Seroprevalence of bovine immunodeficiency virus (BIV) antibodies in the cattle population in Germany. *Zoonoses and Public Health.* 1994,**41**(1–10): 679–84.
10. *Polack B, Schwartz I, Berthelemy M, Belloc C, Manet G, Vuillaume A, Baron T, Gonda MA, Lévy D.* Serologic evidence for bovine immunodeficiency virus infection in France. *Vet Microbiol.* 1996;**48**(1–2):165–73.
11. *Brujeni GN, Poorbazargani TT, Nadin-Davis S, Tolooie M, Barjesteh N.* Bovine immunodeficiency virus and bovine leukemia virus and their mixed infection in Iranian Holstein cattle. *J Infect Dev Ctries.* 2010;**4**(9):576–9.
12. *Whetstone CA, VanDerMaaten MJ, Black JW.* Humoral immune response to the bovine immunodeficiency-like virus in experimentally and naturally infected cattle. *J Virol.* 1990;**64**(7):3557–61.
13. *Juliarena MA, Gutierrez SE, Ceriani C.* Determination of proviral load in bovine leukemia virus-infected cattle with and without lymphocytosis. *Am J Vet Res.* 2007;**68**(11):1220–5.
14. *Rola-Łuszczak M, Finnegan C, Olech M, Choudhury B, Kuźmak J.* Development of an improved real time PCR for the detection of bovine leukaemia provirus nucleic acid and its use in the clarification of inconclusive serological test results. *J Virol Methods.* 2013;**189**(2):258–64.
15. *Moody CA, Pharr GT, Murphey J, Hughlett MB, Weaver CC, Nelson PD, Coats KS.* Confirmation of vertical transmission of bovine immunodeficiency virus in naturally infected dairy cattle using the polymerase chain reaction. *J Vet Diagn Invest.* 2002;**14**(2):113–9.
16. *Materniak M, Sieradzki Z, Kuzmak J.* Detection of bovine foamy virus in milk and saliva of BFV seropositive cattle. *Bull. Vet. Inst. Pulawy.* 2010;**54**(4):461–5.