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# Population and biological preconditions for the cattle retroviruses' expansion

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This research was aimed at studying of population and biological aspects of cattle retroviruses' expansion, such as breed and age of animals, form of head ownership, retroviruses biology. *Bovine leukemia virus* (*BLV*) and *bovine immunodeficiency virus* (*BIV*) are retroviruses which cause chronic incurable diseases of cattle. These agents have a phylogenetic relationship with similar pathogens in humans. There is a possible danger of viral entry to humans through the consumption of infected foodstuffs. 773 blood samples from Black-and-White, Holstein, Simmental, Kazakh White-headed and crossbred cattle of different districts of the Saratov region were analyzed by polymerase chain reaction (PCR). Studies reveal that *bovine immunodeficiency* and *bovine leukemia viruses* are spread widely in cattle of the Saratov region: 30.5% and 39.8% on average, respectively. The infection rate varies considerably depending on age-sex group affiliation and cattle ownership. *BIV* and *BLV* infection rates increase with the animals age, especially among farm herd. Significant epizootic feature of retroviral infections in cattle in the Saratov region is a high level of retroviral coinfection – 25.2% on average. The clinical complications of *BIV* infection, confirmed by laboratory studies, were most frequently recorded in cattle aged 5–10 years. For analysis of diagnostic accuracy of serological and molecular genetic methods for enzootic bovine leucosis diagnosis, 271 cattle blood samples were studied by PCR and AGIDT (agar gel immunodiffusion test) in parallel. The comparative analysis shows that diagnostic efficiency of AGIDT in comparison to PCR is 30.8%. Taking into account the retroviruses biological features, the PCR assay can be recommend as a screening method for *BLV*-infection revealing, especially when the imported cattle are quarantined at the place of keeping. In herds with high *BLV*-infection level, cattle should be tested for *BIV* presence to stop the cattle retroviruses' expansion.

***Keywords:*** polymerase chain reaction, agar gel immunodiffusion test, retroviruses, enzootic bovine leucosis, bovine immunodeficiency, expansion, diagnostics.

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# Популяционно-биологические предпосылки экспансии ретровирусов крупного рогатого скота

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Целью настоящих исследований явилось выявление популяционных и биологических предпосылок распространения ретровирусных инфекций среди крупного рогатого скота. Особое внимание уделялось таким аспектам как порода и возраст животных, форма владения, биологические особенности ретровирусов. *Bovine leukemia virus* (*BLV*) и *bovine immunodeficiency virus* (*BIV*) являются ретровирусами, вызывающими хронические неизлечимые заболевания крупного рогатого скота. Данные возбудители имеют филогенетическую связь с подобными патогенами человека. Существует вероятность передачи вирусов от животных человеку через контаминированные продукты питания. Методом полимеразной цепной реакции (ПЦР) были исследованы 773 пробы крови Черно-пестрого, Голштинского и Симментальского скота, коров породы Казахская белоголовая и беспородных животных из различных районов Саратовской области. Исследования показали, что *BLV* и *BIV* широко распространены среди

крупного рогатого скота Саратовской области: 30,5 и 39,8% в среднем, соответственно. Уровень инфицированности значительно варьирует в зависимости от половозрастной группы и формы владения скота. Степень *BIV* и *BLV*-инфицирования увеличивается с возрастом животных, особенно среди фермерского скота. Немаловажной особенностью ретровирусных инфекций крупного рогатого скота в Саратовской области является высокий уровень ретровирусной коинфекции – в среднем 25,2%. Клинические проявления *BIV*-инфекции, подтвержденной лабораторными исследованиями, наиболее часто регистрировались среди скота в возрасте 5–10 лет. Для выяснения диагностической ценности серологического и молекулярно-генетического методов диагностики энзоотического лейкоза крупного рогатого скота, 271 проба крови была исследована параллельно методами ПЦР и РИД (реакция иммунодиффузии). Сравнительный анализ показал, что диагностическая эффективность РИД относительно ПЦР составляет 30,8%. Учитывая биологические особенности ретровирусов, ПЦР-анализ может быть рекомендован в качестве скринингового метода выявления *BLV*-инфекции, особенно при карантинировании ввозимого из-за границы скота. В стадах с высоким уровнем *BLV*-инфекции, крупный рогатый скот необходимо исследовать на наличие *BIV*, чтобы остановить экспансию ретровирусов крупного рогатого скота.

***Ключевые слова:*** полимеразная цепная реакция, реакция иммунодиффузии, ретровирусы, энзоотический лейкоз крупного рогатого скота, иммунодефицит крупного рогатого скота, распространение, диагностика.

According to the International Committee on Taxonomy of Viruses (ICTV) database, currently 96 families of viruses are described and the *Retroviridae* family is one of particular importance among them. This is due not only to the unique biological properties and structure, but also to the fact that retroviruses cause chronic incurable diseases, which tend to widespread, such as enzootic bovine leucosis and bovine immunodeficiency. The causative agents of these diseases, *bovine immunodeficiency virus* (*BIV*) and *bovine leukemia virus* (*BLV*) were isolated in 1969. These agents have a phylogenetic relationship with similar pathogens in humans: *human immunodeficiency virus* (*HIV*) and *human T-lymphotropic virus* (*HTLV*) and a high degree of genetic homology between them [1]. Under experimental conditions sheep, rabbits, pigs and monkeys turned to be susceptible to the leukemia virus [2]. It is known that retroviruses are capable of overcoming the interspecific barrier, including the transition from animals to humans [3]. Although the pathogenicity of *BLV* to humans is not proven, it is considered as a breast cancer risk factor, other researchers have a different opinion [4]. *BIV* and *BLV* are revealed in the milk and meat of sick and infected animals, accordingly there is a possible danger of viral entry to humans through the consumption of infected foodstuffs. Moreover, it was found that the infected animals milk contains the hazardous to human health metabolites [5].

*BIV* and *BLV*, similarly *HIV* and *HTLV*, infect the immune system cells (lymphocytes, monocytes and macrophages), which is designed to fight them and ensure the organism homeostasis. The viruses turn the lymphocytes into the "viral particles cloning factories". As a result, the adaptive capacity of the organism, specific and non-specific resistance are sharply lowered, which inevitably leads to the development of the pathological process [6].

Enzootic bovine leucosis is one of the most pressing and urgent problems of livestock husbandry. The cattle stock with hematological malignancies is rather high, especially in countries with highly developed dairy cattle. It leads to significant economic loss in connection with reducing quantity and quality of products, cattle death rates or emergency slaughter of animals, receiving less young stock, loss of the breeding value and limited sales of cattle, additional costs for anti-epizootic preventive measures, animals' treatment and milk pasteurization [7].

According to the researchers' data, these infections are widespread. *BLV* registered in Japan from 28.6 to 68.1% in different regions, in South America from 34 to 50%, in Canada up to 89%, in USA up to 83.9%, in Brazil to 50%, in Korea from 50 to 86.8%, in Turkey and Iran 48.3% and 64.7%, respectively [8], in Chile 29.1%, in Peru and Paraguay 42.3% and over 50% of samples, respectively, In Bolivia 30% [9], in Argentina from 32.8 to 84% (up to 90.9% by some accounts) [8, 9], in Philippine 4.9–23.1% [10], as well in Bulgaria, Croatia, Estonia, Latvia, Poland, Romania, Ukraine, New [8], Lithuania [11]. Enzootic bovine leucosis is spread in many regions of the Russian Federation [12, 13]. According to the data of the information-analytical center of the Rosselkhoznadzor of the Russian Federation, enzootic bovine leucosis is the disease with a subclinical case. At the present time are 138 affected with leukemia points in Russia. Moreover, it was revealed 31256 suffering from leukemia cattle heads in 2017. This is the highest rate in the nosological profile of cattle infectious diseases.

*Bovine immunodeficiency virus* is recorded in Australia (15.9%) [14], in the USA (21–

30%) [15], in South Korea (33%) [16], in Brazil (11.7%) [17], in Zambia (11.4%) [18], in Turkey (12.3%) [19], in Germany (6.6%), in Japan (7.5%), in Italy (2.5–5.1%), in India (24%), as well as in French and Louisiana [1]. On the Russian Federation, such studies are sporadic. According to some data, infection with bovine immunodeficiency virus in the Moscow region constitutes from 11 to 67%, and in the Stavropol region this figure is 11–33% among the examined cattle [20, 21].

There are a number of serological tests that determine viral structural proteins and glycoproteins. In Russia, according to the officially approved rules, anti-epizootic measures against bovine leucosis are based on the identification and removal of infected with *BLV* animals, basing on the data of agar gel immunodiffusion test (AGIDT) and hematologic studies. AGIDT is prescribed for international animals' trade, in spite of its relatively low sensitivity [22]. For the diagnosis of *BIV* there are no certified sets and approved instructions. There is an opinion that polymerase chain reaction has higher test-sensitivity, specificity and informative value than other methods for viruses detecting [12].

Saratov region is an important agricultural area with developed dairy cattle. According to official statistics, the enzootic bovine leucosis infection rate is 9.5%, and there are 9 epizootic focuses of leukemia in the Saratov region in 2017. Thus, the problem of cattle retrovirus infection is very relevant at the moment and requires a specific attention. The purpose of the research was studding of epizootic situation of retroviral infection in cattle of the Saratov region and comparative analysis of standard serological method (AGIDT) and contemporary molecular genetic method (PCR) for enzootic bovine leucosis diagnosis.

## Material and methods

Cattle from 5 dairy farms and 299 cows in private ownership were tested over a five-year period using AGIDT and PCR. A total, for analyzing the epizootic situation of retrovirus infection in cattle, 773 blood samples from Black-and-White, Holstein, Simmental, Kazakh White-headed and crossbred cattle of different districts of the Saratov region were analyzed (Table 1). FEEVT "Krasnokutsky Veterinary College" is a structural subdivision of the Saratov State Vavilov Agrarian University. All the tested samples were divided into 4 groups: I group samples were getting from 3–6 months' calves; II group samples were obtained from 7 18 months' youngstock; III group samples – from 1.5–5 years old cattle and IV group samples – from older 5 years old animals (Table 2). *BLV* provirus carriers were considered the animals, which confirmed their positive status in PCR twice for 2 weeks.

For comparative analysis of diagnostic accuracy of serological and molecular genetic methods for enzootic bovine leucosis diagnosis, 271 cattle blood samples from disadvantaged by leukemia "Yagodnopolyanskoe" LTD. of Tatishchev district were studied by standard serological method (AGIDT) and contemporary molecular genetic method (PCR) in parallel. All AGIDT positive samples used for the comparative analysis were confirmed by PCR.

**Molecular genetic method.** DNA extraction and purification was performed using the kit of DNA-Sorb-B (Amplisens, Russia) according to the manufacturer's instructions.

The blood samples were analyzed by PCR method. For amplification of *BLV* and *BIV* proviruses DNA, the amplifier T 100 ("Bio-Rad", USA) was used.

In the study of enzootic bovine leucosis, to identify provirus DNA, the "LEUKOS" kit (InterLabService, Russia) was used according to the kit instruction.

*BIV* infection in cattle was determined using the PCR mix and buffer solution ("Lytech" LTD., Russia) with the adding of the primers to the *BIV* gag gene (synthesized by JSC "Syntol", Russia). The structure of the oligonucleotide primers is: the forward primer (5'-GTCTTCCCA-CATCCGTAACATCTCCT-3') and the reverse primer (5'-CCCCAGGTCCCATCAACATTCAT-CAG-3'). Samples were initially denatured at 95 °C for 2 min, then amplified by using 45 cycles of 95 °C for 20 s, 58 °C for 20 s, and 72 °C for 40 s. A final extension of 1 min at 72 °C was added at the end of the program to ensure complete amplification of the target region.

Detection of the amplification products was performed by method of gel electrophoresis in a 2% agarose gel with 0.5 mkg/L ethidium bromide under standard conditions accompanied by the photographic recording of results using BioRad ChemiDoc MP equipment ("Bio-Rad", USA).

**Serological method.** For comparative analysis the statistical data of "Tatishchev regional veterinary laboratory" LTD. (Saratov region) were used. Sera were tested for anti-p24 antibody in AGIDT.

## Results and Discussion

***BIV* infection in cattle.** The test results revealed that presence of *BIV* provirus in cattle varies widely (Table 3). From 79 the I group animals' blood samples, 8 (10.1%) showed positive results, in private ownership only 7.7%, 3 out of 39 calves, were *BIV*-positive while in farm herds infection rate was 12.5% (5/40). Of 139 young stock' blood samples (II group), 24 (17,3%) were positive and infection rate in private ownership cattle was less again – 12.8% (6/47), than in farm herds – 22.5% (18/80). In III group animals' blood samples *BIV* carriers were revealed in 15.4% (39 samples out of 254). Only 12 examined animals (11.8%) of this group in private ownership showed positive results. The infection rate in local herd at the age of 1.5–5 years was averaged 15.4% (39/245) and varied from 9.4% (8/85) of "Yagodnopolyanskoe" LTD to 17.7% (2/12) of "Ozernoe" LTD and 25.0% (5/20) of FEEVT "Krasnokutsky

**Table 1**

Tested cattle head

| The Saratov region district | Ownership | Number of cattle heads | Cattle age, yars | Cattle gender | Cattle breed |
|---|---|---|---|---|---|
| Tatishchev district | "Yagodnopolyanskoe" LTD | 271 | 0.6–10 | F/M | Black-and-White |
| Krasnokutsky district | FEEVT* "Krasnokutsky Veterinary College" | 40 | 4–8 | F | Kazakh White-headed |
| Atkarsky district | "Ozernoe" LTD | 32 | 4–8 | F | Kazakh White-headed |
| Marksovsky district | "Trudovoe" LTD | 17 from locale cattle | 5–6 | F | Black-and-White |
| | | 15 from Slovakia and Estonia | 5–6 | F | Holstein |
| | | 72 from Canada | 4–7 | F | Holstein |
| Penza district | EPF** "Zarya" | 27 | 1–3 | F | Simmental |
| Dukhovnitsky district | Private ownership | 299 | 0.3–10 | F/M | Simmental, Black-and-White and crossbred cattle |
| Total | | 773 | x | x | x |

*Note: M – masculine, F – feminine, * – Federal Educational Establishment of Vocational Training, ** – Experimental Production Farm.*

**Table 2**

Blood simples number

| Ownership | Cattle age group | | | |
|---|---|---|---|---|
| | 3–6 months | 7–18 months | 1.5–5 years | > 5 years |
| "Yagodnopolyanskoe" Ltd | 40 | 80 | 85 | 66 |
| FEEVT "Krasnokutsky Veterinary College" | – | – | 20 | 20 |
| "Ozernoe" LTD | – | – | 12 | 20 |
| "Trudovoe" LTD: Locale cattle | – | – | – | 17 |
| European cattle | – | – | – | 15 |
| Canadian cattle | – | – | 20 | 52 |
| EPF "Zarya" | – | 12 | 15 | – |
| Private ownership | 39 | 47 | 102 | 111 |
| Totale | 79 | 139 | 254 | 301 |

*Note: "–" – studies have not been conducted.*

**Table 3**

The incidence of viral immunodeficiency in cattle

| Ownership | Cattle age groups | | | |
|---|---|---|---|---|
| | 3–6 months (I) | 7–18 months (II) | 1.5–5 years (III) | > 5 years (IV) |
| | BIV+, No/% | BIV+, No/% | BIV+, No/% | BIV+, No/% |
| "Yagodnopolyanskoe" Ltd. | 5/12.5 | 18/22.5 | 8/9.4 | 38/57.6 |
| FEEVT "Krasnokutsky Veterinary College" | – | – | 5/25.0 | 13/65.0 |
| "Ozernoe" LTD | – | – | 2/16.7 | 12/60.0 |
| "Trudovoe" LTD: Locale cattle | – | – | – | 6/35.3 |
| European cattle | – | – | – | 8/53.3 |
| Canadian cattle | – | – | 14/70.0 | 38/73.1 |
| EPF "Zarya" | – | – | – | – |
| Private ownership | 3/7.7 | 6/12.8 | 12/11.8 | 48/43.2 |
| Totale | 8/10.1 | 24/17.3 | 39/15.4 | 163/54.1 |

*Note: "–" – studies have not been conducted.*

Veterinary College". The most commonly *BIV*-infected animals occurred among imported from Canada cattle – 70.0% (14/20). The prevalence of *BIV*-provirus in IV cattle group was 54.1% (163/301) and oscillated between 35.5% (6/17) in local cattle of satisfactory by leukemia farm and 73.1% (38/52) in imported from Canada cattle. In cattle of disadvantaged by leukemia farms, infection rate was within the order of 60 percent. Less infected animals were detected among private ownership cattle – 43.2% or 48 out of 111 tested animals. All the 27 blood samples of EPF "Zarya" were negative. Our research results correlate with the St Cyr Coats et al (1994) data, which revealed that the BIV-positive cattle are mainly recorded among adult animals: 29% of cases among cattle of 3–4 years and 70% among cattle of 7–10 years.

Other researchers' studies correlate with our data. In Canada, the highest retrovirus infection rate was achieved in farm herds (up to 89%) and in private ownership animals it was 20.8–37.4%, as well as in Argentina individual and herd prevalence levels was scale up to 32.8% and 84%, respectively, and in Japan it was 28.6% and 68.1% at the individual and herd levels, respectively. In Iran retrovirus infection prevalence rate in herds constituted 64.7%, while in private ownership cattle it was from 17 to 24.6% [8]. It may be due to closer contact between farmers' cattle, the possibility of iatrogenic spread of infection when carrying out therapeutic and diagnostic activities, predisposition to disease of highly productive animals and other factors determined by the peculiarities of the acquisition, feeding and housing of animals.

Clinical manifestations of an immunodeficiency state were observed in 29.7% of the examined animals. The most commonly *BIV*-infected animals had evidence of mastitis, metritis, placenta retention, respiratory syndromes and gastrointestinal tract dysfunction, as well as regional lymphadenitis. It should be noted that in most cases there was a combined development of symptoms, and clinical manifestations were recurrent. According to [23] data, there is enough experimental evidence that *BIV* can cause the immune system dysfunction in animals, which makes them vulnerable to secondary infections. It is explained by the diversity and not the specificity of the *BIV* infection clinical manifestation [1].

***BLV infection in cattle.*** Results of cattle blood PCR testing have been summarized in Table 4. The incidence of enzootic bovine leucosis among cattle of the Saratov region is sufficiently high. According to results of IV group animals' blood testing, PCR analysis allows to identify the *BLV* carrier state in 2 samples out of 17 (11.8%), even when the AGIDT negative cattle have not shown the antibodies presence. Among the imported from America (Canada) and Europe (Slovakia and Estonia) cattle only a few respondents have been positively to AGIDT, when conducting research into a period of quarantine (within 1%), most of the animals were latent carriers of infection. However, in accordance with the results of PCR studies the imported cattle of this group, *BLV* infection was reviled 33.3% (5 samples out of 15 in European cattle) and 26.9% (14 samples out of 52 in Canadian cattle). In cattle of disadvantaged by leukemia farms, *BLV*-infection rate was within the order of 70.0 75.0%, while

among in private ownership cattle *BLV* provirus was identified in 42 samples out of 111 (37.8%). It can be explained by higher resistance of the cattle, due to both genetic factors and favorable conditions of livestock feeding, maintenance and operation. The infection rate of III group animals was slightly below. *BLV* carriers were detected in 5 out of 25 Canadian cows (25.0%) and 27 out of 102 in local private ownership cattle (26.5%). The *BLV* prevalence of disadvantaged by leukemia herds varies between 40.0% (6/15) in cattle of EPF "Zarya" and 66.7% (8/12) in cattle of "Ozernoe" LTD. In cattle of "Yagodnopolyanskoe" LTD 38 samples out of 85 (44.7%) and 65.0% tested samples (13/20) of FEEVT "Krasnokutsky Veterinary College" were positive. The infection rate in herd of 7–18 months varied from 50.0% (40/80) in "Yagodnopolyanskoe" LTD to 33.3% (4/12) in EPF "Zarya". From 47 of private ownership animals of this group, 8 (17.0%) showed positive results. In I group only 10.3%, 4 out of 39 calves, were *BLV*-positive while in farm herds infection rate was 32.5% (13/40). Thus, according to our data, the most commonly *BLV*-infected animals occurred among cattle aged 5–10 years – 47.2% (142/301). The *BLV*-infected animals among cattle at the age of 1.5–5 years and 7–18 months were revealed in 38.2% (97/254) and 37.4% (52/139) of cases, respectively. The least infected were calves aged 3–6 months – 21.5% (17/79).

Our research results reveal that enzootic bovine leucosis is widespread among the farms livestock of the Saratov region, and *BLV* seroprevalence rates in cattle are generally lower than PCR analysis data. A number of researchers are also noted the discrepancy between the results of AGIDT and PCR analysis [12].

**Retrovirus coinfection in cattle.** As it follows from the data of Table 5, coinfection with both retroviruses were detected in 10.0% of 3 6 months' herd calves (4/40) and in only 5.1% (2/39) of private ownership calves, on the average it was 7.6% (6/79). Among the II group cattle coinfection was identified in 21.5% (17/80) and 10.6% (5/47) of cases in herd and private ownership, respectively, and on the average it was 15.8% (22/139). In III group animals' blood samples *BLV-BIV* carriers were revealed in 11.4% (28 samples out of 254). Eleven examined animals out of 102 (10.8%) of this group, in private ownership, showed positive results. And the infection rate in local herd of III group varied from 9.4% (8/85) in "Yagodnopolyanskoe" LTD to 20.0% (4/20) in FEEVT "Krasnokutsky Veterinary College" as well as in Canadian cattle. In "Ozernoe" LTD it was 16.6% (2/12). The most commonly *BLV-BIV* coinfected animals occurred among IV group of cattle – 40.5% (122/301). The prevalence both of *BLV* and *BIV* proviruses in cattle of disadvantaged by leukemia farms was 65.0, 57.6 and 50% in FEEVT "Krasnokutsky Veterinary College" (13/20), in "Yagodnopolyanskoe" LTD (38/66) and in "Ozernoe" LTD (10/20), respectively. In imported from Canada and Europa cattle it was 25.0% (13/52) and 26.7% (4/15), respectively. In local cattle of satisfactory by leukemia farm the infection rate was 11.8% (2/17). Forty-two out of 111 samples (37.8) of cattle in private ownership showed both *BIV* and *BLV* proviruses presence.

**Table 4**

The incidence of enzootic bovine leucosis in cattle

| Ownership | Cattle age groups | | | |
|---|---|---|---|---|
| | 3–6 months (I) | 7–18 months (II) | 1.5–5 years (III) | > 5 years (IV) |
| | BLV+, No/% | BLV+, No/% | BLV+, No/% | BLV+, No/% |
| "Yagodnopolyanskoe" Ltd. | 13/32.5 | 40/50.0 | 38/44.7 | 50/75.8 |
| FEEVT "Krasnokutsky Veterinary College" | – | – | 13/65.0 | 15/75.0 |
| "Ozernoe" LTD | – | – | 8/66.7 | 14/70.0 |
| "Trudovoe" LTD: Locale cattle | – | – | – | 2/11.8 |
| European cattle | – | – | – | 5/33.3 |
| Canadian cattle | – | – | 5/25.0 | 14/26.9 |
| EPF "Zarya" | – | 4/33.3 | 6/40.0 | – |
| Private ownership | 4/10.3 | 8/17.0 | 27/26.5 | 42/37.8 |
| Totale | 17/21.5 | 52/37.4 | 97/38.2 | 142/47.2 |

*Note: "–" – studies have not been conducted.*

**Table 5**

The incidence of retrovirus coinfection in cattle

| Ownership | Cattle age groups | | | |
|---|---|---|---|---|
| | 3–6 months (I) | 7–18 months (II) | 1.5–5 years (III) | > 5 years (IV) |
| | BLV+BIV+, No/% | BLV+BIV+, No/% | BLV+BIV+, No/% | BLV+BIV+, No/% |
| "Yagodnopolyanskoe" Ltd. | 4/10.0 | 17/21.3 | 8/9.4 | 38/57.6 |
| FEEVT "Krasnokutsky Veterinary College" | – | – | 4/20.0 | 13/65.0 |
| "Ozernoe" LTD | – | – | 2/16.6 | 10/50.0 |
| "Trudovoe" LTD: | | | | |
| Locale cattle | – | – | – | 2/11.8 |
| European cattle | – | – | – | 4/26.7 |
| Canadian cattle | – | – | 4/20.0 | 13/25.0 |
| EPF "Zarya" | – | – | – | – |
| Private ownership | 2/5.1 | 5/10.6 | 11/10.8 | 42/37.8 |
| Totale | 6/7.6 | 22/15.8 | 28/11.4 | 122/40.5 |

*Note: "–" – studies have not been conducted.*

**Table 6**

The diagnostic accuracy of AGIDT and PCR

| Groups of animals | Tested samples, No | AGIDT | | PCR | |
|---|---|---|---|---|---|
| | | Positive samples | | Positive samples | |
| | | No | % | No | % |
| Calves aged 6–8 months (I) | 40 | 2 | 5.0 | 13 | 32.5 |
| Heifers aged 9–12 months (II) | 40 | 6 | 15.0 | 19 | 47.5 |
| Heifers aged 13–18 months (III) | 40 | 7 | 17.5 | 21 | 52.5 |
| Heifers in milk (IV) | 40 | 9 | 22.5 | 14 | 35.0 |
| Cow aged 3–5 years (V) | 40 | 8 | 20.0 | 23 | 57.5 |
| Cow aged 6–10 years (VI) | 63 | 11 | 17.5 | 50 | 79.4 |
| Yearling bulls (VII) | 5 | – | 0.0 | 1 | 20.0 |
| Stud bulls (VIII) | 3 | – | 0.0 | – | 0.0 |
| Totale | 271 | 43 | 16 | 141 | 52 |

*Note: "–" – studies have not been conducted.*

Our data are comparable with the results of both domestic and foreign researchers, which showed, that in Stavropol region coinfection was recorded in 33.1% of the retroviruses infected cattle [21], and in England in 43% of cases bovine immunodeficiency was associated with bovine leukemia [15]. In our opinion, a high level of retroviral coinfection indicates that the immunodeficiency virus can occur resulting in transplacental animals, weakens the immune system and promotes infection of cattle leukemia virus.

**The diagnostic accuracy of AGIDT and PCR for enzootic bovine leucosis diagnosis.** A further stage of our research was to compare the effectiveness of the classical serological method (AGIDT) and modern molecular genetic methods (PCR) for detection of bovine leukemia virus. For this purpose, 271 blood samples of cattle blood were studied simultaneously using AGIDT and PCR methods. To identify characteristic trends, blood samples were obtained from animals of different age and gender groups (Table 6). It is known that cloistral antibodies are founded in the calves' blood, moreover there is a report, that the use of same brand of *BLV* antibody-positive colostrum replacers may also lead to false-positive serological diagnostics [24]. Consequently, for the comparative analysis, we used samples from animals older than 6 months. Serological and PCR tests results, when considered in all tested cattle (n = 271), indicated that positive results (presence of antileukemic antibodies) were observed in 16% of samples using AGIDT with the blood serum of cattle, and the presence of provirus DNA was detected in 52% of animals by PCR. For comparative analysis, only confirmed

by AGIDT in the PCR the results were used. The PCR test results were significantly different from serological test results, but in different groups it was expressed to varying degrees. The results of AGIDT with 40 sera of I group calves showed the antibodies presence in 2 samples (5.0%), while PCR analysis reviled the provirus presence in 13 samples (32.5%). Similar results were recorded in VI animals' group: the proportion of revealed by PCR positive animals increased to 50 out of 63 (79.4%), when by AGIDT method it only was 11 out of 63 (17.5%). Our data correlates with Jacobs et al. results, which show the higher efficacy of PCR for early diagnosis of enzootic leucosis in calves and detection of *BLV*-infection in cows older than 8 years [25]. It can be caused by lack diagnostic antibodies titers in both young and old cattle [22], as well as a high level of retrovirus coinfection [1]. The *BLV*-antibodies prevalence, determining with AGIDT, in II, III and V groups of animals oscillated between 15.0% (6/40), 17.5% (7/40) and 20.0% (8/40), respectively. The PCR study allowed to identify in these groups an additional 13, 14 and 15 animals, which increased the positive samples number to 47.5%, 52.5% and 57.5%, respectively. In IV animals' group the diagnostic results were the most comparable: seroprevalence level was 22.5% (9/40) and provirus carriage was 35.0% (14/40). Among bulls only 1out of 5 stud bulls showed a PCR positive result (20.0%). There were no seropositive bulls among animals of VII and VIII groups. Our results show that it is essential to conduct a screening study among the calves and old cattle using both PCR and AGIDT. It allows to identify as early stages of *BLV*-infection in cattle, and animals with reduced immune reactivity. In all, PCR studies allowed to identify 36% more infected animals than AGIDT. Parallel studies revealed an additional 70 infected cattle heads. Thus, PCR diagnostic efficacy is 1.63 times higher than that of AGIDT.

## Conclusions

Thus, our findings revealed a high prevalence of retroviral infections among cattle in the Saratov region, especially in farm herds. The *BIV* and *BLV* infection rates increase with the animals age 5.5 and 2.2 times, respectively. *BIV* and *BLV* are revealed in farmers' animals 1.5 and 1.7 times, respectively, frequently than in private ownership cattle. Significant epizootic feature of retroviral infections in cattle in the Saratov region is a high level of retroviral coinfection. An important role in the spread of retroviral infections belongs to imported livestock. Despite the lack of specific clinical signs, it is possible to ascertain the presence of signs of reducing the overall resistance in *BIV*-infected cattle. The frequency of development of clinical complications of *BIV* infection correlates with increasing age of the animals. Diagnostic efficiency of AGIDT in comparison to PCR is 30.8%. Our studies data allows us to recommend the PCR assay as a method of screening studies for *BLV*-infection diagnosis along with AGRIT, especially when the imported cattle are quarantined at the place of keeping. It is not desirable to be limited to only one method of diagnosis.

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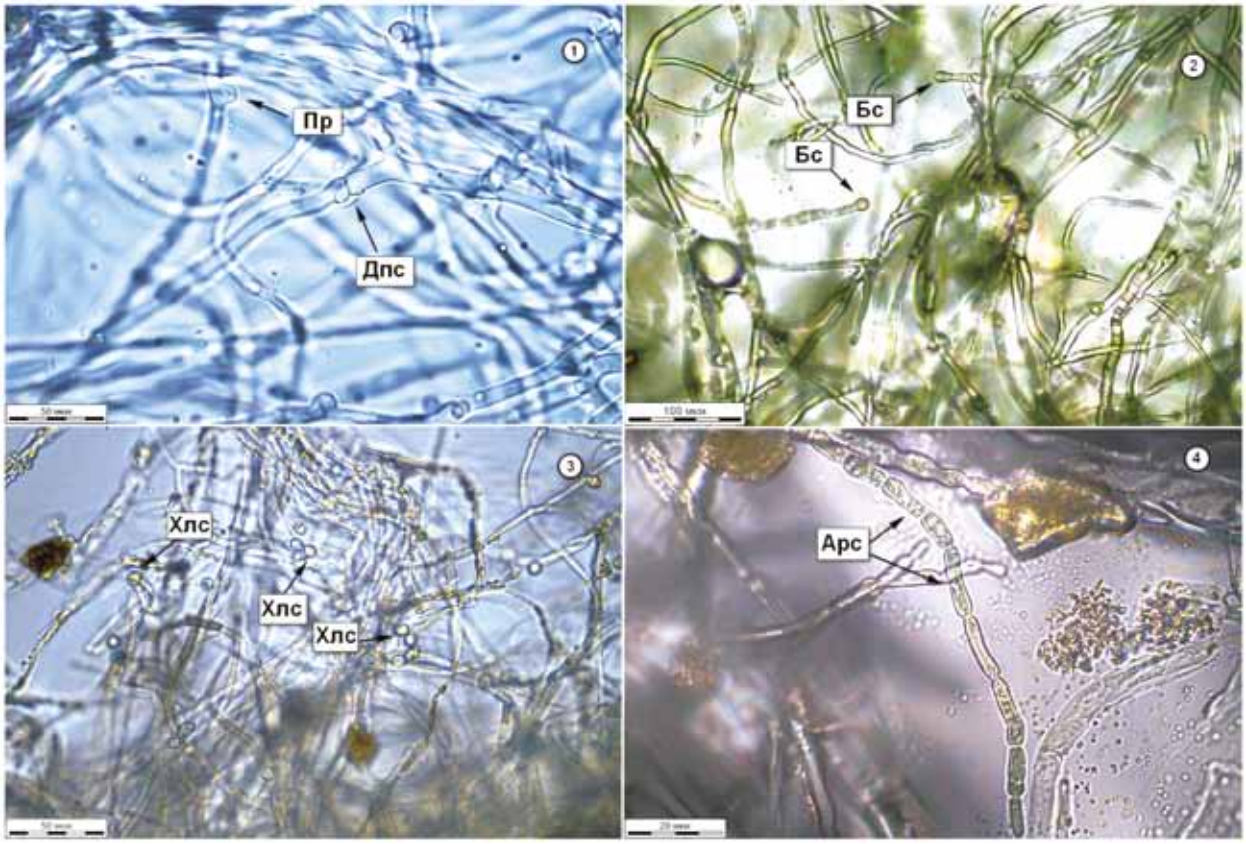


**Рис. 1**. Наблюдаемые в мицелии микроскопические структуры *Hericium erinaceum:* 1 – пряжки (Пр), долипоровые септы (Дпс); 2 – бластоспоры (Бс); 3 – хламидоспоры (Хлс); 4 – артроспоры (Арс)
**Fig. 1.** Microscopic structures of *Hericium erinaceum* observed in the mycelium: 1 – buckles (Пр), dolipore septa (Дпс); 2 – blastospores (Бс); 3 – chlamydospores (Хлс); 4 – arthrospores (Арс)

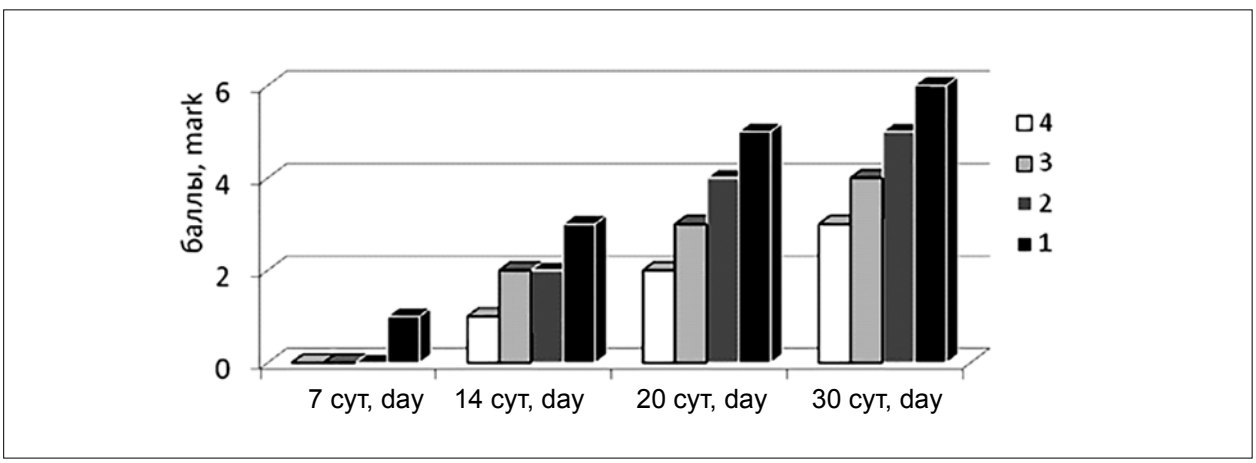


**Рис. 2.** Динамика колонизации субстрата мицелием *H. erinaceus*. Варианты: 1) опилки 50 об.%: солома 50 об.%; 2) опилки 50 об.%:зерно 10 об.%:солома 40 об.%; 3) опилки 20 об.%:зерно 20 об.%:солома 60 об.%; 4) опилки 10 об.%: зерно 30 об.%: солома 60 об.%
**Fig. 2.** Dynamics of colonization of the substrate by the mycelium of *H. erinaceus*. Variants: 1) sawdust 50% vol.: straw 50% vol.; 2) sawdust 50 vol.%: grain 10 vol.%: straw 40 vol.%; 3) sawdust 20% by volume: grain 20% by volume: straw 60% by volume; 4) sawdust 10 vol.%: grain 30 vol.%: straw 60 vol.%

**РЕЦЕНЗИЯ НА МОНОГРАФИЮ А. А. ШИРОКИХ «МИКСОМИЦЕТЫ ЗАПОВЕДНИКА “НУРГУШ”» (КИРОВ: ООО «ТИПОГРАФИЯ «СТАРАЯ ВЯТКА», 2018. 95 С.) С. 125**

*Hemitrichia calyculata*

*Licogala conicum*

*Physarum album*

*Stemonitis fusca*

*Trichia varia*