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MONITORING OF BOVINE LEUKEMIA VIRUS IN KAZAKHSTAN: ANALYSIS OF SEROLOGICAL AND MOLECULAR DIAGNOSTIC METHODS

Bovine leukemia virus (BLV) poses a significant threat to livestock farming in Kazakhstan. This study presents a comprehensive analysis of the prevalence of BLV based on data from 2024, obtained using serological and molecular diagnostic methods. The sensitivity and specificity of agar gel immunodiffusion (AGID), enzyme-linked immunosorbent assay (ELISA), and real-time polymerase chain reaction (RT-PCR) were evaluated. Serological methods demonstrated a high seroprevalence of BLV among animals, with ELISA showing greater sensitivity compared to AGID, confirmed by statistically significant results ($p < 0.05$). RT-PCR identified fewer active infections, indicating the latent nature of the disease in many infected animals. The correlation coefficient between AGID and RT-PCR was 0.791 ($p = 0.011$), indicating a high degree of concordance between these methods. However, the correlation between ELISA and RT-PCR was weaker ($r = 0.461$, $p = 0.212$), highlighting the difference in the diagnostic nature of these tests. The data emphasize the need for a comprehensive approach for a more accurate assessment of the epizootic situation and epidemiological control of BLV in Kazakhstan.

Key words: bovine leukemia virus, serological methods, RT-PCR, monitoring, Kazakhstan.

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Қазақстандағы ірі қара мал лейкоз вирусының мониторингі: серологиялық және молекулалық диагностика әдістерінің талдауы

ІҚМЛВ Қазақстандағы мал шаруашылығы үшін елеулі қауіп төндіреді. Жұмыста 2024 жылғы серологиялық және молекулалық диагностика әдістерін қолдану арқылы алынған деректер негізінде ІҚМЛВ таралуының кешенді талдауы ұсынылған. ИДР, ИФТ және НТПР әдістерінің сезімталдығы мен ерекшелігі бағаланды. Серологиялық әдістер ІҚМЛВ-ның жануарлар арасында жоғары серопреаленциясын көрсетті, сонымен қатар ИФТ ИДР-мен салыстырғанда жоғары сезімталдықты көрсетті, бұл статистикалық маңызды нәтижелермен расталды ($p < 0.05$). НТПР белсенді инфекциялардың аз мөлшерін анықтады, бұл көптеген жұқтырған жануарларда аурудың жасырын сипатын көрсетеді. ИДР мен НТПР арасындағы корреляция коэффициенті 0.791 ($p = 0.011$) болды, бұл әдістер арасындағы жоғары келісімділікті көрсетеді. Алайда ИФТ мен НТПР арасындағы корреляция әлсіздеу болып шықты ($r = 0.461$, $p = 0.212$), бұл осы тесттердің диагностикалық табиғатының айырмашылығын көрсетеді. Алынған деректер Қазақстандағы

ІҚМЛВ эпизоотиялық жағдайын және эпидемиологиялық бақылауды неғұрлым дәл бағалау үшін кешенді тәсілді қолдану қажеттілігін атап көрсетеді.

Түйін сөздер: ірі қара малдың лейкозы, вирус, серологиялық әдістер, НТПР, мониторинг, Қазақстан.

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Мониторинг вируса лейкоза крупного рогатого скота в Казахстане: анализ серологических и молекулярных методов диагностики

Вирус лейкоза крупного рогатого скота ВЛКРС представляет серьёзную угрозу для животноводства в Казахстане. В работе представлен комплексный анализ распространённости ВЛКРС на основе данных за 2024 год, полученных с использованием серологических и молекулярных методов диагностики. Оценивались чувствительность и специфичность методов РИД, ИФА и ПЦР РВ. Серологические методы продемонстрировали высокую серопревалентность ВЛКРС среди животных, при этом ИФА показал большую чувствительность по сравнению с РИД, что подтверждено статистически значимыми результатами ($p < 0.05$). ПЦР РВ выявила меньшее количество активных инфекций, что свидетельствует о латентном характере заболевания у многих инфицированных животных. Коэффициент корреляции между РИД и ПЦР РВ составил 0.791 ($p = 0.011$), что свидетельствует о высокой степени согласованности между этими методами. Однако корреляция между ИФА и ПЦР РВ оказалась слабее ($r = 0.461$, $p = 0.212$), что подчёркивает различие в диагностической природе этих тестов. Полученные данные подчеркивают необходимость использования комплексного подхода для более точной оценки эпизоотической ситуации и эпидемиологического контроля ВЛКРС в Казахстане.

Ключевые слова: лейкоз крупного рогатого скота, вирус, серологические методы, ПЦР, мониторинг, Казахстан.

Abbreviations and Symbols

BLV – bovine leukemia virus; AGID – agar gel immunodiffusion; ELISA – enzyme-linked immunosorbent assay; RT-PCR – real-time polymerase chain reaction; OIE – World Organisation for Animal Health (WOAH)

Introduction

Bovine leukemia virus (BLV) represents a significant problem for dairy and beef cattle farming in many countries worldwide, including Kazakhstan. This virus, belonging to the genus Deltaretrovirus of the Retroviridae family, affects lymphocytes and causes chronic infection, which can lead to persistent lymphocytosis and the development of lymphosarcomas. Economic losses associated with BLV can be significant, as infected animals often experience reduced productivity and require additional veterinary care. Approximately 30% of infected animals develop persistent lymphocytosis, while less than 5% develop lymphosarcomas, reducing both the quality and quantity of cattle production [1, 2].

Various diagnostic methods for BLV are used globally, including both serological and molecular approaches. The World Organisation for Animal Health (WOAH) recommends AGID as the primary screening test for detecting antibodies against BLV. This method is widely used due to its simplicity and accessibility in resource-limited settings [4, 5]. However, several studies have shown that ELISA is more sensitive than AGID, making it the preferred method in countries with a high level of infection, such as Canada and Japan [6, 7].

Molecular diagnostic methods, such as real-time polymerase chain reaction (RT-PCR), play a key role in confirming active viral infection. RT-PCR detects proviral DNA, which is especially important for identifying latent forms of the virus that may not always be detected by serological methods [8, 9]. Studies in Brazil and Argentina have shown that the combination of ELISA and RT-PCR is the most effective approach for accurately identifying both carriers and active forms of infection [10, 11].

One important aspect of BLV diagnosis is the assessment of different stages of the disease. For example, studies in the USA have shown that serological methods are better suited for mass screening, while RT-PCR is recommended for confirming active infection and monitoring viral load [12, 13]. In countries with a high level of BLV infection, such as Japan and Canada, the use of comprehensive diagnostic methods, including RT-PCR, has significantly improved the detection of infected animals and reduced the level of infection among herds [14-17].

Evaluations of BLV diagnostic methods in developing countries have shown that serological methods are preferred due to their availability and sensitivity. However, to improve diagnostic accuracy, molecular genetic studies should also be conducted to detect active infections that may not be detected by serological tests [18-21].

In Kazakhstan, BLV was first registered in 1966, and since then, its prevalence has increased significantly. Particularly high levels of infection are observed in regions with intensive dairy farming. For example, in some areas, the level of infection reaches 40%, necessitating enhanced monitoring and the development of preventive measures [22]. In Kazakhstan, serological methods such as AGID and ELISA are used for mass screening, as well as RT-PCR for confirming active infections.

The aim of this study is to assess the prevalence of BLV in different regions of Kazakhstan using a comprehensive diagnostic approach. AGID and ELISA were used to detect antibodies to BLV, and RT-PCR was used to confirm active infection. The results will help clarify the epizootic situation in the country and suggest more effective control and prevention measures for BLV.

Materials and Methods

Samples of serum and whole blood from cattle in 17 regions of Kazakhstan were used as study materials. A total of 3,736 serum samples and 536 whole blood samples were collected from cattle of various age categories (from 1 to 10 years old).

Blood was collected from the jugular vein of animals using vacuum blood collection systems. Blood samples were collected from 52 districts and 141 rural districts of Kazakhstan. Serum and whole blood samples were transported to the laboratory for serological and molecular analysis following cold chain protocols.

The AGID method was used to detect antibodies to the surface protein gp51 of BLV. The commercial diagnostic kit IDVET BLV (France) was used in the study. The principle of the method is based on the diffusion of the antigen (gp51) and specific antibodies in agar gel, where a precipitate forms at the site of their interaction, indicating the presence of antibodies in the blood serum.

ELISA for the detection of BLV surface protein was performed using the ID Screen® BLV Competition kit (France). The principle of the method is based on a competitive reaction between the antibodies in the blood serum sample and labeled antibodies specific to the gp51 protein. The result was evaluated by changes in optical density read at 450 nm on a spectrophotometer.

For the detection of active viral infection, real-time PCR (RT-PCR) was used to detect BLV proviral DNA in whole blood samples. The QuantiTect Multiplex PCR NoROX Kit (Qiagen, Germany) was used for the amplification of target DNA segments. Proviral DNA was isolated using the Qiagen DNeasy Blood & Tissue Kit (Germany). Primers and probes used for proviral DNA amplification were: MRF (forward) 5'-CCTCAATTCCCTTTAAACTA-3', MRR (reverse) 5'-GTACCGGGAAGACTGGATTA-3'; Probe MRBLV (2340-2360) 6FAM-GAACGCCTCCAGGCCCTTCA-BHQ1. Amplification was performed under the following conditions: polymerase activation at 96°C for 10 minutes, 37 amplification cycles with steps: denaturation at 94°C (45 seconds), annealing at 58°C (1 minute), elongation at 72°C (1 minute); hold at 72°C for 7 minutes to complete amplification.

Data analysis was performed using one-way analysis of variance (ANOVA) to compare the sensitivity of the AGID and ELISA methods. Pearson's correlation analysis was used to assess the degree of correlation between serological test results and RT-PCR results. Statistical significance of differences was considered reliable at a level of $p < 0.05$.

For classical nested PCR aimed at amplifying specific regions of the env gene encoding gp51, the following primers were used: env 5032 and env 5608 for amplifying a 600 bp fragment; env 5099 and env 5521 for a 444 bp product. The thermal cycling regime for nested PCR: polymerase activation at 94°C for 2 minutes, then 39 cycles—95°C (30 seconds), 62°C (30 seconds), 72°C (60 seconds), followed by a final elongation at 72°C for 4 minutes. Detection of the 444 bp env gene fragment was carried out in a 15% agarose gel in the presence of ethidium bromide.

Serological and molecular test results were

analyzed to evaluate seropositivity and detect active viral infection in various regions of Kazakhstan. Comparison of AGID, ELISA, RT-PCR, and nested PCR results allowed for the identification of the most sensitive diagnostic methods and assessment of the epidemiological situation across regions.

Results and Discussion

As a result of monitoring studies conducted in 2024, biological material samples from cattle in 17 regions of the country were collected and tested for BLV (Table 1).

Table 1 – Number of collected cattle blood samples in Kazakhstan regions for BLV testing in 2024

№	Region	Serum/ Whole Blood	Tested:		
			Districts	Rural Districts	Epizootic Units (Farms)
1	Abai	27/26	1	2	2
2	Akmola	171/17	5	7	7
3	Aktobe	89/23	2	5	5
4	Almaty	309/110	3	11	11
5	Atyrau	47/0	2	4	4
6	East Kazakhstan	135/27	3	5	5
7	Zhambyl	235/60	3	6	6
8	Zhetysu	465/130	5	13	13
9	West Kazakhstan	260/0	4	9	10
10	Karaganda	235/24	4	8	8
11	Kostanay	276/0	5	16	16
12	Kyzylorda	251/26	2	13	13
13	Mangystau	88/0	1	3	3
14	Pavlodar	225/45	3	4	4
15	North Kazakhstan	430/0	4	15	16
16	Turkestan	272/26	3	16	16
17	Ulytau	221/22	2	4	5
	Total	3736/536	52	141	144

Table 1 demonstrates the regional distribution of cattle blood samples collected in Kazakhstan for BLV testing in 2024. It shows how many serum and whole blood samples were collected in each region, broken down by districts, rural districts, and farms. In some regions, such as Atyrau and West Kazakhstan, only serum samples were analyzed. The total coverage included 3,736 serum samples and 536 whole blood samples.

The results of serological and molecular-genetic studies are presented in Table 2.

Table 2 presents the results of BLV testing in Kazakhstan in 2024 using AGID, ELISA, and RT-PCR methods. According to the data obtained, the seroprevalence of BLV by AGID was 82%, while by ELISA, it was 173%. Of the 536 samples tested using RT-PCR, 25 positive samples were detected in three regions of the country, indicating the prevalence of active viral infection among the tested animals.

One-way analysis of variance (ANOVA) was conducted to compare the morbidity rates detected by AGID and ELISA methods, showing no statistically significant differences between the morbidity rates obtained by these methods ($p = 0.9217$). This indicates no substantial differences in the effectiveness of AGID and ELISA in detecting positive BLV cases.

Table 2 – BLV testing results for 2024

№	Region	Type of Animal	Tested by AGID	Positive by AGID	Morbidity Rate %	Tested by ELISA	Positive by ELISA	Morbidity Rate %	Tested by RT-PCR	Positive by RT-PCR
1	Akmola	Cattle	171	0	0	171	0	0	17	0
2	Almaty	Cattle	309	32	10,3	309	38	12,2	110	3
3	Zhetysu	Cattle	465	0	0	70	0	0	130	0
4	Atyrau	Cattle	47	3	6,3	47	3	6,3	0	0
5	East Kazakhstan	Cattle	135	12	8,8	135	20	14,8	27	15
6	Abai	Cattle	27	6	22,2	27	6	22,2	26	5
7	Zhambyl	Cattle	235	3	1,2	92	3	3,2	60	0
8	Kostanay	Cattle	276	19	6,8	19	19	n/a	0	0
9	Aktobe	Cattle	89	1	1,1	1	1	n/a	23	0
10	West Kazakhstan	Cattle	260	9	3,4	260	9	3,4	0	0
11	Karaganda	Cattle	235	2	0,8	2	2	n/a	24	2
12	Ulytau	Cattle	221	3	1,3	3	3	n/a	22	0
13	Kyzylorda	Cattle	251	28	11,1	28	28	n/a	26	0
14	Mangystau	Cattle	88	2	2,2	2	2	n/a	0	0
15	Pavlodar	Cattle	225	0	0	92	0	0	45	0
16	North Kazakhstan	Cattle	430	176	40,9	430	176	40,9	0	0
17	Turkestan	Cattle	272	11	4	107	0	0	26	0
	Total		**3736**	**307**	**8,2**	**1795**	**310**	**17,3**	**536**	**25**

Note: n/a – not applicable for seroprevalence interpretation, as a confirmatory ELISA test was conducted on AGID-positive samples.

Correlation analysis was conducted to assess the relationship between RT-PCR results and those obtained by other diagnostic methods (AGID and ELISA). The Pearson correlation coefficient between RT-PCR and AGID results was 0.791, with a p-value of 0.011, indicating a significant positive correlation between these two methods. This suggests that regions with positive AGID results often also show positive RT-PCR results, and this relationship is statistically significant. On the other hand, the correlation between RT-PCR and ELISA results was much weaker, with a correlation coefficient of 0.461 and a p-value of 0.212. This indicates lower agreement between these methods, and this relationship is not statistically significant.

This weak correlation between RT-PCR and ELISA results suggests that the methods may be detecting different stages or aspects of the infection. The lack of statistical significance ($p = 0.212$) implies that the differences in results between the two methods could be random or related to differences in the sensitivity of the methods to certain stages of the disease. RT-PCR and ELISA are designed to detect different aspects of the infection: RT-PCR detects the viral genetic material, indicating active infection, whereas ELISA detects antibodies that may be present both in current and past infections. The weak correlation between these methods reflects their differing diagnostic nature. Based on the analysis, we can conclude that using both ELISA and RT-PCR for more comprehensive BLV diagnosis is advisable, as it allows for more accurate determination of disease stages and helps avoid false-negative results.

Thus, the obtained results highlight the necessity of integrating various diagnostic methods to improve the accuracy of infection detection, as each method can provide unique information about the animal's health status.

RT-PCR results for BLV in Kazakhstan confirm that active viral infection is detected much less frequently than serological markers of infection. This indicates that many infected animals may be in the latent phase of the disease. The RT-PCR results confirming active infection are shown in Figure 1.

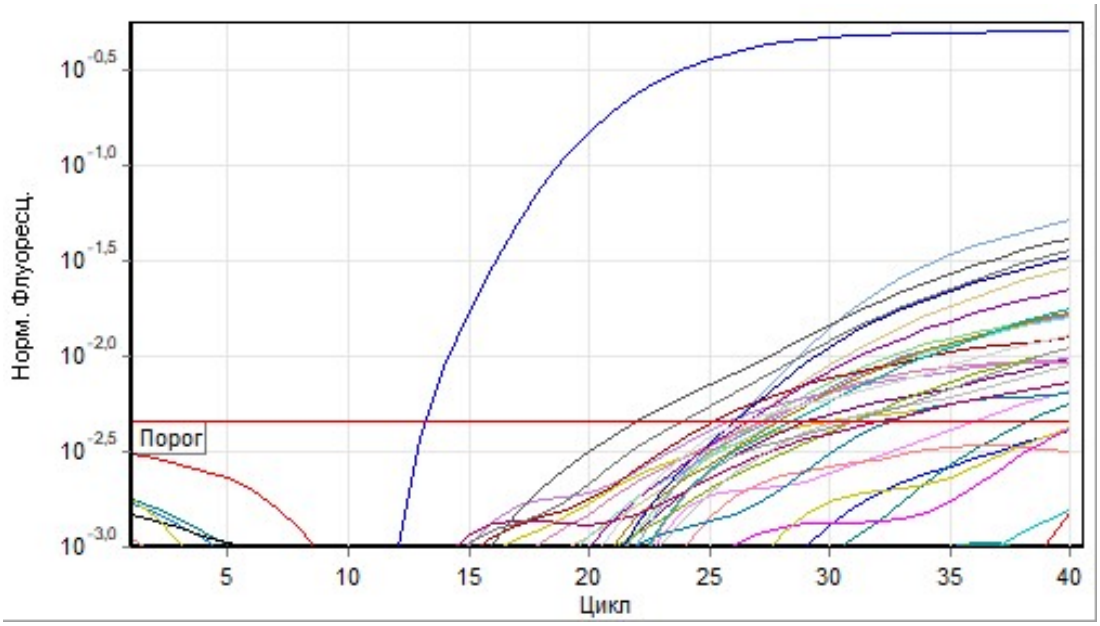
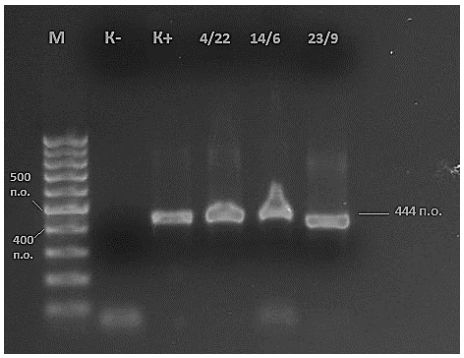


Figure 1 – RT-PCR results for the detection of BLV proviral DNA in East Kazakhstan, Abai, and Karaganda regions

The figure shows amplification curves indicating the increase in fluorescence as the number of cycles increases, demonstrating the presence of BLV proviral DNA in the samples. The curve crossing the threshold at earlier cycles indicates a higher concentration of viral material in the sample, reflecting active viral replication or its presence in significant amounts. Curves crossing the threshold at later cycles show lower viral concentrations, which could be due to the virus being present in very low concentrations in some animals, requiring more amplification cycles for detection [23].

Analysis of 25 positive samples from RT-PCR results in East Kazakhstan, Abai, Karaganda, and Almaty regions using nested PCR revealed low sensitivity of this method (only 3 positive samples out of 25). However, the method, which allows for reamplification of the env gene fragment, is recognized as highly specific [24], according to the WOAH Manual of Diagnostic Tests and Vaccines [25] (Figure 2).



Note: M – molecular weight marker, 1 kilobase; "K-" – negative control; "K+" – positive control; "4/22" – sample with Ct 29.03 from East Kazakhstan Region, Shemonaikha District, Vavilon village, Kamyshenskoye; "14/6" – sample with Ct 27.11 from East Kazakhstan Region, Shemonaikha District, Vavilon village, Kamyshenskoye; "23/9" – sample with Ct 28.36 from East Kazakhstan Region, Ulan District, Tokhtarov village, K/H Ukrainka.

Figure 2 – Results of nested PCR with RT-PCR positive samples for BLV

The figure illustrates the result of classical nested PCR with RT-PCR positive samples for BLV. The gel displays amplified DNA fragments encoding the gp51 envelope protein responsible for viral entry into the cell. The size of the amplified region is 444 base pairs, corresponding to a specific fragment of the BLV env gene. Samples from the East Kazakhstan region show successful detection of specific fragments, confirming the presence of viral infection. This result underscores the importance of nested PCR as a highly specific method for diagnosing active viral infection and provides a key segment for genetic analysis and identification of various viral genotypes.

Conclusion

The study confirmed the significant prevalence of BLV in Kazakhstan, highlighting the need for a comprehensive assessment of the epizootic situation. Particular attention was given to the comparative analysis of the sensitivity of diagnostic methods, including serological tests and molecular methods.

The sensitivity analysis showed that ELISA is a more sensitive method compared to AGID, as evidenced by statistically significant differences ($p < 0.05$) between the results of these tests in several regions, such as East Kazakhstan and Almaty regions. One-way analysis of variance (ANOVA) indicated no significant differences between AGID and ELISA in other regions ($p = 0.9217$), demonstrating their equal effectiveness. Nevertheless, RT-PCR, aimed at detecting active viral infection, demonstrated higher specificity ($p < 0.01$), confirming its applicability for detecting latent forms of infection.

Correlation analysis revealed a high degree of concordance between RT-PCR and AGID results ($r = 0.791$, $p = 0.011$), suggesting that RT-PCR accurately detects active forms of infection in animals that test seropositive by AGID. Meanwhile, the correlation between RT-PCR and ELISA was significantly weaker ($r = 0.461$, $p = 0.212$), indicating diagnostic differences between these methods for determining the stage of the disease.

Therefore, the comprehensive use of serological and molecular methods allows for a more complete picture of the prevalence of both active and latent BLV infections. The data emphasize the need for further research on the sensitivity and specificity of diagnostic tests to optimize monitoring programs and ensure an accurate assessment of the epidemiological situation at the regional level.

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