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Comparative assessment of immunodiffusion and enzyme-linked immunosorbent assay used for bovine leukosis diagnosis

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SUMMARY
The implementation of animal health improvement and disease prevention activities with respect to bovine leukosis involves a need for timely detection of cattle infected with bovine leukaemia virus (BLV) on livestock farms. That is why early diagnosis using more sensitive and highly accurate methods is of particular importance. The paper presents the results of cattle serum tests for bovine leukosis with enzyme-linked immunosorbent assay (ELISA) and immunodiffusion (ID), as well as the comparative assessment of their effectiveness. A total of 440 cattle blood samples were subjected to serological testing with immunodiffusion; 37 (8.4%) of them tested positive for bovine leukaemia virus. The cattle blood samples were submitted from the Kumtorkalinsky (127), Karabudakhkentsky (122), Buynaksky (89) Raions, from Makhachkala (56) and Kaspiysk (46). Seropositivity was 17 (13.4%), 8 (6.6%), 5 (5.6%), 4 (7.1%) and 3 (6.5%), respectively. For the comparative assessment of the diagnostic tests, 100 (5 ID-positive and 95 ID-negative) serum samples were taken and tested with ELISA. As a result, specific antibodies against BLV gp51 antigen were detected in 4 ID-negative serum samples. All ID-positive serum samples also tested positive with ELISA. All in all, 9 virus carriers were detected with ELISA, that is 44.4% more than with immunodiffusion. Thus, enzyme-linked immunosorbent assay is characterized by a higher sensitivity, as compared with immunodiffusion, and allows for improved detection of infected animals. However, alongside the advantages, this technique has certain disadvantages, one of which is the high price of the diagnostic test kit for anti-BLV antibody detection and the equipment required.

Keywords: bovine leukaemia virus, specific antibodies, seropositivity, comparative assessment, immunodiffusion, enzyme-linked immunosorbent assay, sensitivity and specificity of method

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Сравнительные аспекты диагностики лейкоза крупного рогатого скота при применении реакции иммунодиффузии и иммуноферментного анализа

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РЕЗЮМЕ
При проведении оздоровительно-профилактических мероприятий при лейкозе крупного рогатого скота возникает необходимость своевременного выявления инфицированного вирусом лейкоза поголовья в животноводческих хозяйствах. Поэтому важное значение имеет ранняя диагностика с применением более чувствительных и высокоточных методов. В статье приводятся результаты исследования сывороток крови животных на лейкоз крупного рогатого скота методом иммуноферментного анализа и в реакции иммунодиффузии и изучения их эффективности в сравнительном аспекте. Всего серологическим методом с применением реакции иммунодиффузии было исследовано 440 проб крови крупного рогатого скота, из них 37 (8,4%) оказались сероположительными к вирусу лейкоза. Пробы крови животных были получены из Кумторкалинского (127), Карабудахкентского (122), Буйнакского (89) районов, г. Махачкалы (56) и г. Каспийска (46). Серопозитивность соответственно составила 17 (13,4%), 8 (6,6%), 5 (5,6%), 4 (7,1%) и 3 (6,5%). С целью сравнительного анализа диагностических тестов было отобрано 100 проб сывороток крови: 5 – РИД-положительных и 95 – РИД-отрицательных, которые

исследовали методом иммуноферментного анализа. В результате в четырех РИД-отрицательных пробах выявлены специфические антитела к антигену gp51 вируса лейкоза крупного рогатого скота. При этом все РИД-позитивные пробы сывороток крови методом иммуноферментного анализа также определены как серопозитивные. В общей сложности иммуноферментным анализом было выявлено 9 вирусоносителей, что на 44,4% больше, чем обнаружено с помощью реакции иммунодиффузии. Таким образом, метод иммуноферментного анализа в сравнении с реакцией иммунодиффузии характеризуется более высокой чувствительностью и позволяет дополнительно выявлять инфицированных животных. Однако данный метод кроме преимуществ имеет и ряд недостатков, одним из которых является высокая стоимость диагностического набора для выявления антител к вирусу лейкоза крупного рогатого скота и используемого оборудования.

Ключевые слова: вирус лейкоза крупного рогатого скота, специфические антитела, серопозитивность, сравнительный анализ, реакция иммунодиффузии, иммуноферментный анализ, чувствительность и специфичность метода

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INTRODUCTION

Under current conditions of animal husbandry, a continuous control over the movement of cattle latently infected with bovine leukemia virus (BLV) is largely absent. Despite measures taken to combat bovine leukosis caused by this virus, the disease tends to be widely spread worldwide, including in the Russian Federation Subjects [1–5]. The main reasons for this include factors such as late slaughter of BLV infected animals, lack of animal health improvement and disease prevention activities, untimely diagnostic testing, etc. That is why early diagnosis using more sensitive and highly accurate tests and methods is of particular importance [6–11].

At present, immunodiffusion (ID) being a standard and proven method is commonly used for bovine leukosis diagnosis at all the veterinary laboratories, diagnostic units, veterinary clinics of the Republic of Dagestan. The advantage of immunodiffusion over other techniques is that ID testing of one sample is 5–7 times cheaper as compared with similar test systems. Other advantages are that it is easy to perform and does not require any special equipment, thus being easily applicable at the diagnostic units in the distant areas of the Republic. However, immunodiffusion has certain disadvantages such as non-specific reactions, low sensitivity of anti-BLV antigen antibody detection, test reading not earlier than after 48 hours, as demonstrated by multiple studies on bovine leukosis diagnosis [12–14].

Given these drawbacks, animal serum samples should be tested for bovine leukosis using enzyme-linked immunosorbent assay (ELISA); besides, there is a need for the comparative assessment of both techniques for their effectiveness. Many authors provide in their papers the evidence of ELISA being a more sensitive method when it comes to the detection of specific antibodies against BLV antigen as compared with immunodiffusion [15–16]. ELISA can be applied within animal health improvement and disease prevention activities with respect to bovine leukosis, since they necessitate the timely detection of BLV infected cattle on livestock farms [16–20].

In view of the above, the aim of the study was to perform the comparative assessment of ELISA and immunodiffusion test systems used for animal serum tests for detection of specific antibodies against BLV antigen in accordance with methodical guidelines.

MATERIALS AND METHODS

Animal serum samples submitted from different farms of the Karabudakhkentsky, Kumtorkalinsky and Buynaksky Raions, as well as from Makhachkala and Kaspiysk were used as material for diagnostic tests for bovine leukosis with immunodiffusion and ELISA.

Immunodiffusion tests were carried out using the test kit for serological diagnosis of bovine leukosis manufactured by the FKP "Kursk Biofactory – BIOK Company" (Russia). This test system is designed for detection of antibodies against BLV glycoprotein antigen with agar gel precipitation test.

ELISA tests involved the use of the test kit for detection of specific antibodies against BLV gp51 in the individual and pooled samples of cattle blood serum or plasma and milk manufactured by the OOO "Vetbiokhim" (Russia).

All the diagnostic tests were performed in accordance with the "Methodical guidelines for diagnosis of bovine leukosis"[1].

RESULTS AND DISCUSSION

The diagnostic tests of 440 cattle serum samples for bovine leukosis with immunodiffusion were carried out at the Laboratory of Infectious Pathology of Farm

[1] Methodical guidelines for diagnosis of bovine leukosis: approved by the Veterinary Department of the Ministry of Agriculture of the Russian Federation on 23 August 2000 No. 13-7-2/2130. Available at: http://docs.cntd.ru/document/1200118749.

Table
Results of diagnostic tests of animal serum samples submitted from farms of the Republic of Dagestan for bovine leukosis with immunodiffusion and ELISA

Raions and municipalities	Tested with immunodiffusion (ID)			Tested with ELISA		
	number of samples	ID (+)	%	out of ID (–)	out of ID (+)	ELISA (+)
Kumtorkalinsky	127	17	13.4	48	2	3 (+1)
Karabudakhkentsky	122	8	6.6	29	1	3 (+2)
Buynaksky	89	5	5.6	8	2	2
Makhachkala	56	4	7.1	5	0	1 (+1)
Kaspiysk	46	3	6.5	5	0	0
Total	440	37	8.4	100 (95 + 5)		9 (9.0%)

Animals of the Caspian Regional Research Veterinary Institute – Branch of Dagestan Agriculture Science Center in 2022. Of these, 37 samples (8.4% of the test animals) tested positive for bovine leukemia virus. The cattle serum samples were submitted from the following raions and municipalities: the Kumtorkalinsky Raion – 127, the Karabudakhkentsky Raion – 122, the Buynaksky Raion – 89, Makhachkala – 56 and Kaspiysk – 46. The seropositivity in these raions and municipalities was 17 (13.4%), 8 (6.6%), 5 (5.6%), 4 (7.1%), 3 (6.5%), respectively (Table).

At the next stage of the study, 100 cattle serum samples with different serological statuses (based on the immunodiffusion test results) were taken from the 440 samples for ELISA testing: from the Kumtorkalinsky Raion – 50 (48/2), from the Karabudakhkentsky Raion – 30 (29/1), from the Buynaksky Raion – 10 (8/2), from Makhachkala – 5 (5/0) and from Kaspiysk – 5 (5/0). ELISA revealed the presence of specific antibodies against BLV gp51 in 4 samples that had tested negative with immunodiffusion. ELISA tests also detected specific antibodies against BLV antigen in all the ID-positive serum samples.

Thus, a total of 9 virus carriers were detected with ELISA, i.e. 44.4% more than with immunodiffusion (Figure).

The results presented in the Table and the Figure show that ELISA is a more sensitive method. It should be noted that non-specific reactions were observed in some animal serum samples tested with ELISA and the test results for 6 samples were found to be inconclusive. This is probably due to the fact that ELISA is associated with certain limitations; in particular, hemolyzed and contaminated animal serum samples, as well as those subjected to multiple freezing and thawing are not suitable for testing. However, as regards bovine leukosis diagnosis, this technique has certain advantages such as high sensitivity in detection of specific antibodies against BLV gp51, fast availability of test results, the use of a minimal amount of test material (4 µL of serum). The main disadvantages of ELISA include the high price of the diagnostic test kit, as well as the need for availability of a spectrophotometer (reader)

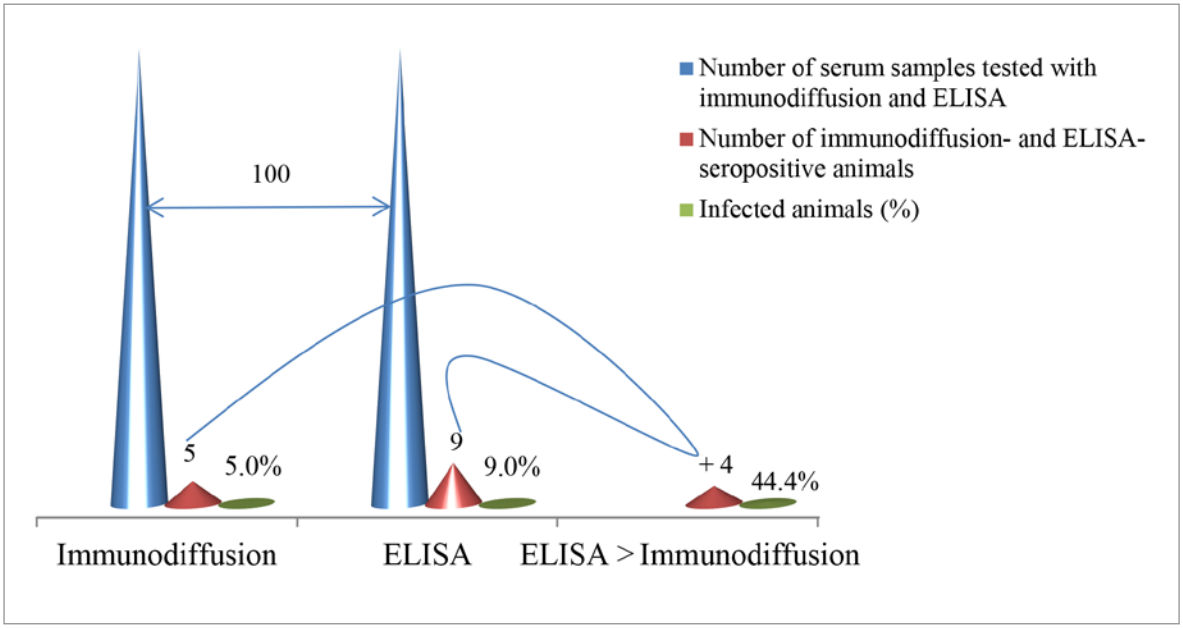

Fig. Comparative assessment of the results of immunodiffusion and ELISA tests of animal serum samples for antibodies against bovine leukemia virus

at the laboratory to be used to measure optical density at a wavelength of 450 nm.

In the light of the above, it can be concluded that ELISA is characterized by a higher sensitivity, as compared with immunodiffusion, and allows for improved detection of BLV infected animals.

CONCLUSION

The comparative assessment of immunodiffusion and ELISA used for bovine leukosis diagnosis shows that ELISA is characterized by higher specificity and sensitivity than immunodiffusion. The ELISA tests of 100 cattle serum samples detected 9 BLV-carriers, whereas only 5 samples had tested positive with immunodiffusion. Thus, the ELISA tests detected about 44.4% more reactors. Such high percentage can be possibly attributed to a small size of the sample of cattle serum samples out of those tested with immunodiffusion. In case of a large-scale ELISA testing for bovine leukosis, the percentage of detected BLV infected animals would probably be lower, ranging between 15 and 30% and thus being consistent with the data provided by other researches in their papers [11, 18].

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