IMMUNOFERMENT ANALYSIS AND COMPARATIVE ASSESSMENT OF IMMUNODIFFUSION REACTION IN THE DIAGNOSIS OF LARGE HORNED ANIMAL LEUKOSIS

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Annotation:
When carrying out wellness and preventive measures against cattle leukosis, the need arises for timely early detection of livestock infected with the leukosis virus on livestock farms. Therefore, early diagnosis using ultra-sensitive and high-resolution methods is considered important. The article presents the results of an examination of animal serum for cattle leukosis using immunoferment analysis and immunodiffusion reaction, as well as a comparative study of the effectiveness of these methods.

Keywords: large horned animal leukosis virus, special antibodies, seropositivity, comparative evaluation, immunodiffusion reaction, immunoferment analysis, method specificity and sensitivity.

Introduction

In modern animal husbandry conditions, constant monitoring of the movement of livestock infected with the giant horned bovine leukemia virus (BLV) is largely non-transferable. Despite measures aimed at combating cattle leukosis, the causative agent of which is this virus, the disease can spread on a large scale in the world, as well as in breeding farms of the Republic of Uzbekistan. [1,2,3]. The main reasons for this are the lack of timely delivery for slaughter of animals infected with BLV, the lack of compliance with health and preventive measures, the absence of timely diagnostic tests and other similar factors. Therefore, it is considered important to make an early diagnosis and follow a complex set of measures using extremely sensitive, high-precision reactions and methods. [4,5,6].

Currently, standard, approved immunodiffusion reaction (IDR) is used to diagnose all animal diseases and diagnose mainly cattle leukemia in food safety centers, veterinary clinics. The advantage of the method is that the cost of studying one sample is 5-7 times less than that performed by other methods. Also, the advantage of this reaction is the simplicity of the formula, easily applied in diagnostic rooms located in remote regions of the Republic and the absence of the need for special equipment. Nevertheless, IDR has a number of disadvantages: nonspecific reactions, low sensitivity, reaction duration (48 hours), which is confirmed by many researchers in the field of diagnosis of cattle leukosis [7,8,9].

Due to the aforementioned disadvantages of IDR, it will be necessary to carry out a diagnostic examination of animal blood serum for large horned animal leukosis using immunoferment analysis (ELISA – enzyme linked Immuno Sorbent Assau) and a comparative study of the effectiveness of both methods. In scientific research work, many authors prove that ELISA is a much more sensitive method than IDR to detect antibodies specific to the BLV antigen [10,11,12]. The Immunoferment analysis (IFA) method can be used to carry out health and preventive measures against cattle leukosis, since it requires timely detection of animals infected with BLV on livestock farms [13-14].

Based on the above, the purpose was set to conduct a comparative analysis of the ELISA and IDR test systems in the study of animal blood serum to identify antibodies specific to the yshhlv antigen based on methodological guidelines.

Materials and methods

Blood serum samples of animals from various farms of the Kiziltepa, Konimex and Karmana districts, as well as from the Jondor and Romitan districts, served as a material for diagnosing cow leukemia using IDR and IFA.

A set of serological diagnostics of cow leukemia was used to conduct IDR. Kursk Biofabrics diagnostic kit made by the BIOK company (Russia) was used. This testing system is designed to detect yshhlv antibodies against the animal serum glycoprotein 51 antigen in agar gel.

For ELISA – immunoferment analysis, a kit was used to identify antibodies specific to the yshhlv gp 51 antigen in blood and cattle milk serum in samples produced by Kursk Biofabrics (Russia), intended for screening and validation.

All diagnostic studies were according to 'the indications for the diagnosis of cow leukemia.'

Results and discussions

In 2021-2022, diagnostic studies were carried out in 440 blood serum samples in the conditions of the virology laboratory of the Veterinary Research Institute of Uzbekistan. An Immunoferment analysis (ELISA) reaction found 9 head virus carriers, which showed that 44.4% more samples had anti-viral antibodies than immunodiffusion. Thus, the immunoferment analysis method is characterized by hypersensitivity compared to the immunodiffusion reaction and allows for improved detection of virus-infected animals. However, in addition to the advantages, this method has certain disadvantages, one of which is the high cost of a diagnostic test kit for the detection of antibodies against BLV, as well as the cost of the necessary reagent and equipment.

37 of the animals ' blood gave a seropositive result to BLV, which was 8.4% of the number of Animals examined. These samples of cattle blood serum were taken from the following districts: Kiziltepa – 127, Konimex – 122, Karmana – 89, Jondor – 56 and Romitan – 46.

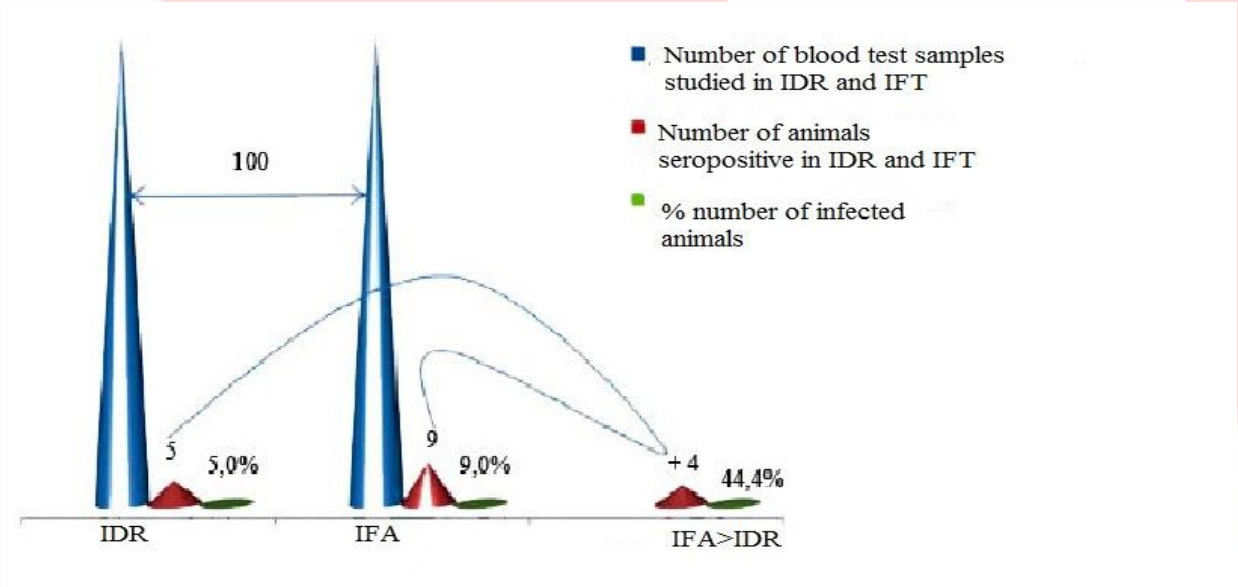
Seropositivity in these districts 17 (13,4%), 8 (6,6%), 5 (5,6%), 4 (7,1%), 3 (6,5%) accordingly (Table 1).

Table 1. Results of diagnostic tests of samples of animal blood serum taken from certain farms of the Republic of Uzbekistan on cow leukosis using IDR and ELISA

Subdistricts	Checked at IDR			Checked at IFA		
	Sample number	IDR +	%	From IDR (-)	From IDR (+)	IFA (+)
Kiziltepa	127	17	13,4	48	2	3 (+1)
Konimex	122	8	6,6	29	1	3 (+2)
Karmana	89	5	5,6	8	2	2
Jondor	56	4	7,1	5	0	1 (+1)
Romitan	46	3	6,5	5	0	0
Total	440	37	8,4	100 (95+5)	5	9 (9,0%)

In the next step, samples with serological status were selected from 440 cattle serum samples for IFA examination: from Kiziltepa District – 50 (48/2), Konimex -30 (29/1), Karmana – 10 (8/2), Jondor -5 (5/0), as well as from Romitan - 5 (5/0). As a result, antibodies against the specific GP 51 antigen of Yshhlv were detected in 4 samples that reacted negatively in IDR, in the ELISA – immunoferment analysis reaction. At the same time, all IDR - positive serum samples using the ELISA method also showed antibodies specific to the yshhlv antigen.

Thus, a total of 9 virus carriers were detected in Elisa, which accounted for 44.4% more than those detected using IDR (fig.1).



Picture1. Results of comparative serological tests of animal serum samples for the presence of antibodies against BLV by IDR and IFA methods.

The results presented in the table and figure show that immunoferment analysis is a more sensitive method. It is important to note that when immunoferment analysis was performed, nonspecific reactions were observed in the blood serum of some animals, and 6 samples were assessed as suspicious. Perhaps this is due to the fact that immunoferment analysis has a number of limitations, for example, blood serums of hemolyzed and contaminated animals are unsuitable for research and cannot be used in their repeated frozen and dissolved state. However, this method has a number of advantages in the diagnosis of cow leukemia: hypersensitivity in the detection of antibodies specific to the GP51 antigen of gp51 BLV, rapid results, the use of a minimum amount of test material (4 ml of blood serum). Among the main disadvantages of IFA is the high cost of the diagnostic kit, as well as the need for spectrophotometers (result reading) in the laboratory to measure optical density with a wavelength of 450 nm.

Based on the above, it can be concluded that IFA is characterized by hypersensitivity compared to IDR and allows additional identification of animals infected with large horned animal leukosis.

Conclusion

When comparing two methods for diagnosing giant horned animal leukosis, immunoferment analysis (IFA) showed higher results of specificity and sensitivity than immunodiffusion reaction (IDR). In IFA, 100 serum samples of large horned animals were tested to reveal 9 virus carriers, while only 5 positive samples were taken in IDR. That is, the number of animals additionally identified using IFA was 44.4%. Such a high percentage may be associated with a low number of cow blood serum samples from among those studied using IFA. The percentage of detection of animals infected with BLV in mass research of livestock farms for cattle leukosis by the IFA IDR method can be low and ranges from 15 to 30%, as indicated in the scientific articles of other researchers [4,6]

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