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Diagnostic Value of IHR in Comparison with RBT and Other Serological Reactions in Small Cattle Brucellosis

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Abstract

The article presents statistical data on the prevalence of infectious diseases of small cattle on the territory of the Republic of Dagestan, describes the epizootic situation for sheep and goats brucellosis, indicates the number of animals studied, as well as the number of disadvantaged areas and affected animals. To study the diagnostic value of the indirect hemagglutination reaction (IHR), in comparison with the rose bengal test (RBT) and other serological methods (Agglutination reaction (AR), Complement fixation test (CFT), and IDR with O-polysaccharide (OPS) antigen), 496 ewes from a brucellosis-free farm and 589 heads from affected farms were examined. The rose-bengal test revealed brucellosis in 114 (20.4%) ewes, second only to the indirect hemagglutination reaction. Studies have confirmed the specificity, high sensitivity and suitability of the rose-bengal test for the diagnosis of brucellosis in small cattle.

JEL Classification: D71, D72, H76, J13, L31, Z13.

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1 Introduction

One of the main tasks of the Agro-industrial complex in the field of agriculture is to achieve a high level of animal husbandry, which is necessary to fully meet the needs of the population in animal products, as well as raw materials (leather, wool) for consumer goods industry.

Ensuring the well-being of animal husbandry for infectious diseases and, in particular, for such a disease as brucellosis, since this infection causes significant economic damage to the industry, is an important criterion for solving this problem. The damage caused to farms consists of such factors as: a decrease in animals productivity, a violation of breeding work and reproduction of the herd, youngness of sheep, postpartum complications, abortions and a birth of non-viable litter. To the material costs, it is necessary to add funds aimed at carrying out veterinary-sanitary and quarantine-restrictive measures.

The elimination of animal brucellosis is very important in epidemiological terms, since affected animals pose a great threat to human health, being a source and reservoir of infection.

The most dangerous for humans is brucellosis of sheep and goats caused by B. melitensis, for this reason, the recovery of sheep from brucellosis is not only economic, but also of great social importance (Nicolleti, 2005). In humans, brucellosis is characterized by a tendency to a chronic course with a long-term persistence of the pathogen and a high risk of disability, which determines the social significance of this infection.

The current monitoring system for brucellosis is aimed at controlling the appearance and development of the brucellosis process, for this purpose, various diagnostic methods are used, mainly serological (IHR, RBT, AR, complement fixation test (CFT) and immunodiffusion reaction (IDR) and bacteriological studies. The effectiveness of the measures taken depends on the specificity and sensitivity of the diagnostic tools and methods used.

In our country, for many years, for the diagnosis of brucellosis, along with the use of AR and CFT, an accelerated (plate) agglutination reaction (pl. AR) on glass was tested, proposed by Huddlson (1927) and many authors have established its diagnostic value. Since 1963, pl. AR has been officially adopted as an express method for the diagnosis of brucellosis in small cattle and reindeer. In animals of other species, this reaction has not found application, since cases of non-specific reactions have been noted in obviously healthy animals (Gulyukin et al., 2019; Abdelbaset et al., 2018; Purcell & Rivard, 2012).

In an acidic environment, the inhibition of non-specific AR occurs, while the severity of a specific reaction does not change. Piettz and Nicoletti (1967) developed a plate AR on a cardboard card with blood plasma and acid buffered antigen stained with rose bengal (card test) (Ducrotoy & Bardosh, 2017).

A modification of this reaction with blood serum, proposed by an English researcher, was called the rose bengal sampling or rose Bengal test (RBT).

On the recommendation of the FAO/WHO Committee of Experts on Brucellosis, the rose bengal test is used in many countries around the world.

In our country, the RBT was adopted for implementation in veterinary practice in 1978. Industrial technology of manufacturing, standardization and control of antigen for RBT was developed by Kasyanov (1987). The antigen for this reaction is prepared from a culture of a highly agglutinable weakly virulent strain of B. abortus 19, inactivated by heating and phenol, colored with rose bengal and suspended in a lactic acid buffer solution with a pH of 3.65 (Gulyukin et al., 2018; Turdiev et al., 2019).

The diagnostic value of this reaction has been comprehensively studied by domestic and foreign researchers, who have established that RBT is a specific and highly sensitive method for brucellosis diagnostics. Most authors believe that the advantage of this reaction also lies in its ability to detect brucellosis patients in animals at an earlier time after infection (Arakelyan & Dimov, 2013; Gunashev et al., 2020; Kabardiev & Yusupov, 2018; Orakbay et al., 2015; Rasulov et al., 2017; Turdiev et al., 2019).

When testing RBT in a wide production experience, in comparison with other generally accepted methods (AR, CFT) for brucellosis diagnostics in animals of different species, it was found that this test is highly specific and gives negative results in all cases with blood serum of animals healthy for brucellosis or affected with other diseases. Moreover, in most animals with brucellosis, RBT appeared earlier and captured specific antibodies for a longer time than other serological reactions (Turdiev et al., 2019).

It was found that with the help of RBT in chronic and fresh foci of bovine brucellosis, more infected animals can be detected than with AR and CFT, and it was recommended to use this reaction as the main method for diagnosing brucellosis.

Many authors have concluded that RBT is a highly specific and sufficiently sensitive response to the detection of anti-brucellosis antibodies. Nevertheless, in the early stages of antibody formation, it can be inferior in the sensitivity of AR and especially IHR, which is associated with the characteristics of specific antibodies formed during this period (Arakelyan et al., 2010; Arakelyan & Dimov, 2013; Rasulov et al., 2017).

In 2010-2015, blood serum from 16,147 sheep and goats was tested for brucellosis using RBT in comparison with AR, CFT, and EIA in the Republic of Tajikistan. To clarify the diagnosis, 183 sera from those who responded positively to brucellosis and 59 samples from those who did not respond when tested by C-ELISA were subjected to a selective PCR study. The analysis of the results of the conducted studies showed that the rose Bengal test (RBT) is the most sensitive among the traditional methods of serological diagnosis of brucellosis, but less specific and sensitive, in comparison with C-ELISA (Rasulov et al., 2017; Rahman et al., 2013)

At the same time, in the Russian Federation, a new method for brucellosis diagnostics was adopted in veterinary practice – the indirect hemagglutination reaction (IHR) with the use of an erythrocyte antigen, produced as a Set for serological diagnostics of brucellosis in large and small cattle in IHR (Kabardiev & Yusupov, 2018; Khalikov, 2017; Yusupov et al., 2015, Yusupov et al., 2018).

At present, based on a large amount of factual material, it has been established that IHR with the use of this Set can detect brucellosis in animals that react both in AR and CFT. IHR is of particular value for conducting studies to control the well-being of herds for brucellosis, as it allows to identify animals infected with brucellosis in fresh cases of infection that do not respond to AR, CFT and other serological reactions (Arakelyan et al., 2010; Kabardiev & Yusupov, 2018; Ulasevich et al., 1980; Khalikov, 2017).

Given the above, the test of the IHR diagnostic value using a Set for serological diagnosis of brucellosis of large and small cattle in indirect hemagglutination reaction, in comparison with RBT and other diagnostic methods for brucellosis of sheep and goat is of a great scientific and practical interest.

2 Materials and Methods

To study the nosologic profile of infectious diseases of small cattle on the territory of the Republic of Dagestan, an analysis of the reporting data of the Veterinary Committee of the Republic of Dagestan for 2020 was carried out.

In order to test the diagnostic value of IHR, in comparison with RBT, AR, CFT, and IDR with O-polysaccharide (OPS) antigen in sheep brucellosis, the blood sera of 496 ewes of the brucellosis-free farm, 6 aborted and kept with them in the same flock, 24 normally lambed ewes, and 559 ewes of the brucellosis-affected flock were examined.

RBT, AR, CFT were set according to the "Manual for the diagnosis of brucellosis of animals" (2003), IHR – in accordance with the "Instructions for the use of a set for serological diagnostics of brucellosis of

large and small cattle in the reaction of indirect hemagglutination (IHR)", approved by the Rosselkhoznadzor (2006) with the use of brucellosis erythrocyte antigen, manufactured by Vetmedservice LLC (the Republic of Dagestan, city of Makhachkala) according to the method developed by Caspian zonal NIVI, VGNKI and VNIIBTZH.

3 Results and Discussion

In the nosologic profile of infectious diseases of small cattle, brucellosis takes the first place in the prevalence in the Republic of Dagestan and is 87% (Figure 1).

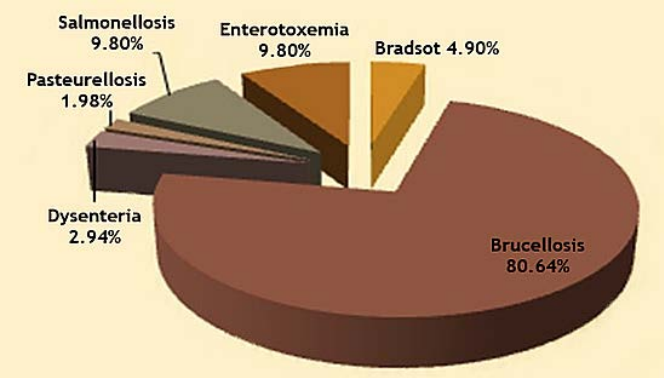

Figure 1: Nosologic profile of infectious diseases of small cattle in the Republic of Dagestan (by the number of affected animals) in 2020.

The epizootic situation of small cattle brucellosis in the Republic of Dagestan continues to be complex and tense. During serological diagnostics for 2020, 82 sick animals were identified out of the 411.6 thousand heads examined in 5 unfavorable localities.

When studying the blood sera of 496 healthy brucellosis ewes of the brucellosis-free farm, negative results were obtained in all samples (Table 1). Table 2 shows the results of the blood sera of six aborted and 24 normally lambed ewes.

Table 1: Results of a study in IHR, RBT, AR, CFT of blood sera of sheep of the brucellosis-free farm.

Blood sera examined	Quantity	IHR	RBT	AR	CFT
From healthy sheep of brucellosis-free farms	496	-	-	-	-

Note: "-" refers to negative reaction

Table 2: Results of the IHR test, in comparison with RBT and other diagnostic methods for the study of sheep blood serum brucellosis in a fresh focus of infection.

Ewe No.	Lambing results	IHR	RBT	AR	CFT
3212	abortion	800+++	+++	200+++	- # #
5434	abortion	800+++	+++	50+++	- # #
8658	abortion	-	-	-	-
3706	abortion	200+++	-	25+++	- # -
6793	abortion	800+++	#	200#	- # #
2973	abortion	800#	-	200#	- # #
1991	norm. lamb	-	++	-	-
1155	norm. lamb	100+++	+++	25+++	-
3935	norm. lamb	-	++	-	-
8806	norm. lamb	-	++	-	-
7647	norm. lamb	100+++	-	25++	- ++ -

Note: "- " indicates a negative result; "+" positive result; "#" positive result of 4 cross.

In 19 others normally lambed sheep, negative results were obtained for all reactions.

Table 2 shows that all aborted ewes, except for one that did not react in any reaction, received positive IHR results in diagnostic titers, which in all cases coincided with positive CFT. In two of the five aborted sheep that responded to IHR, AR, and CFT, the RBT readings were negative.

At the same time, in three out of 24, normally lambed sheep RBT was positive with negative indications of all other reactions.

In the brucellosis-affected farm, where an acute outbreak of small-bovine brucellosis was registered in the previous year, accompanied by mass abortions and a large number of isolated animals with brucellosis, the blood sera of 559 ewes were examined in order to study the diagnostic value of IHR in comparison with RBT and other serological reactions (AR, CFT, IDR with OPS antigen) (Table 3).

Table 3: Results of the IHR test for the diagnosis of sheep brucellosis, in comparison with IDR and other serological reactions, in an acute focus of brucellosis infection

IHR		RBT	AR, ME						CFT					AR+CFT complex	IDR
titre	qty		25	50	100	200	400	Total	1:5	1:10	1:20	1:40	Total		
1:25	26	24							5	2			7	7	1
1:50	62	33	1	5	1			7	12	6	4	6	28	33	3
1:100	26	14		7	1			8	3	5	3	9	20	20	1
1:200	23	12	2	6	4	7		19	2	2	4	10	18	23	2
1:400	27	20		7	11	6	1	25			2	21	23	26	11
1:800	11	8		2	5	3	1	11				10	10	11	8
1:1600	6	3		-		2	4	6				6	6	6	4
Total	181	114	3	27	22	18	6	76	22	15	13	62	112	129	30
incl. positive	155	114				73		73	112				112	126	30
%	27.7	20.4						13.1					20.0	22.5	5.4
controvers.	26		3					3					-	3	
negative	378	445						483					447	430	529
In total	559	559						559					559	559	559

The comparative analysis results showed that, despite the absence of abortions, the infection in the flock was active, as evidenced by a high percentage of animals responding positively to brucellosis and high titers of IHR, AR and CFT (Table 3). Of the 559 sheep heads studied, positive IHR was obtained with blood sera of 155 (27.7%), positive RBT – 114 (20.4%), AR – 73 (13.1%), CFT – 112 (20.0%), and IDR – 30 (5.4) heads. The AR+CFT complex (considering the matching reactions) revealed brucellosis in 126 (22.5%) ewes.

The most sensitive diagnostic response was IHR, which revealed 27.7% of patients with sheep brucellosis, while with the help of RBT, the diagnosis of brucellosis was established only in 20.4% of animals.

All the sheep, both with positive and doubtful indications of RBT, AR, CFT, and IDR, reacted in the IHR as well. At the same time, in comparison with these reactions, IHR revealed in addition to RBT – 41(7.3%), AR+CFT – 29 (5.2%), IDR – 125 (22.4%) animals that responded positively to brucellosis. The results of 26 ewes that reacted doubtfully in IHR in a titer of 1:25 coincided with other serological reactions: in 24 cases - with the results of RBT, 7-CFT, one - IDR.

4 Conclusion

From this study, it is found that the vast majority of sheep (23 heads out of 30), in whose serum precipitins were detected using the IDR with OPS antigen, reacted in IHR in the titer of 1:400 and CFT - 1:40 (22 heads), 1: 20 (1 head), the rest (7 heads) reacted in IHR and CFT in diagnostic titers.

AR with a single brucellosis antigen was less sensitive for the diagnosis of sheep brucellosis, which does not show 82 infected sheep compared to IHR, which is 52.9% of the number of animals that responded positively to IHR. AR was used to identify 73 sheep that responded positively to brucellosis (13.1%), while the use of IHR allowed to establish brucellosis in 155, which is 27.7%.

The rose bengal test established brucellosis in 114 (20.4%) ewes, second only to the indirect hemagglutination reaction in the number of isolated affected animals. The studies have shown the specificity, high sensitivity of the RBT and its suitability for the diagnosis of brucellosis in small cattle.

This study has shown that of all the serological reactions tested in the diagnosis of brucellosis in sheep and goats, the indirect hemagglutination reaction (IHR) is the most effective and allows to identify the maximum number of infected animals in the acute focus of infection.

5 Availability of Data and Material

Data can be made available by contacting the corresponding authors.

6 References

Abdelbaset E. Abdelbaset, Mostafa F.N. Abushahba, Maha I. Hamed, Mohamed S. Rawy, (2018). Sero-diagnosis of brucellosis in sheep and humans in Assiut and El-Minya governorates, Egypt. *International Journal of Veterinary Science and Medicine*, 6, s63-s67.

Arakelyan, P.K., Dimov, S.K. (2013). Optimization of measures for brucellosis of farm animals in modern conditions. *Veterinary medicine*, 4, 23-27.

Arakelyan, P.K., Oschepkov, V.G., Dimov, S.K., Donchenko, A.S. (2010). Modern problems of control of the epizootic process of brucellosis of small cattle. *Veterinary doctor*, 6, 22-25.

Cutler, S.J., Zygmunt, M.S., Gapin – Bastuji, B. (2012). Brucella species: brucellosis. *BSL 3 and BSL 4 agents: Epidemiology, microbiology and prartical gvidelines*, 19, 35.

Ducrotoy, M.J., Bardosh K.L. (2017). How do you get the Rose Bengal test at the point – of – care to diagnose brucellosis in Africa? The importance of systems approach. *Acta tropica*, 165, 33 – 39.

Gulyukin, M.I., Zarebezhny, A.D., Iskandarov, M.I., Fedorov, A.I., Gulyukin, A.M. et al. (2019). Efficiency of measures against brucellosis of agricultural animals. *Veterinary Medicine*, 11, 20-24.

Gunashev, Sh.A., Dzhambulatov, Z.M., Musiev D.G, [et al.] (2020). Dynamics of infectious diseases spread in the farms of the Republic of Dagestan in 2019. *Bulletin of the Dagestan SAU*, 2(60), 64-67.

Kabardiev, S.Sh., Yusupov, O.Yu. (2018). Scientific achievements and main results of research of the Caspian zonal NIVI on the problem of brucellosis. *Veterinary medicine and feeding*, 2, 47-50.

Khalikov, A.A. (2017). Diagnostic value of HAI in sheep and goat brucellosis. *Veterinary medicine and feeding*, 5, 13.

Nicolleti, P. (2005). Epidemiology in brucellosis. Br. res. conf. Merida, Yucatan, Mexico, pp.19-23.

Nielsen, K. (2002). Diagnosis of brucellosis with serology. *Veterinary microbiology*, 90, 447-459.

Orakbay L.Zh., Cherepanova, L.Yu., Denisova, T.G. (2015). Modern aspects of the epidemic process of brucellosis. *Modern problems of science and education*, 6, 12.

Purcell, B.K., Rivard, R. (2012). Brucellosis. *Biodefense research methodology and animal models*, 2nd Ed., pp.197-222.

Rahman, A. A., Saegerman, C., Berkvens, D., Fretin, D., Gani, M. O., Ershaduzzaman, M., ... & Emmanuel, A. (2013). Bayesian estimation of true prevalence, sensitivity and specificity of indirect ELISA, Rose Bengal Test and Slow Agglutination Test for the diagnosis of brucellosis in sheep and goats in Bangladesh. *Preventive Veterinary Medicine*, *110*(2), 242-252.

Rasulov, S.A. Mirzoev, D.M., Iskandarov, M.I. (2017). Diagnostic value of modern and traditional research methods in brucellosis of small cattle. *Veterinary medicine and feeding*, 2, 45-46.

Turdiev, Sh.A., Iskandarov, M.I., Gulyukin, A.M. (2019). *Brucellosis of small cattle*. 426p.

Ulasevich, P.S., Kasyanov, A.N., Malakhova, T.I., Klimanov, A.I. (1980). Serological diagnostics of brucellosis of roses – bengal breakdown. *Poster pamphlet VDNKH USSR*, M, pp. 7.

Yanikova, E.A. Yusupov, O.Yu., Kabakhova P.M., et al. (2016). Express method for detecting anti-brucellosis antibodies in blood serum and milk. *Veterinary doctor*, 5, 16.20.

Yusupov, O.Yu., Khairov, S.G., Kabardiev, S.Sh., Sklyarov, O.D., Klimanov, A.I., Oschepkov, V.G., Degtyarenko, L.V., Devrishov D.A. (2015). HAI efficiency in brucellosis of cattle, sheep and goats. *Veterinary medicine*, 11, 22-25.

Yusupov, O.Yu., Mikailov, M.M., Yanikova, E.A., et al. (2018). A new express method for diagnosing brucellosis in sheep and goats. Makhachkala: Caspian zonal NIVI, pp. 21.

Zhanbyrbaev, M., Zamankhanbaeva, B., Meirbekova, M. (2018). The efficiency of modern methods of serological diagnosis of brucellosis in animals. *ICITE*, pp. 287-289.

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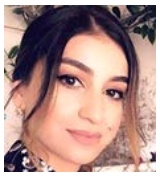

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