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New brucellosis erythrocyte diagnosticum for IHT in comparison with other serological methods (SAT, CFT, RBT and IDT)

ABSTRACT

Relevance. Brucellosis is registered in many countries of the world, including Russia. The most unfavorable situation in the Russian Federation has developed in the Southern and North Caucasus Federal Districts. In the first place among the number of identified sick animals is the Dagestan Republic, where, despite the efforts of veterinary specialists, it is not possible to reduce the number of oci of brucellosis.
With the help of classical methods (SAT, CFT), only 70% of animals infected with brucellosis can be detected. In this regard, domestic and foreign researchers have done a lot of work to improve existing and develop new diagnostic tools and methods.

Methods. One of the most sensitive methods for the diagnosis of brucellosis is the indirect hemagglutination reaction (IHT). In comparative tests in IHT, with an improved antigen, good results were obtained.

Results. The possibility of obtaining a highly active erythrocyte diagnosticum has been established with the combined use of two new surfactants: Progress products manufactured by AMC Media LLC (Losino-Petrovsk, Moscow region) and sodium dodecyl sulfate.
The most rational parameters of the use of detergents for the treatment of brucella suspension and their optimal concentrations have been determined.
It was found that IHT with a new antigen is specific and highly sensitive, compared with other serological reactions.

Key words: brucellosis, diagnostics, serological methods, cattle, antigen, IHR

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Новый бруцеллезный эритроцитарный диагностикум для РНГА в сравнении с другими серологическими методами (РА, РСК, РБП и РИД)

РЕЗЮМЕ

Актуальность. Бруцеллез регистрируется во многих странах мира, в том числе и в России. Самая неблагоприятная ситуация РФ сложилась в Южном и Северо-Кавказском федеральных округах. На первом месте по числу выявленных больных животных находится Республика Дагестан, где, несмотря на усилия ветеринарных специалистов, не удается сократить число очагов бруцеллеза.
С помощью классических методов (РА, РСК) можно выявить только 70% инфицированных бруцеллезом животных. В связи этим отечественными и зарубежными исследователями проведена огромная работа по совершенствованию существующих и разработку новых диагностических средств и методов.

Методы. Одним из наиболее чувствительных методов для диагностики бруцеллеза является реакция непрямой гемагглютинации (РНГА). При сравнительных испытаниях в РНГА (с усовершенствованным антигеном) были получены хорошие результаты.

Результаты. Установлена возможность получения высокоактивного эритроцитарного диагностикума при сочетанном применении двух новых поверхностно-активных веществ — средства «Прогресс» производства ООО «АМС “Медиа”» (г. Лосино-Петровск, Московская обл.) и додецилсульфата натрия.
Определены наиболее рациональные параметры применения детергентов для обработки суспензии бруцелл и их оптимальные концентрации.
Установлено, что РНГА с новым антигеном является специфичной и высокочувствительной по сравнению с другими серологическими реакциями.

Ключевые слова: бруцеллез, диагностика, серологические методы, крупный рогатый скот, антиген, РНГА

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Введение/Introduction

Brucellosis is registered in many countries of the world, including Russia. The most unfavorable situation of the Russian Federation has developed in the Southern and North Caucasus Federal Districts. In the first place among the identified sick animals is Dagestan Republic, where, despite the efforts of veterinary specialists, it is not possible to reduce the number of disadvantaged points [1–3].

Antibrucellar measures include diagnostics, prevention and disinfection of infected farms and subsidiary farms. For the effectiveness of these measures, it is necessary to use modern brucellosis vaccines and highly sensitive diagnostic tests. To improve existing and develop new diagnostic tests and methods, domestic and foreign researchers have developed and put into practice test systems: enzyme-linked immunosorbent assay (ELISA) with S and R antigens, polymerase chain reaction (PCR) and for differential post-vaccination diagnostics — immunodiffusion test (IDT) [4, 5].

According to many authors, one of the promising diagnostic methods is indirect hemagglutination test (IHT) using a specific and highly sensitive erythrocyte antigen [4–6].

The performed studies and the comparative analysis of IHT in laboratory, technological enviroment showed the specificity and its sensitivity in the diagnosis of brucellosis of animals, compared with CFT, SAT, RBT and IDT [7, 8].

The main purpose of our research was to develop a method for manufacturing an antigen for IHT, through the combined use of Progress preparations manufactured by AMC Media LLC and sodium dodecyl sulfate, to study the diagnostic value of this antigen in comparison with other serological reactions.

Материалы и методы исследований / Materials and methods

The studies were conducted from 2021–2022 in the laboratory of infectious pathology of farm animals of the Caspian zonal research veterinary institute — a branch of federal state budgetary scientific institution «Federal agrarian scientific center of Dagestan Republic» and the sheep breeding farm of Dagestan Republic, which is disadvantaged by brucellosis.

Sensitin for loading (sensitization) of stabilized erythrocytes was extracted from a suspension (bakmass) of brucelles of a weakly virulent highly antigenic strain of *B. abortus 19* grown on a dense nutrient medium — meat-peptone liver-glucose-glycerin agar (MPLGGA) by salt extraction, to compound a brucellosis erythrocyte diagnosticum.

One of the main factors of manufacturing an antigen for IHT is the treatment of brucella suspension in the production process with a detergent (surfactant — secondary sodium alkyl sulfate). At the same time, the extraction of antigenic complexes improves. For this purpose, the preparation «Progress» of the Novocherkassk Factory of Synthetic Products (NFSP) (Russia) was used [9–12].

Due to the closure of this factory, we decided to replace the preparation «Progress» of NFSP (Russia) with the most affordable. Optimal results were obtained using the preparation «Progress» produced by LLC «AMS “Media”» (Losino-Petrovsky, Moscow region, Russia) and sodium dodecyl sulfate.

The determination of the necessary concentrations of detergents required for maximum extraction of antigenic complexes from brucella strain B.abortus19 was carried out by treating brucella suspension in various modes with increasing doses of «Progress» produced by «AMS “Media”» LLC (Losino-Petrovsky, Moscow region, Russia) and sodium dodecyl sulfate. The optimal concentrations are: for «Progress» — 4.0–4.5%, sodium dodecyl sulfate — 0.2–0.25% to the volume of 70–80 billion (in 1 ml) brucella suspension.

Erythrocyte formalinization was performed using the R. Weinbach method in the modification of the Caspian zonal RVI[1]. In the manufacture of the diagnosticum used in IHT, the erythrocyte sensitin was extracted from a brucella suspension of the Br. abortus 19 strain, with autoclaving in a hypertonic 12% *NaCl* solution.

Studies of blood sera in SAT, CFT, RBT and IHT were carried out according to all-Union State Standard 34105-2017[2].

Результаты и обсуждение / Results and discussion

Diagnosticums made with the use of the preparation «Progress» — produced by «AMC “Media”» LLC (Losino-Petrovsky, Moscow region, Russia) and sodium dodecyl sulfate did not have sufficient activity (Tables 1, 2).

Table 1. **Results of checking the activity and specificity of IHT manufactured using the «Progress» tool — produced by «AMC “Media”» LLC (Losino-Petrovsky, Moscow region, Russia)**

Amount of antigen per 1 ml	IHT titers						
	with a standard sample of antibrucella abortus serum						Witha negative serum
	1:200	1:400	1:800	1:1600	1:3200	1:50	1:100
1,0	++++	+++	++	–	–	–	–
1,5	++++	++++	+++	+	–	–	–
2,0	++++	++++	+++	+	–	–	–
2,5	++++	++++	++++	+	–	–	–
3,0	++++	++++	++++	+	–	–	–

Note: the highest dilution in which erythrocyte agglutination occurred with a score of 4 or 3 crosses is taken as the titer of antibodies. Reactions with a rating of 2 crosses, 1 cross and minus (–) are considered negative.

Table 2. **The result of checking the activity and specificity of IHT with the use of sodium dodecyl sulfate**

The amount of antigen per 1 ml of erythrocytes	IHT titers						
	with a standard sample of antibrucella abortus serum						With a negative serum
	1:200	1:400	1:800	1:1600	1:3200	1:50	1:100
1,0	++++	++++	++	–	–	–	–
1,5	++++	++++	+++	–	–	–	–
2,0	++++	++++	+++	+	–	–	–
2,5	++++	++++	++++	++	–	–	–
3,0	++++	++++	++++	++	–	–	–

A negative result was obtained in the study of negative serum with all samples (Tables 1, 2).

The reaction titer did not exceed 1:800, when examining a standard sample of antibrucella abortus serum in IHT using these diagnostics whereas according to Technic Specification 9388-001-73917611-2005, its titer should not be lower than 1:1600.

«Progress», manufactured by «AMC “Media”» LLC, was combined with sodium dodecyl sulfate to increase the activity of the antigen.

[1] Modification of the R. Weinbach method is consisted in the following: red blood cells were formalized for 15–16 hours instead of 24 hours in a Shuttel apparatus. The erythrocytes were shaken for 1.5–2 minutes at half-hour intervals, unlike the R. Weinbach method, where it must be done constantly. Due to this, the time was reduced, and more red blood cells were obtained in the process. In the process of obtaining erythrocytes, there was no gluing and no signs of hemolysis, which are usually observed with constant 24-hour shaking. The adsorption properties of stabilized erythrocytes have not changed, and the shelf life has been extended to 2 years or more.

[2] All-Union State Standard 34105-2017 Animals. Laboratory diagnosis of brucellosis. Serological methods.

The optimal ratios of various variants of these detergents were determined, to obtain a highly specific diagnosticum with an activity of 1:1600, with an estimate of four or three crosses (Table 3).

As can be seen from Table 3, the diagnostic for IHT, made in combination with a standard serum, had a titer of 1:1600 and higher, and a negative result was obtained with a negative serum, which corresponds to the requirement of Technic Specification 9388-001-73917611-2005. The preparation made in this way will be used by us for further research.

Table 3. **Results of checking the activity and specificity of the new diagnostic for IHT**

The amount of antigen per 1 ml of erythrocytes	IHT titers						
	with a standard sample of antibrucella abortus serum					With a negative serum	
	1:200	1:400	1:800	1:1600	1:3200	1:50	1:100
1,0	++++	++++	+++	++	–	–	–
1,5	++++	++++	++++	+++	–	–	–
2,0	++++	++++	++++	++++	+	–	–
2,5	++++	++++	++++	++++	++	–	–
3,0	++++	++++	++++	++++	++	–	–

Table 4. **Checking the activity of diagnosticums**

IHT titers with a standard sample of antibrucella abortus serum									
with a diagnosticum No. 1 made by a new method					with a diagnosticum No. 2 made by a previously known method				
1:200	1:400	1:800	1:1600	1:3200	1:200	1:400	1:800	1:1600	1:3200
++++	++++	++++	++++	++	++++	++++	++++	++++	+

Table 4 shows the results of the study of negative serum with these diagnoses, which indicates their specificity.

Both diagnosticums were examined, in comparison with SAT, CFT, RBT and IDT, in the blood sera of 52 goats and sheep from a dysfunctional farm of Dagestan Republic, with an active infection. Data on the comparative test of erythrocyte diagnosticums are given in Table 5.

As can be seen from Table 6, both diagnosticums showed identical results in IHT, at the same time, the new test diagnosticum revealed all animals reacting in other serological reactions. The results of the studies showed that the combined use of the preparation «Progress» and sodium dodecyl sulfate makes it possible to obtain an erythrocyte diagnosticum with high activity and specificity. The patent for the invention RU No. 2667121 dated 31.10.2016 was obtained for this diagnostic.

Table 5. **Test results of two erythrocyte diagnosticums in IHT in comparison with traditional diagnostic methods**

IHT				SAT, ME					CFT					RBT		IDT	
with diagnosticum No. 1, manufactured according to a new method with the combined use of two detergents		with diagnosticum No. 2, manufactured according to a known method															
titre	quantity	titre	quantity	neg.	25	50	100	200	neg.	1:5	1:10	1:20	1:40	pos.	neg.	pos.	neg.
1:400	3	1:400	3	1	–	1	–	1	–	–	–	–	3	3	–	2	1
1:200	5	1:200	5	3	–	1	–	1	–	1	1	2	1	4	1	3	2
1:100	16	1:100	16	8	–	7	1	–	3	–	4	5	4	13	3	5	11
1:50	20	1:50	20	14	–	4	2	–	8	2	6	2	2	8	12	1	19
1:25	4	1:25	5	4	–	–	–	–	4	–	–	–	–	–	4	–	4
neg.	4	neg.	3	4	–	–	–	–	4	–	–	–	–	–	4	–	4
Total	52		52	34	–	13	3	2	19	3	11	9	10	28	24	11	41
pos.	44		44			18					33			28		11	
%	84.6		84.6			34.6					63.4			53.8		21.1	
contr.	4		5														
neg.	4		3	34					19						24		41

Note: pos. — positive result, contr. — controversial result, neg., – — negative result.

Выводы/Conclusion

The possibility of obtaining a highly active erythrocyte diagnosticum has been established with the combined use of two surfactants — «Progress» products manufactured by «AMC “Media”» LLC (Losino-Petrovsk, Moscow region) and sodium dodecyl sulfate.

The most rational parameters of the use of detergents for the treatment of brucellar suspension and their optimal concentrations have been determined.

In the study of blood sera of small cattle, brucellosis was diagnosed in IHT in 44 samples (84.6%), SAT — 18 (34.6%), CFT — 33 (63.4%), RBT — 28 (53.8%) and IDT — 11 (21.1%), respectively.

It was found that IHT with a new antigen is specific and highly sensitive, compared with other serological reactions.

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All authors made an equal contribution to the work.
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