OPEN ACCESS
Research Journal of Veterinary Practitioners

Research Article

Comparative Evaluation of the Reaction of Indirect Hemagglutination, Enzyme Immunoassay and Serological Tests at the Diagnosis of Brucellosis in Cattle

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Abstract | The results of comparative diagnostic studies of blood serum from cattle for brucellosis from farms with different epizootic situations, carried out in 2019 . The diagnostic efficacy of the indirect hemagglutination reaction (RNGA) was studied in comparison with the enzyme-linked immunosorbent assay (ELISA), agglutination reaction (RA), complement fixation reaction (CSC), rose-bengal test (RBP) and immunodiffusion reaction with O-PS antigen (RID with O -PS antigen). The specificity of these serological tests was confirmed by the negative results of studies of 40 blood serum samples from animals from a safe farm not vaccinated against brucellosis. Determination of the sensitivity of the tested diagnostic tools was carried out using 46 blood serum samples from cows immunized with a vaccine from the B. abortus 82 strain from a farm that is unfavorable for brucellosis. Studies have shown that RNGA is one of the most sensitive tests, which allows to identify in a dysfunctional economy a high percentage of animals with brucellosis (39.1% of those studied) in the early stages after infection and surpasses the results of most other serological methods. Data from comparative serological studies of cattle blood serum in RNGA, ELISA, RA, RSK, RBP and RID with O-PS, confirmed the specificity of the tests and high sensitivity of RNGA with brucellosis erythrocyte antigen.

Keywords | Brucellosis, Cattle, Laboratory diagnostics, Methods, Blood serum.

Received | May 29, 2022; **Accepted** | June 25, 2022; **Published** | October 15, 2022
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Citation | Mikailov MM, Chernykh OY, Yanikova EA, Nurlygayanova GA, Sklyarov OD (2022). Comparative evaluation of the reaction of indirect hemagglutination, enzyme immunoassay and serological tests at the diagnosis of brucellosis in cattle. Res J. Vet. Pract. 10(4): 46-51.
DOI | http://dx.doi.org/10.17582/journal.rjvp/2022/10.4.46.51
ISSN | 2308-2798

INTRODUCTION

Brucellosis is a zoonotic chronic disease caused by bacteria of the genus Brucella, which is widespread among animals and humans in several countries. The main source of the causative agent of the disease for humans is animals sick with brucellosis. Russian and foreign scientists consider brucellosis as a national and international problem of humans and animals (Bahri, Dmitriev, 2018; Iskandarov et al., 2017; Manturle, et al., 2007).

In the territory of the Russian Federation (RF), a complex epizootic situation of brucellosis in cattle has been recorded in recent years (Novikova et al., 2019). Thus, according to the Information and Analytical Center of the Veterinary Supervision Directorate of the Federal State Budgetary Institution "Federal Center for Animal Health" (IAC), in 2017 the country had 538 were identified, in 2018 - 593, according to the data for the III quarter of 2019 - 306 new unfavorable points for brucellosis of cattle. The number of cattle that fell ill with brucellosis in this period amounted

to 7706 and 5854 heads, respectively, according to the results for the third quarter of 2019 - 5537. According to the IAC data for the third quarter of 2019, brucellosis of cattle was registered in 30 constituent entities of Russia. The largest number of new unfavorable points for brucellosis of cattle was revealed: in the Karachay-Cherkess Republic - 78, in the Republic of Dagestan - 42, Stavropol Territory - 25, Krasnodar Territory - 21, Republic of North Ossetia (Alania) - 18, Chechen Republic - 28, Krasnodar Territory - 21, Astrakhan Region - 18, Republic of Kalmykia - 12. The epizootic situation in animal brucellosis that has developed in the Russian Federation in recent years requires constant monitoring, including serological methods. In order to control the effectiveness and optimization of anti-brucellosis measures, several means of serological diagnostics have been introduced into the country's veterinary practice, in particular, RA, RSK (RDSK), RBP, RID with O-PS antigen, CR with milk, etc. In addition to the indicated diagnostic tools for brucellosis, in recent For years, different versions of enzyme-linked immunosorbent assay (ELISA) have been used (Dimova, 2015). In 2006, in 17 constituent entities of the Russian Federation, a wide testing in production conditions of the indirect hemagglutination reaction (RHA) with the use of erythrocyte antigen, proposed by the FGBNU VNIIBTZh, the Caspian zonal NIVI and the FGBU VGNKI, was successfully completed. According to the results of approbation, in a comparative test of RNGA with other serological tests, it was found that the first absorbs all the dubious and positive research results obtained in RA and RSK. Nevertheless, the "Kit for the diagnosis of brucellosis in cattle and small ruminants in the reaction of indirect hemagglutination" has not received wide practical application (Degtyarenko, 2015; Yusupov et al., 2015).

Purpose: to conduct a comparative test of the RNGA method and other serological tests (ELISA, RA, RSK, RBP and RID with O-PS antigen) using domestic diagnostic test systems for the diagnosis of animal brucellosis.

MATERIALS AND METHODS

In 2019, in the laboratory of the Caspian zonal NIVI - a branch of the FANTS RD FGBNU, a study of the diagnostic efficiency of the Kit for serological diagnosis of brucellosis in cattle and small ruminants in the reaction of indirect hemagglutination (RNGA) was carried out in comparison with other serological methods (ELISA, RA, RSK, RBP and RID with O-PS antigen) using diagnostics made in the Russian Federation. The material was blood serum samples from cattle from farms with different epizootic situations of brucellosis located in the Republic of Dagestan. The specificity of the reactions was assessed based on the results of studies of 40 blood serum samples from cattle not immunized with anti-brucellosis vaccines from a farm without brucellosis. The sensitivity of RNGA was assessed by examining 46 blood serum samples of cattle from a farm with a problem with brucellosis after at least 6 months after immunization of animals with a vaccine from the B. abortus 82 strain. Serological examination of animals in RA, RSK, RID and RBP was carried out in accordance with the Manual on the diagnosis of brucellosis in animals, approved. Veterinary Department of the Ministry of Agriculture of the Russian Federation on September 29, 2003 No. 13-0502 / 0850. Determination of the amount of immunoglobulins (in the same serum samples) was performed in ELISA and RNGA, respectively, using the "Diagnostic kit for the detection of individual specific antibodies of class G to bacteria of the genus Brucella in the serum (plasma) of the blood of farm animals by the enzyme immunoassay (ELISA)", manufacturer OOO NPF "Sibbotest", the setting of the RNGA was carried out according to the "Instructions for the use of a kit for serological diagnosis of brucellosis of cattle and small ruminants in the reaction of indirect hemagglutination (RNGA)", produced by OOO Vetmedservice of Dagestan (Makhachkala) (Novikova et al., 2019).

RESULTS AND DISCUSSION

At the first stage of the work, in order to determine the specificity of the serological tests selected for testing, a study was made for brucellosis of 40 blood serum samples from cattle not vaccinated against brucellosis, and belonging to the Keger Agrofirm (Republic of Dagestan), brucellosis-free cattle (Table 1).

According to the data presented in Table 1, all blood serum samples were tested in RNGA, ELISA, RA, RSK, RBP and RID with O-PS antigen with a negative result.

Determination of the sensitivity of the tested diagnostic tools was carried out using 46 blood serum samples from cattle, after at least 6 months after immunization of animals with a vaccine from the B. abortus 82 strain from farms of the private sector of the private sector of the village of Moksob, Khasayurt district of the Republic of Dagestan (Table 2).

According to the data (Table 2), out of 46 samples of cattle blood serum, 18 (39.1%) were tested with a positive result in RNGA, 29 (63%) by IFA (OOO NPF Sibbotest), 18 RSK (39, 1%), RA - 15 (32.6%), RBP - 15 (32.6%), in RID with O-PS antigen - 7 (15.2%) samples. In the course of the studies, it was found that RNGA in sensitivity is inferior to IFA (LLC NPF "Sibbotest"), is not inferior to RSC and exceeds RA and RBP in the sum - 3 (6.5%), as well as RID - 11 (23.9%) .A significant percentage of an

Table 1: Results of studies on brucellosis in cattle from a prosperous farm

Sample No.	Diagnostic methods								
	RNGA		ELISA (OOO NPF "Sibbotest")	RA, ME		RSK		RBP	RID
	1:50	1:100		50	100	1:5	1:10		
1	-	-	neg.	-	-	-	-	neg.	neg.
2	-	-	neg.	-	-	-	-	neg.	neg.
3	-	-	neg.	-	-	-	-	neg.	neg.
4	-	-	neg.	-	-	-	-	neg.	neg.
5	-	-	neg.	-	-	-	-	neg.	neg.
6	-	-	neg.	-	-	-	-	neg.	neg.
7	-	-	neg.	-	-	-	-	neg.	neg.
8	-	-	neg.	-	-	-	-	neg.	neg.
9	-	-	neg.	-	-	-	-	neg.	neg.
10	-	-	neg.	-	-	-	-	neg.	neg.
11	-	-	neg.	-	-	-	-	neg.	neg.
12	-	-	neg.	-	-	-	-	neg.	neg.
13	-	-	neg.	-	-	-	-	neg.	neg.
14	-	-	neg.	-	-	-	-	neg.	neg.
15	-	-	neg.	-	-	-	-	neg.	neg.
16	-	-	neg.	-	-	-	-	neg.	neg.
17	-	-	neg.	-	-	-	-	neg.	neg.
18	-	-	neg.	-	-	-	-	neg.	neg.
19	-	-	neg.	-	-	-	-	neg.	neg.
20	-	-	neg.	-	-	-	-	neg.	neg.
21	-	-	neg.	-	-	-	-	neg.	neg.
22	-	-	neg.	-	-	-	-	neg.	neg.
23	-	-	neg.	-	-	-	-	neg.	neg.
24	-	-	neg.	-	-	-	-	neg.	neg.
25	-	-	neg.	-	-	-	-	neg.	neg.
26	-	-	neg.	-	-	-	-	neg.	neg.
27	-	-	neg.	-	-	-	-	neg.	neg.
28	-	-	neg.	-	-	-	-	neg.	neg.
29	-	-	neg.	-	-	-	-	neg.	neg.
30	-	-	neg.	-	-	-	-	neg.	neg.
31	-	-	neg.	-	-	-	-	neg.	neg.
32	-	-	neg.	-	-	-	-	neg.	neg.
33	-	-	neg.	-	-	-	-	neg.	neg.
34	-	-	neg.	-	-	-	-	neg.	neg.
35	-	-	neg.	-	-	-	-	neg.	neg.
36	-	-	neg.	-	-	-	-	neg.	neg.
37	-	-	neg.	-	-	-	-	neg.	neg.
38	-	-	neg.	-	-	-	-	neg.	neg.
39	-	-	neg.	-	-	-	-	neg.	neg.
40	-	-	neg.	-	-	-	-	neg.	neg.

Note: neg. - negative reaction.

Table 2: Results of studies on brucellosis of cattle from a dysfunctional economy

№ P/p.	Tag number	Diagnostic methods											
		RNGA (blood serum dilution)						nterpreting Results RNGA	ELISA (OOO NPF "Sibbotest")	Titer		RBP	RID
		1: 50	1: 100	1: 200	1: 400	1: 800	1: 1600			RA, ME	RSK		
1	4785	#	#	+++	+++	++	-	positive	dubious	50	1:40+++	positive	negativ
2	4639	#	#	+++	+++	-	-	positive	positive	100	1:20#	positive	пол
3	4634	#	#	#	#	#	+++	positive	positive	400	1:40#	positive	negativ
4	4589	-	-	-	-	-	-	negativ	positive	100	-	negativ	negativ
5	4681	-	-	-	-	-	-	negativ	negativ	-	-	negativ	negativ
6	4547	#	#	#	#	#	#	positive	positive	400	1:40#	positive	positive
7	4637	#	#	#	#	#	#	positive	positive	100	1:40#	positive	positive
8	4984	-	-	-	-	-	-	negativ	positive	200	-	negativ	negativ
9	4523	#	#	#	+++	+++	++	positive	positive	100	1:20+++	negativ	positive
10	4903	+++	-	-	-	-	-	negativ	positive	-	-	negativ	negativ
11	4539	++	+	-	-	-	-	negativ	positive	-	-	negativ	negativ
12	4827	-	-	-	-	-	-	negativ	negativ	-	-	negativ	negativ
13	4782	#	#	#	#	#	+++	positive	positive	400	1:80#	positive	negativ
14	4894	#	#	#	+++	-	-	positive	positive	100	1:80+++	positive	negativ
15	4961	-	-	-	-	-	-	negativ	dubious	-	-	negativ	negativ
16	4522	#	#	#	#	#	#	positive	positive	100	1:40#	positive	positive
17	4626	#	#	#	#	+++	++	positive	positive	200	1:40#	positive	negativ
18	4562	#	+++	+++	++	-	-	positive	positive	50	1:10+++	positive	negativ
19	4801	-	-	-	-	-	-	negativ	negativ	-	-	negativ	negativ
20	4600	#	#	#	#	#	#	пол	positive	200	1:80#	positive	negativ
21	4188	+++	+++	-	-	-	-	dubious	dubious	50	1:5 +	negativ	negativ
22	4443	-	-	-	-	-	-	negativ	negativ	-	-	negativ	negativ
23	4532	-	-	-	-	-	-	negativ	negativ	-	-	negativ	negativ
24	4580	#	#	#	+++	++	-	positive	positive	-	1:20+++	positive	negativ
25	4795	+++	+++	++	-	-	-	dubious	positive	-	-	negativ	negativ
26	4620	+++	-	-	-	-	-	negativ	dubious	-	-	negativ	negativ
27	4828	+++	-	-	-	-	-	negativ	positive	-	1:5+	negativ	negativ
28	2897	++	-	-	-	-	-	negativ	dubious	-	-	negativ	negativ
29	4882	+++	+++	++	-	-	-	dubious	positive	-	1:5 +	negativ	negativ
30	4758	+++	++	-	-	-	-	negativ	positive	50	-	negativ	negativ
31	4587	+++	++	-	-	-	-	negativ	dubious	50	-	negativ	negativ
32	4898	+++	++	-	-	-	-	negativ	positive	50	-	negativ	negativ
33	4550	+++	+++	++	-	-	-	dubious	positive	50	1:10+++	negativ	negativ
34	4360	-	-	-	-	-	-	negativ	negativ	-	-	negativ	negativ
35	4763	-	-	-	-	-	-	negativ	negativ	-	-	negativ	negativ
36	4798	-	-	-	-	-	-	negativ	negativ	-	-	negativ	negativ
37	4855	+++	+++	+++	-	-	-	positive	positive	50	1:10+++	negativ	negativ
38	4858	+++	+++	+++	-	-	-	positive	positive	-	1:5 +	negativ	negativ
39	4940	#	+++	-	-	-	-	dubious	positive	-	-	negativ	negativ
40	4844	-	-	-	-	-	-	negativ	dubious	-	-	negativ	negativ
41	2910	#	#	#	#	#	#	positive	positive	400	1:10#	positive	positive
42	4822	#	#	#	#	#	#	positive	positive	400	1:80#	positive	positive

43	4709	#	+++	+++	-	-	-	positive	positive	100	1:10++	positive	negativ
44	4805	+++	++	-	-	-	-	negativ	positive	-	1:5 +	negativ	negativ
45	4989	-	-	-	-	-	-	negativ	negativ	-	-	negativ	negativ
46	4988	+++	++	+	-	-	-	negativ	negativ	-	-	negativ	negativ
ИТОГО:							negativ	23	10	23	23	31	39
							dubi-ous	5	7	8	5	-	-
							positive	18	29	15	18	15	7

Note: neg. - negative reaction. catfish. - dubious reaction.floor. - positive reaction.

imals responding positively to brucellosis according to the results of a serological study of WRID with O-PS antigen and high titers of antibodies in RNGA, RA, and RSC suggest that brucellosis infection in the herd under study is acute. According to the results of comparative serological studies of blood sera of cattle from farms with different epizootic situations for brucellosis, the specificity and high sensitivity of the RNGA method with brucellosis erythrocyte antigen, produced by LLC Vetmedservice, Republic of Dagestan (Makhachkala), was established.

CONCLUSION

The results of comparative serological studies of cattle in RNGA, ELISA, RA, RSK, RBP and RID with O-PS antigen, confirmed the specificity and high sensitivity of most of the tests tested. High sensitivity - 63% (to the tested samples) was established by the enzyme immunoassay (ELISA), manufactured by OOO NPF "Sibbotest". At the same time, RNGA turned out to be one of the most sensitive tests, which made it possible to identify a significant part of animals with brucellosis in a dysfunctional economy (39.1% of the total number of animals studied), which exceeds the results of other serological methods: RA - 32.6%, RBP - 32.6 %, RID with O-PS antigen - 15.2%. Our data are consistent with the conclusions of researchers who studied the diagnostic value of the RNGA method in populations of other animals, in regions with different intensity of the epizootic process for brucellosis (Vinokurov, 2010; Nurlygayanov, 2013).

CONFLICT OF INTEREST

The authors declared no conflict of interest.

NOVELTY STATEMENT

The authors declare that the results obtained on the topic of the article were obtained empirically, and the reflected information is new for science in the field of bacteriology and immunology.

AUTHORS' CONTRIBUTION

All authors took part in a comparative assessment of the reaction of indirect hemagglutination, enzyme immunoassay and serological tests in the diagnosis of brucellosis in cattle, collected materials, analyzed the material, participated in writing the manuscript. Collectively reviewed the manuscript. All authors read and approved the final version of the manuscript.

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