Diagnostic effectiveness of serological tests for the detection of brucellosis antibodies

E.I. Kasymov*, *B.K.* Otarbayev, *G.D.* Ilgekbayeva, *I.N.* Galymzhan, and *T.M.* Kydyrkhanov

Kazakh National Agrarian Research University, Abai st. 8, Almaty, Kazakhstan

Abstract. The article reflects the results of testing various diagnostic tests (ST, RA, CFT, BRT, IHA and ELISA) in the diagnosis of brucellosis in cattle, where animals are subjected to serological tests without the use of a vaccine. Antibodies against brucellosis were detected in diagnostic titer in ST in 100% and in ELISA in 96% of cases. The difference between the rates of these reactions was statistically insignificant (P> 0.1). ICA is significantly inferior to these two tests. This difference turned out to be significant, that is, significant (P <0.01). The lowest rates are set for RA, RBT and CFT. For the diagnosis of brucellosis, of all the tests tested, ST and ELISA turned out to be the most sensitive, and of these two tests, the most accessible for practice is ST.

1 Introduction

Bovine brucellosis is a widespread zoonotic disease in many countries of the world that causes great economic damage to livestock and poses a serious threat to human health [1-3]. One of the main methods of rehabilitation of unsuccessful foci of brucellosis in cattle in our republic and some countries of the CIS and far abroad is the systematic conduct of serological studies followed by the slaughter of reacting animals and appropriate sanitary measures [1, 2, 4]. The success of this recovery method depends mainly on the early detection of all infected animals. Therefore, the largest number of studies has been devoted to the study of the diagnostic value of the agglutination reaction (RA), the complement fixation reaction (CFT) and its long-term variant (LTV), the Bengal rose test (BRT). Over time, it turned out that none of them, even the complex application of these tests, does not always allow the identification of all infected animals in a short time. In addition, the complex application of these methods when carrying out health-improving activities has become very difficult and time-consuming [2, 5, 6].

As a result of the search for highly sensitive diagnostic methods available for wide practice, E.Engval and P. Perlman in 1972 developed an enzyme-linked immunosorbent assay (ELISA) [7], and in 1981 T. Saiduldin – a conglutinating complex fixation test (CCFT) [5, 6, 8]. Currently CCFT is renamed after the author's name – Saiduldin Test (ST) [9-11].

According to the author and his followers, the ST fully confirms the overall positive results of RA, CFT, CLFT, RBT for brucellosis. In addition, ST can additionally identify infected animals in a herd [2, 5, 6, 9-11]. In a special experiment on 46 cows with similar

* Corresponding author: yerken.kassymov@mail.ru

signs of serological reactions, biological analysis in guinea pigs revealed brucellosis in 54.3% of cases [5, 6]. The specificity of ST was confirmed by DPT with the O-PS antigen produced by "Vector", Novosibirsk [12], as well as by studying the dynamics of indications for additional reoperation in animals with negative AT and CFT. In the last experiment, 92 animals with current results from 4912 cows and heifers studied (with heifers at the age of 4-6 months and before mating are immunized with the anti-brucellosis vaccine from strain 82, and the cows are systematically vaccinated. Serological test for AT, CFT and ST) isolated and kept separate from the main herd. On one farm, during 136-154 days, they were examined 6 times (every 15-30 days), as a result of which the diagnosis of brucellosis was confirmed in 91.3% of cows and 83% of heifers. On another farm, 70% of the isolated animals were diagnosed within 90 days. In these dysfunctional farms, all herds for AT, CFT and ST were studied with isolation of animals that reacted with ST, which allowed the second and third diagnostic tests to be negative for AT, CFT and RBT. Twofold negative results of these tests allowed for a short period of time (8 months) to cure the herd of brucellosis [9]. In terms of sensitivity, ST in the diagnosis of brucellosis is not inferior to ELISA with an immunoperoxidase conjugate produced by the RRIEM named Gamaley [2].

ST uses the same components and equipment as CFT, except for the replacement of guinea pig complement with bovine serum containing conglutinin, which actively reacts with complement, a fixed antigen-antibody complex. Since complement and conglutinin act as a single functional system, the serum that is the source of these factors, T.Saiduldin called it “gluing” [8]. To introduce ST into veterinary practice, industrial production of dry conglutinating serum was established [13], and its production tests were carried out in veterinary laboratories by examining 81056 samples of bovine serum for brucellosis in ST in in four republics of the CIS. At the same time, ST with the use of a dry preparation was highly effective and accessible for practice. The diagnosis of brucellosis by AT, CFT and RBT was confirmed by ST in 95.6%, 99.3% and 70.7%, respectively [14].

Recently, an immunochromatographic assay (ICA) and an enzyme-linked immunosorbent assay (ELISA) have been proposed by “Bru Ltflow Ab” to detect antibodies to the causative agent of brucellosis. The diagnostic value of ST in comparison has not previously been studied.

The purpose of the research. Testing of the diagnostic values of serological methods (ST, AT, RBT, CFT, ICA and ELISA) in the diagnosis of brucellosis of cattle in farms and settlements unfavorable for this disease, where animals have not been subjected to anti-brucellosis vaccinations.

2 Materials and methods

Complex serological methods were used to study 310 blood serum samples from cattle.

BRT, RA, CFT for brucellosis were carried out according to the existing guidelines. ST according to the manual for setting and recording the Saiduldin Test (ST) in the diagnosis of brucellosis in cattle, approved by Director of the Department of Veterinary Surveillance of the Ministry of Agriculture of the Republic of Kazakhstan dated December 27, 2000. We used the ICA kit for the detection of antibodies to the causative agent of brucellosis "Bru Ltflow Ab" (UAB "LT Biotech" IK 302303586 Rugiq 21-24, LT-08419, Vilnius Reg. Nr. 127918, VI Registry centras Vilniaus fillialas), as well as B. Brucella Ab ELISA (Anigen enzyme immunoassay kit for the presence of antibodies to Brucella. South Korean production BioNote 22 SamsungIro 4-gil, Hwaseong-si, Gyeonggi-do 445-170 Republic of Korea).

Statistical processing of the results of serological studies was carried out according to the method of T. Saiduldin [8].

3 Research results and discussion

When determining the diagnostic effectiveness of serological reactions, titers of 1:5 in four crosses and higher were taken as a positive ST result. In a brucellosis-unfavorable site, the results of complex serological tests (ST, AT, CFT, RBT, ICA and ELISA) examined 310 blood serums of cattle aged 1 year and older. The results are shown in the table and in Figure 1.

Table. 1. Comparative results of serological reactions at a brucellosis problem

№	Name of the reaction					
	ST	CFT	RBT	AT	ICA	ELISA
1	1:320	1:80	+	1:400	++++	1:800
2	1:160	1:40	–	–	++++	1:200
3	1:20	–	–	–	–	1:50
4	1:20	–	+	–	++	1:50
5	1:40	1:10	+	–	–	1:100
6	1:80	1:20	–	–	++	1:200
7	1:40	1:10	–	–	+++	1:100
8	1:160	1:40	+	1:200	++++	1:400
9	1:20	1:10	–	–	–	1:50
10	1:10	–	–	–	–	–
11	1:20	–	+	–	++	1:50
12	1:80	1:20	–	–	++	1:200
13	1:320	1:80	+	1:400	++++	1:800
14	1:160	1:40	+	1:200	++++	1:400
15	1:40	1:10	+	–	–	1:100
16	1:20	-	-	-	-	1:50
17	1:40	1:10	+	-	++	1:100
18	1:10	-	-	-	-	1:50
19	1:640	1:80	+	1:200	+++	1:800
20	1:320	1:80	+	1:200	++++	1:800
21	1:40	1:20	+	-	+++	1:200
22	1:320	1:40	+	1:100	+++	1:400
23	1:80	1:80	+	1:200	++++	1:200
24	1:320	1:80	+	1:100	++++	1:400
25	1:20	-	-	-	-	1:50
26	1:160	1:20	+	-	++++	1:200
27	1:40	1:10	-	-	++	1:50
Middle Title	260 +22,3 -18,2	15 +18,1 -15,3		50 +14,1 -12,3		150 +18,1 -15,3

By all reactions, brucellosis was diagnosed in 27 animals. In conventional reactions, brucellosis was diagnosed in RBT - 16; AT - 6; CFT - 20, and in ICA - 19. Summarized positive results of these tests were confirmed by ELISA (22), and 4 sick animals were additionally identified. In ST, these positive results are 100% confirmed. In addition, they additionally reacted to brucellosis in ST - in one animal. As a result of statistical processing, the average titer in ST was 1: 260, and in CFT - 1:15, AT -1: 50, this difference turned out to be significant ($P < 0.01$). In ELISA, the average titer was 1:150, this difference between the ST result turned out to be statistically insignificant ($P > 0.1$).

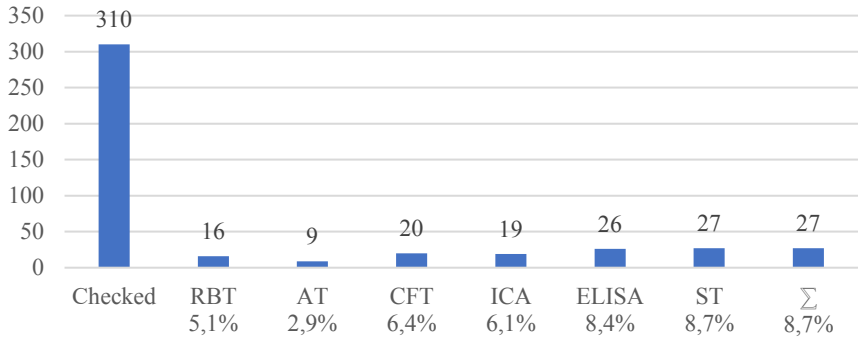

Fig. 1. Percentage of positive results in various serological tests

As can be seen from Figure 1, positive results as a percentage were registered in the AT - 2.9% (9), in the CFT - 6.4% (20), in the RBT - 5.1% (16), in the ICA - 6,1% (19), ELISA - 8.4% (26) and in ST - 8.7% (27) cases. The total positive results of complex tests as a percentage was 8.7%.

4 Conclusion

Anti-brucellosis antibodies were found in diagnostic titers for ST in 100%, and in ELISA in 96% of cases. The difference between the indicators of these reactions was statistically insignificant (P> 0.1). ICA was somewhat inferior to these tests. The lowest rates were established for AT, RBT and CFT. Thus, the results of ST and ELISA significantly exceeded the indicators of other reactions (ICA, AT, RBT and CFT). This difference turned out to be significant, that is, significant (P <0.01). For the diagnosis of brucellosis, of all the tests tested, ST and ELISA turned out to be the most effective, and of these two tests, ST is the most effective and accessible for practice.

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