International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 12 (9), pp. 001-004, September, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Comparative efficacy of Rose Bengal plate test, standard tube agglutination test and Dot ELISA in immunological detection of antibodies to *Brucella abortus* in sera

Deepti Chachra, Hari Mohan Saxena*, Gurpreet Kaur and Mudit Chandra

Department of Veterinary Microbiology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, 141004 India.

Accepted 25 May, 2018

Comparative efficacy of 3 serodiagnostic tests (RBPT, STAT and Dot ELISA) in detecting anti - *Brucella* antibodies in sera was evaluated on a total of 28 serum samples which included 18 samples from brucellosis suspected and 10 from normal healthy (brucellosis unaffected) cattle. Out of 18 sera from suspected cases, only 1 (5.55%) sample was found positive by STAT and 9 (50%) samples were positive by RBPT, whereas Dot ELISA could detect antibodies in all the 18 (100%) samples. Interestingly, RBPT could detect antibodies in 10 out of 17 (58.82%) samples found negative by STAT. The entire 9 samples positive by RBPT (100%) showed positive results with Dot ELISA also. Of the 9 RBPT negative samples, 11.25% showed positive and 88.88% showed negative results by STAT while all the 9 samples (100%) showed positive results with Dot ELISA. All the 10 sera from normal healthy animals were negative by RBPT, STAT and ELISA. Thus, Dot ELISA was found to be the most sensitive of the 3 tests used. It is, however, suggested that in order to get a fool proof diagnosis of *Brucella* infection, a combination of RBPT and Dot ELISA should be used, especially in case of samples found negative by either RBPT or STAT used alone or in combination.

Key words: Brucellosis, RBPT, STAT, Dot ELISA, *Brucella.*

INTRODUCTION

Brucellosis is an important and widely prevalent zoonotic disease of man and animals (cattle, buffaloes, sheep, goats, dogs etc) caused by *Brucella* organisms (Schelling et al., 2003) . The common serological test used for the diagnosis of brucellosis is Rose Bengal plate test (RBPT) based on agglutination of colored particulate antigen (killed *Brucella* organisms) by the antibodies present in the patient's serum. Although it is a simple, cheap and effective test, the RBPT is generally considered to be less sensitive than other tests like standard tube agglu-tination test (STAT), complement fixation test (CFT) and enzyme linked immunosorbant assay (ELISA). ELISA has been claimed to be a good screening test whether used alone or in combination with the RBPT (Jacques et al., 1998).

However, few studies have been conducted on the comparative sensitivity of the 3 tests. In particular, the negative tests given by RBPT and STAT when used alone need to be further confirmed by other tests like ELISA, to avoid any possibility of wrong diagnosis owing to false negative reactions by these tests. The present study was therefore, undertaken to explore this aspect of serodiagnosis of brucellosis.

MATERIALS AND METHODS

Samples

A total of 28 serum samples from cattle including 18 from cattle suspected of brucellosis and 10 samples from brucellosis - free cattle from different parts of the Punjab state of India were included in the study. All the serum samples were subjected to the common serological tests RBPT, STAT and Dot ELISA, respectively. The

*Corresponding author. Email: hmsaxena@yahoo.com. Tel.: +919417147813.

Table 1. Comparative sensitivity of RBPT, STAT and Dot ELISA.

S. no.	ELISA Comb and Lane no.	Sample no.	RBPT	STAT	ELISA
1	C1L2	J15	Positive	Negative	Positive
2	C1L8	J-7	Positive	1:20	Positive
3	C1L10	J18	Positive	1:20	Positive
4	C2L2	4-S	Positive	1:20	Positive
5	C2L3	4-C	Positive	Negative	Positive
6	C2L4	07-1222	Positive	Negative	Positive
7	C4L1	5-C	Positive	Negative	Positive
8	C4L6	07-1258	Positive	Negative	Positive
9	C4L9	47	Positive	Negative	Positive

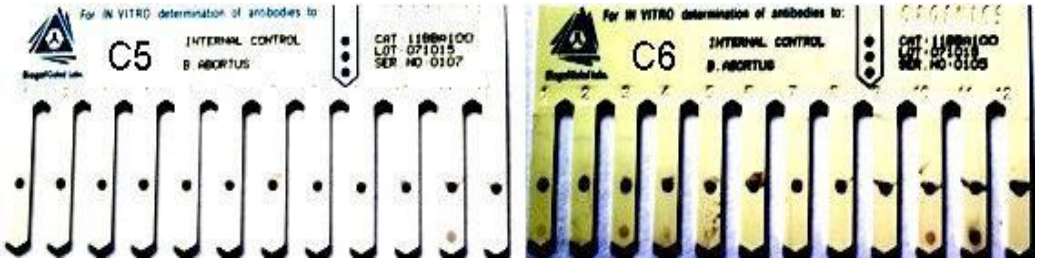

Figure 1. Dot ELISA of cattle sera for antibodies to *Brucella abortus.*

cattle included in the study had not been vaccinated against Brucellosis. Serum samples positive by all the 3 tests, that is, RBPT, standard tube agglutination test (STAT) and Dot ELISA were considered as positive controls. The 18 sera included in the study were selected out of 70 serum samples screened for Brucellosis. The 18 sera were positive by Dot ELISA but negative by RBPT and / or STAT. The animals positive by Dot ELISA were taken as positive since the results of Dot ELISA have been found to correlate well with the clinical picture and microbiological as well as molecular (PCR) analysis. However, the specimens could not be cultured for *Brucella* organisms.

In the absence of culture of *Brucella* organisms, the results of ELISA and PCR were considered as the gold standard.

Serological tests

Rose Bengal plate test (RBPT):

Equal volumes (10 µl of each) of RBPT colored antigen (IVRI, Izatnagar, India) and test serum were mixed on a clean glass slide (Morgan et al., 1978) with the help of a clean sterilized toothpick. The slide was observed after 1 min for the formation of clumps. The formation of clear clumps was considered a positive test while the absence of clear clumps was considered a negative reaction.

Standard tube agglutination test (STAT):

Plain antigen of *Brucella abortus* S99 (IVRI, Izatnagar) was used as per the method of Alton et al. (1975). Two fold serial dilutions (1:20 to 1:640) of the sera were prepared in phenol saline and equal quantity (0.5 ml) of antigen was added to each tube. All the tubes were incubated at 37^{o}C for 24 h. The results were compared with the antigen control tube showing 50% agglutination. A titer of 1:40 or above was considered positive.

Dot ELISA:

ImmunoComb Bovine *Brucella* antibody test kit (Biogal-Galed Labs, Israel) was used for conducting Dot ELISA with the serum samples of cattle. The samples to be tested were mixed with the diluent in the first row of wells of a multichamber developing plate. The test spots on the comb were then incubated with the serum samples in the developing plate. The comb was then transferred to a well containing buffer to wash the unbound antibodies from the antigen spots. The comb was then allowed to react with an anti -cattle IgG alkaline phosphatase conjugate. After 2 more washes, the comb was moved to the last well. Color was developed by an enzymatic reaction. The intensity of color of the test spots corresponded directly to the antibody level in the test sample.

RESULTS

In the present study on 18 serum samples from cattle suspected of brucellosis, 9 (50%) samples were found to be positive while 9 (50%) samples were found to be negative by RBPT. Only 1 (5.55%) out of 18 samples was positive by STAT. All the 9 RBPT positive samples (100%) showed negative results by STAT. All the 18 samples (100%) showed positive results with Dot ELISA. (Table 1, Figure 1).

Among the RBPT negative samples, 8 out of 9 (88.88%) showed negative results by STAT while 1 out of 9 (11.11%) showed a titer of 1:40. However, all the samples negative by RBPT or STAT showed positive results with Dot ELISA. (Table 2, Figure 1).

All the serum samples from normal healthy cattle were negative by RBPT, STAT and Dot ELISA (Table 3, Figure 1).

Table 2. Detection of RBPT and STAT false negative cases by Dot ELISA.

S. no.	ELISA Comb and Lane no.	Sample no.	RBPT	STAT	ELISA
1	C1L7	5P	Negative	1:40	Positive
2	C1L9	J-4	Negative	1:20	Positive
3	C3L3	M-9	Negative	Negative	Positive
4	C3L10	M-18	Negative	Negative	Positive
5	C6L1	J-6	Negative	Negative	Positive
6	C6L3	J-19	Negative	Negative	Positive
7	C6L4	M-19	Negative	Negative	Positive
8	C6L5	M-20	Negative	Negative	Positive
9	C6L10	M-17	Negative	Negative	Positive

Table 3. Serological evaluation of sera from Brucellosis - free cattle.

S. no.	ELISA Comb and Lane no.	Sample no.	RBPT	STAT	Dot ELISA
1	C1L3	136P/J-26	Negative	Negative	Negative
2	C2L2	4	Negative	Negative	Negative
3	C3L7	M3	Negative	Negative	Negative
4	C4L5	A4	Negative	Negative	Negative
5	C5L3	D3	Negative	Negative	Negative
6	C5L6	2-P	Negative	Negative	Negative
7	C5L7	1-P	Negative	Negative	Negative
8	C5L8	6-P	Negative	Negative	Negative
9	C5L10	A-5	Negative	Negative	Negative
10	C6L2	J-16	Negative	Negative	Negative

DISCUSSION

The present study has shown that the commonly used conventional serodiagnostic tests for brucellosis, RBPT and STAT, may not be absolutely reliable. RBPT detected antibody in the sera of 50% of the animals suspected for brucellosis whereas, STAT could detect only 5.55% cases. Interestingly, RBPT, commonly believed to be cruder and less sensitive test could detect 58.82% samples negative by STAT. On the other hand, all the samples from suspected cattle shown to be negative by RBPT or STAT, were found positive by Dot ELISA.

Other workers have also reported comparable results with RBPT, STAT and ELISA in the serodiagnosis of brucellosis. Otlu et al. (2008) studied the seroprevalance of brucellosis in cattle and humans. Among the cattle sera, 32.92 and 34.64% were found positive by RBPT and SAT, respectively. Of the human samples, 13, 14.22 and 17.88% of the sera were found positive for brucellosis by RBPT, SAT and ELISA respectively. Kanani (2007) studied the seroprevalance of brucellosis by ELISA and compared its efficacy with RBPT and STAT. The tests revealed 5.94, 9.90 and 9.90% of the bulls positive for brucellosis by RBPT, STAT and ELISA, respectively. The sensitivity was reported to be 50% in case of RBPT and 62.5% in case of STAT considering ELISA as a gold standard test. However, Ofukwu et al. (2008) found a non - significant ($p < 0.05$) difference in the sensitivity for the RBPT and SAT tests. Jacques et al. (1998) assessed the efficacy of indirect ELISA in comparison with RBPT and CFT on sera from ewes infected with *Brucella melitensis*. The indirect ELISA was shown to be a good screening test to be used alone or in addition to RBPT. However, for an accurate and fool proof diagnosis of brucellosis, it is suggested on the basis of our findings that a combination of RBPT and ELISA should be followed in case of samples found negative by either RBPT or STAT used singly or in combination.

Conclusions

Comparative efficacy of 3 serodiagnostic tests (RBPT, STAT and Dot ELISA) in detecting anti - *Brucella* antibodies in sera was evaluated on a total of 28 serum samples which included 18 samples from brucellosis suspected and 10 from normal healthy (brucellosis unaffected) cattle. Only 50% of the suspected samples were positive by RBPT, whereas Dot ELISA could detect antibodies in

all the samples. RBPT could detect antibodies in 58.82% samples found negative by STAT while all the 9 RBPT negative samples showed positive results with Dot ELISA. It is, therefore, suggested that for an accurate and fool proof diagnosis of brucellosis, a combination of RBPT and ELISA should be followed in case of samples found negative by either RBPT or STAT used singly or in combination.

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