Journal of Microbiological Methods 97 (2014) 25–28

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Note

Innovative modifications to Rose Bengal plate test enhance its specificity, sensitivity and predictive value in the diagnosis of brucellosis

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ARTICLE INFO

Article history:
Received 19 October 2013
Received in revised form 8 December 2013
Accepted 8 December 2013
Available online 15 December 2013

Keywords:
Superagglutination test
Brucellosis
Sensitivity
Specificity
False negative
False positive

ABSTRACT

Current agglutination tests occasionally yield false results. Superagglutination test reduced false results, had higher sensitivity (95.88%) and negative predictive value (95.83%) than Rose Bengal plate test (RBPT), Standard Tube Agglutination test (STAT), ELISA, and Complement Fixation test and specificity (89.32%) and positive predictive value (89.42%) higher than RBPT and STAT.

Brucellosis is an important zoonotic disease caused by *Brucella* organisms. It is of public health significance and causes huge economic losses to the livestock sector due to reproductive losses in animals, abortions, placentitis, epididymitis and orchitis. Brucellosis is endemic in India (Aulakh et al., 2008) where it is estimated to cause a loss of US $58.8 million per year (Kollannur et al., 2007).

The Rose Bengal plate test (RBPT) is often used as a rapid screening test in the diagnosis of brucellosis (Ruiz-Mesa et al., 2005). Although the sensitivity of RBPT is reported to be very high, the specificity can be disappointingly low (Barroso et al., 2002). As a result, the positive predictive value of the test is low and a positive test result thus requires confirmation by a more specific test (Smits and Kadri, 2005).

The RBPT could sometimes give a false positive result. Suitable modifications of the RBPT are, therefore, required to get accurate results. We have developed a novel Superagglutination test to enhance the sensitivity and minimize false positive and false negative results of RBPT (Saxena and Kaur, 2013). The present study was undertaken to compare the sensitivity and specificity of the novel Superagglutination test with the available serodiagnostic tests RBPT, STAT, CFT, and ELISA to evaluate its efficacy on serum samples that may be either false positive or false negative by RBPT.

Guidelines of the Institutional Animal Ethics Committee were followed in the study.

A total of 200 bovine (181 cattle and 19 buffalo) serum samples were derived from the animals in veterinary clinics, dairy farms and gaushalas (animal shelters) in and around Ludhiana. All the animals were of age two years or more. Brucellosis suspected herds were selected for sampling primarily based on the history of abortions in the herd while normal healthy animals were sampled from the herds of the university dairy farm without the history of abortions and repeatedly RBPT negative status. Common serological tests i.e. the RBPT, STAT, iELISA and CFT along with the Superagglutination test were applied on all the serum samples.

RBPT was done as per the standard method (Morgan et al., 1978).

For performing Superagglutination test, equal volumes (2.5 μl each) of RBPT colored antigen, test serum stained with 0.1% Coomassie Blue dye, biotinylated anti-bovine IgG (Sigma) and streptavidin (Sigma) were mixed thoroughly on a clean glass slide in the abovementioned sequence. The slide was observed for 4 min for the formation of clumps. Ordinary hand lens was used occasionally for better resolution. The slides were viewed under low power (10×) of an inverted microscope to visualize the composition of clumps in case of doubt. Formation of clear agglutination, within which the blue color (due to the Coomassie Blue dye staining the serum antibodies) and the pink color (due to the Rose Bengal dye stained RBPT antigen) could be differentiated on magnification, were considered as positive, while absence of clear agglutinates was considered as negative.

The standard method recommended by the Office International des Epizootes (OIE, 2004) was followed for Standard Tube Agglutination test (STAT).

To perform Indirect ELISA (iELISA), a commercial kit was procured from Immunobiological Laboratories IBL-America (Minneapolis, USA).

Abbreviations: RBPT, Rose Bengal plate test; STAT, Standard Tube Agglutination test; iELISA, Indirect enzyme linked immunosorbent assay; CFT, Complement fixation test.

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0167-7012/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.mimet.2013.12.005

The test was performed according to the instructions provided in the kit manual.

Complement Fixation test (CFT) was performed as per the OIE Manual.

The positive predictive value (PPV) and negative predictive value (NPV) for each diagnostic test were calculated using the following formulae:

$$\text{PPV} = \frac{\text{Number of True Positive cases}}{\text{Number of True Positive cases} + \text{Number of False Positive cases}}$$

$$\text{NPV} = \frac{\text{Number of True Negative cases}}{\text{Number of True Negative cases} + \text{Number of False Negative cases}}.$$

Observed proportion of agreement (OPA) and agreement beyond chance (kappa values) were determined using Winepiscope-2 software package with 95% confidence level.

Out of the 200 samples, 97 were found to be positive by RBPT (Table 1). The percent prevalence of brucellosis varied with the test and ranged from 43.50% to 48.50%. The test detected 6% less positive samples than the Superagglutination test and showed a sensitivity of 93.33%, a specificity of 88.18%, a PPV of 86.6% and NPV of 94.17%, respectively (Table 2).

In the case of the Superagglutination test, the clumps on the slide had both blue and pink color. When the slide was viewed under the low power of a light microscope, the agglutinate could be very easily differentiated into two parts, the antibodies were blue in color due to the Coomassie blue dye and the antigen was pink in color due to the Rose Bengal dye (Figs. 1 & 2). A total of 104 out of the 200 serum samples were detected positive by Superagglutination test (Table 1). The test detected more positive samples than ELISA (16.5%), CFT (14.5%), RBPT (6%) and STAT (6%) and showed a sensitivity of 95.88% and a specificity of 89.32%. The positive predictive value (PPV) of this test was found to be 89.42% and Negative Predictive Value (NPV) was 95.83% (Table 2).

STAT could detect 119 out of the 200 samples as positive. A titer of 1:40 and above was considered as positive. A total of 81 samples had titers below 1:40, 36 samples had a titer of 1:40 and 83 samples had titers more than 1:40 (Table 1).

Table 1
Results of analysis of sera by various serological tests.

Sample no.	STAT titer	RBPT	Superagglutination test	iELISA	CFT
69(20/4/11), 80(20/4/11), 83(20/4/11), A, B, F, G, BB, BH, BR, DD, DL, K, L, M, N, S, V, AC, AF, AG, AP, AZ, BG, CB, CK, 68(20/4/11), 70(20/4/11), 71(20/4/11), 77(20/4/11) 78(20/4/11), 81(20/4/11), BK	00	−	−	−	−
AT	00	−	−	−	+
T	00	−	+	−	−
2(1/3/11), 13(1/3/11), 23(1/3/11), 76(T 5/10/11), 10(13/10/11), E, BA, BF, CY, DN, DR, AB,74(20/4/11), 3 (13/10/11)	10	−	−	−	−
AE	10	−	−	−	+
AI, BC, BY	10	+	+	−	−
AN, BW	10	+	+	+	+
23(20/4/11), BP, 102(15/9/11), 7(20/4/11)	20	−	−	−	+
73(20/4/11), 1(1/3/11), 20(1/3/11), 21(T5/10/11), 5(13/10/11), AD, BL, 66(20/4/11), 3(1/3/11), T7, 6(13/10/11), T2061(25/11/11), J	20	−	−	−	−
T6	20	+	−	−	−
CE, AV	20	−	−	+	+
CN, AJ	20	+	+	−	−
CX, AO	20	+	+	+	+
7(13/10/11)	20	−	+	−	−
29(15/9/11), 40(15/9/11), 15(20/4/11), 17(20/4/11), 20(20/4/11), 34(20/4/11), 42(20/4/11), 51(20/4/11), 53(20/4/11), 54(20/4/11), 72(20/4/11), 76(20/4/11), T1, T3, 4(1/3/11), 92(T 5/10/11), 11(13/10/11)	40	−	−	−	−
T8, 79(T 5/10/11), T 85 (25/11/11), O	40	−	−	−	+
DQ, BE, AQ, 2413	40	+	+	+	−
T 81 (25/11/11)	40	−	+	−	+
T 2062 (25/11/11)	40	−	+	+	+
H, W, X, AS, BT	40	+	+	+	+
AL, AY, 2379	40	+	+	−	+
CI	40	+	+	−	−
2218, 2452, 2581, 101(15/9/11), I, AH, CR, DG, DJ, DO	80	+	+	+	+
13(20/4/11), 79(20/4/11), 100(20/4/11)	80	−	+	−	−
11(1/3/11), 19(1/3/11), 82(T 5/10/11)	80	−	−	−	−
DU, BI	80	+	+	+	−
12(13/10/11), CV, 16(20/4/11), BJ, BM	80	+	+	−	−
P	80	+	+	−	+
2308, 2417, 89(T 5/10/11), U, Y, AK, AW, CT, DB, DC	160	+	+	+	+
103(15/9/11), 6(1/3/11), 9(1/3/11)	160	−	+	−	−
T4	160	+	−	−	−
86(T 5/10/11)	160	+	−	+	+
CM, T5, AA	160	+	+	−	+
R, BD	160	+	+	−	−
BX, DP, CZ	160	+	+	+	−
2362, Q, AM, AR, AU, BS	320	+	+	+	+
82(20/4/11)	320	−	+	−	+
BO, BQ	320	+	+	−	+
2490, T2, AX, BZ, 25(20/4/11)	640	+	+	+	+
2574, 2582	640	+	+	+	−
2426, 2467, 2554, 2567, 104(15/9/11), 19(20/4/11), 32(20/4/11), 87(T 5/10/11), CD, CH, CS, 2489, 88(T 5/10/11), 80(T 5/10/11), T 29 (25/11/11), T 77 (25/11/11), T 78 (25/11/11), T 84 (25/11/11)	>1280	+	+	+	+
T 8 (25/11/11)	>1280	+	+	−	+
T 86 (25/11/11)	>1280	+	−	+	+