

Comparative evaluation of the Rose Bengal plate test, standard tube agglutination test and complement fixation test for the diagnosis of human brucellosis

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Summary

In this study, 241 serum samples from individuals exposed to brucellosis were subjected to the Rose Bengal plate test (RBPT); the titre was estimated by standard tube agglutination test (STAT), with positive ≥ 80 IU/ml. Randomly selected sera ($n = 81$) were analysed by complement fixation test (CFT): titre $\geq 1:4$ was considered positive. Of 241 sera subjected to RBPT and STAT, 177 were negative in both tests; 5 samples tested negative by RBPT but positive by STAT. None was positive by RBPT and negative by STAT. Of 81 sera subjected to CFT, 23 (28.4%) were positive. Both RBPT and CFT found 18 samples positive; 5 samples were positive by CFT and negative by RBPT. Comparison of STAT with CFT showed 13 samples positive by STAT but negative by CFT, and 4 positive by CFT but negative by STAT. The sensitivity and specificity of STAT were 82.6% and 77.6%, respectively, with CFT as gold standard. No test is perfect, and the clinical history coupled with a combination of two or more tests will reduce diagnostic errors.

Keywords

Comparative evaluation – Complement fixation test – Human brucellosis – Rose Bengal plate test – Standard tube agglutination test.

Introduction

Brucellosis is a major zoonotic disease that causes considerable economic losses due to reduced productivity, abortions and weak offspring, and impedes trade and export. Almost all domestic species can be affected (9). Human brucellosis is a severely debilitating disease, although the case fatality rate is generally low; it often becomes sub-clinical or chronic, especially if not recognised early and treated promptly. All ages are susceptible, both sexes are affected and congenital cases have been recorded (4, 13).

The clinical picture of brucellosis is not pathognomonic, and the clinical history of the patient is of paramount importance in diagnosis. Unequivocal diagnosis of *Brucella*

infections can be made only by the isolation and identification of *Brucella*, but in situations where bacteriological examination is not practicable, the diagnosis is established using serological methods. Wright and Smith (16) described the first serological test for brucellosis. Since then, a considerable number of serological tests have been developed, and modified in various ways to increase performance (8). Circulating *Brucella* antibodies have been demonstrated by the Rose Bengal plate test (RBPT), standard tube agglutination test (STAT), Coombs test, complement fixation test (CFT), 2-mercaptoethanol test and enzyme-linked immunosorbent assay (ELISA) (5). The World Organisation for Animal Health (OIE) (15) emphasises that no single serological test is appropriate in all epidemiological situations; all have limitations, especially when it comes to screening

individual animals or humans. Therefore, the purpose of this study was to evaluate three serological tests for the diagnosis of human brucellosis.

Materials and methods

In this cross-sectional study, first the purpose of the investigation was explained to the study participants and verbal consent for participation was obtained. Recruitment of participants was based on the selection of individuals occupationally exposed to brucellosis, including dairy workers, animal handlers, veterinary surgeons and veterinary pharmacists, and suspected human cases referred by physicians. Two hundred and forty-one serum samples collected from these individuals were subjected first to RBPT; the titre indicating seropositivity was estimated by STAT to be ≥ 80 IU/ml. Randomly selected sera ($n = 81$) were further analysed by CFT for comparison, and a titre $\geq 1:4$ was considered to be positive (1, 14). The associations between the results from the different diagnostic tests were calculated using Win Episcope 2.0 (12). The antigen for the RBPT and the plain (undyed) *Brucella* antigen for STAT were procured from the Punjab Veterinary Vaccine Institute, Ludhiana, Punjab, and stored at 4°C until use; the plain *B. abortus* antigen required for the CFT was procured from the Biological Products Division of the Indian Veterinary Research Institute (IVRI), Izatnagar.

Results and discussion

Diagnostic methods for brucellosis have been based primarily on serology; the lipopolysaccharide (LPS) from smooth strains induces the greatest immunological responses in various hosts. One of the major diagnostic problems results from the similarity of the O-antigenic side chain of the LPS of *Brucella* to that of other microbes such as *Escherichia coli* O116 and O157, *Francisella tularensis*, *Salmonella* spp. of Kauffmann-White group N (O:30), *Pseudomonas maltophilia*, *Vibrio cholera* and, in particular, *Yersinia enterocolitica* O:9. This cross-reactivity between *Brucella* and other microbes has restricted the specificity of many diagnostic approaches (3, 5).

In the comparison between RBPT and STAT, of 241 sera samples subjected to both tests, 177 were negative in both, whereas five samples tested negative by RBPT but positive by STAT (Table I). None of the samples was positive by RBPT and negative by STAT. By considering STAT as the gold standard, the sensitivity of the RBPT was found to be 92.2%, with a 95% confidence interval (CI) of 0.82–0.97, and the specificity was 100% (CI = 0.97–1.00). The kappa value was 0.95 with a CI of 0.89–0.99, which indicates

excellent agreement between the two tests. In contrast, Chachra *et al.* (2) studied the comparative efficacy of three diagnostic tests on 28 serum samples collected from cattle in different parts of Punjab. Out of 18 serum samples from cattle suspected of brucellosis, 9 samples (50%) were found to be positive by RBPT and only one sample (5.55%) was positive by STAT. Their result also revealed that all 9 RBPT-positive samples (100%) showed negative results by STAT. All 18 samples (100%) showed positive results with dot ELISA. The authors suggested that, for accurate diagnosis of brucellosis, a combination of RBPT and ELISA should be used. This should be followed, when samples were found to be negative, by RBPT and/or STAT.

Table I
Comparison of Rose Bengal plate test, standard tube agglutination test and complement fixation test

(Total number of samples: 241; number of samples analysed by complement fixation test: 81)

	STAT +ve	STAT -ve	CFT +ve	CFT -ve
RBPT +ve	59	0	18	11
RBPT -ve	5	177	5	47
Total	64	177	23	58

CFT: complement fixation test
RBPT: Rose Bengal plate test
STAT: standard tube agglutination test

Out of 81 human serum samples subjected to CFT, 23 (28.4%) (CI = 0.19–0.39) were found to be positive. Both RBPT and CFT were able to detect 18 samples as positive, while five samples were found positive by CFT and negative by RBPT (Table I). The sensitivity and specificity of RBPT were 78.3% (CI = 0.56–0.92) and 81% (CI = 0.69–0.89), respectively, when compared with CFT as a gold standard. The kappa value was 0.55 (CI = 0.36–0.74), which indicates a fair to good level of agreement between the tests. The CFT detects mainly IgG1 antibodies (5, 6).

In acute brucellosis, the first and main immunoglobulin isotype formed is IgM. Subsequently, there is a switch to synthesis of the IgG isotype in patients who have not received treatment. The initial IgM response may not be seen in patients with a slow, insidious onset of disease, in those seen late in the course of the disease, or in those undergoing relapse. The titres of agglutinins (IgM, IgA and IgG), usually decline after successful treatment; if they do not, it is necessary to evaluate the patient for the possibility of a relapse or chronic focal disease. The IgG and IgA titres increase during relapses (6). Given that the present study involved occupationally exposed individuals, the immunoglobulin profile seems to indicate patients with slow onset or a late course of brucellosis infection. This

may be attributed to a delay of the investigation of the first symptoms of brucellosis in these patients because of expectations that the symptoms might improve or the use of antipyretic self-medication.

In the comparison of STAT with CFT, 13 samples were positive by STAT but negative by CFT and 4 were positive by CFT but negative by STAT (Table II). The sensitivity and specificity of STAT were 82.6% (CI = 0.61–0.94) and 77.6% (CI = 0.64–0.87), respectively, when compared with CFT as the gold standard. The kappa value was 0.54 (CI = 0.35–0.73), which indicates a fair to good level of agreement between the tests. Serra and Vinas (11) reported a kappa value of 67% using an optimal break point titre of $\geq 1:160$. In the same study, when patients with previous infection were compared with controls, the sensitivity was found to be 20% at a break point of 1:160 and 33% at $\geq 1:80$, with a specificity of 82%.

Table II
Comparison of standard tube agglutination test and complement fixation test (2x2 contingency table)

	CFT +ve	CFT -ve	Total
STAT +ve	19	13	32
STAT -ve	4	45	49
Total	23	58	81

In the present study of 81 human sera tested by RBPT, STAT and CFT, 18 and 45 samples were found to be positive and negative, respectively, by all the tests (Table III). Four samples were positive only by CFT and 11 samples were positive on both RBPT and STAT but negative on CFT. In the study of Serra and Vinas (11), the results of CFT did not improve the diagnostic accuracy of tube agglutination and IgM ELISA in patients with primary infection. Moreover, in their study, false negative results were reported in two patients with primary infection who had positive blood cultures and high levels of IgM and IgG, which were undetected. From this viewpoint, the 11 CFT negative samples in the present study may in fact have been positive if a more reliable test, such as an IgM and IgG ELISA, had been employed. Lucero *et al.* (7) suggested that CFT mainly identifies IgG antibodies that appear in the later stage of the disease or in the ‘chronic’ form, but this test has several important disadvantages:

- it is unable to detect the acute form of brucellosis
- it is technically complicated to run
- it presents anti-complementary activity
- it requires very labile reagents.

Table III
Discrepancies among the three tests: Rose Bengal plate test, standard tube agglutination test and complement fixation test

CFT	STAT	RBPT	Number
+	+	+	18
+	+	-	1
+	-	-	4
+	+	-	0
-	-	+	0
-	+	+	11
-	-	-	45
-	+	-	2
Total			81

The non-specific clinical picture of human brucellosis emphasises the importance of laboratory-based diagnosis, but no individual test is perfect (8). The Joint FAO/WHO Expert Committee on Brucellosis (5) stated that most patients with acute brucellosis show positive reactions to all four tests considered (STAT, 2-mercaptoethanol, Coombs antihuman globulin test and CFT). However, in some cases, all serological tests remain negative, even in patients with bacteriologically confirmed infection.

In a further study, Saegerman *et al.* (10) compared three different serum indirect ELISAs (i-ELISAs), respectively using 1C8 (anti-bovine IgG1), 3H3 (anti-bovine IgG2 with slight cross-reactions with IgG1) and PG (anti-total IgG) conjugates, with standard serological tests. Their result indicated that, irrespective of the vaccination status of animals and the number of days post infection, the i-ELISAs were more sensitive than traditional tests in the detection of antibodies to *Brucella* in infected pregnant heifers. In the field, the 3H3 conjugate was reported to yield the highest specificity. Moreover, the i-ELISA assays were reported to be more sensitive, to give positive results sooner after infection in non-vaccinated animals and to be more persistent than the traditional serological tests in both experimentally and naturally infected animals. It is logical to conclude that no individual test is perfect, but consideration of the patient’s medical history, coupled with a combination of two or more tests, will reduce diagnostic errors, especially in developing countries where diagnostic facilities and techniques are scarce. In the present study, there was excellent agreement between RBPT and STAT when compared with RBPT and CFT or STAT and CFT. This moderate agreement of CFT with the routine serological tests used in this study and the cumbersome laboratory procedure required for the CFT technique may discourage the use of CFT for routine clinical analysis of suspected cases of human brucellosis.

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Évaluation comparative de l'épreuve à l'antigène tamponné ou rose Bengale, du test standard d'agglutination en tube et de l'épreuve de fixation du complément pour la détection de la brucellose chez l'homme

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Résumé

Pour les besoins de cette étude, 241 prélèvements sériques provenant d'individus exposés à la brucellose ont été soumis à l'épreuve à l'antigène tamponné (EAT) ou rose Bengale ; le test standard d'agglutination en tube (TSAT) a permis d'évaluer les résultats, considérés positifs à partir d'un titre ≥ 80 UI/ml. Des prélèvements sélectionnés au hasard ($n = 81$) ont été soumis à l'épreuve de fixation du complément (FC) : les titres $\geq 1:4$ ont été considérés positifs. Sur les 241 prélèvements soumis à l'EAT et au TSAT, 177 se sont révélés négatifs dans tous les cas ; 5 prélèvements ont été trouvés négatifs à l'EAT mais positifs au TSAT. Aucun prélèvement n'a donné de résultat à la fois positif à l'EAT et négatif au TSAT. Au total, 23 (28,4 %) des 81 sérums soumis à l'épreuve de FC ont donné des résultats positifs. Par ailleurs, 18 prélèvements ont donné des résultats positifs aussi bien à l'EAT qu'à la FC ; 5 autres étaient positifs à la FC mais négatifs à l'EAT. La comparaison entre le TSAT et la FC a montré que 13 prélèvements positifs à la première épreuve étaient négatifs à la seconde, tandis que 4 prélèvements positifs à la FC étaient négatifs au TSAT. La sensibilité et la spécificité du TSAT étaient respectivement de 82,6 % et de 77,6 %, la FC étant l'épreuve de référence (étalon or). Aucun test n'étant parfait, la prise en compte des antécédents cliniques et le recours à deux ou plusieurs tests permettront de réduire le nombre d'erreurs de diagnostic.

Mots-clés

Brucellose humaine – Épreuve à l'antigène tamponné ou rose Bengale – Épreuve de fixation du complément – Évaluation comparative – Test standard d'agglutination en tube. ■

Evaluación comparada de las pruebas de aglutinación en placa de rosa de Bengala, macroaglutinación clásica en tubo y fijación del complemento para diagnosticar la brucelosis humana

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Resumen

Los autores describen un estudio en el que se analizaron por aglutinación en placa de rosa de Bengala (RB) 241 muestras séricas de otras tantas personas expuestas a la brucelosis. El título se calculó por la prueba clásica de macroaglutinación en tubo, estableciendo el valor positivo en ≥ 80 UI/ml. Se analizaron por fijación del complemento (FdC) un conjunto de sueros elegidos aleatoriamente ($n = 81$), para los cuales se consideró positivo todo título $\geq 1:4$. De los 241 sueros sometidos a las pruebas de RB y macroaglutinación en tubo, 177 dieron negativo a ambas pruebas, 5 resultaron negativos a la prueba de RB pero positivos a la macroaglutinación, y ninguno resultó positivo a la primera prueba y negativo a la segunda. De los 81 sueros sometidos a FdC, 23 (un 28,4%) resultaron positivos, 18 muestras resultaron positivas a las pruebas de RB y FdC y 5 dieron positivo a la FdC pero negativo a la prueba de RB. La comparación entre la prueba clásica de macroaglutinación y la de FdC arrojó 13 muestras positivas a la macroaglutinación pero negativas a la FdC, y 4 positivas a la FdC pero negativas a la macroaglutinación. Utilizando la FdC como referencia, o "regla de oro", se cifraron la sensibilidad y especificidad de la prueba de macroaglutinación en 82,6% y 77,6%, respectivamente. Ninguna prueba es perfecta: para reducir errores hay que utilizar simultáneamente la historia clínica y la aplicación combinada de dos o más pruebas.

Palabras clave

Aglutinación en placa de rosa de Bengala – Brucelosis humana – Evaluación comparada – Prueba clásica de macroaglutinación en tubo – Prueba de fijación del complemento.



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