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Rose Bengal test: diagnostic yield and use for the rapid diagnosis of human brucellosis in emergency departments in endemic areas

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ABSTRACT

The aim of the present study was to analyse the diagnostic yield of the rose Bengal test for the rapid diagnosis of human brucellosis in an emergency department in an area where the disease is endemic. The study included 711 patients diagnosed initially with brucellosis and 270 controls. Brucellosis patients were divided into three groups: group I, individuals with no regular exposure to or history of brucellosis; group II, individuals exposed repeatedly to *Brucella* infection; and group III, individuals infected with *Brucella* who had received appropriate treatment during the previous 12 months. Blood cultures were positive for 445 (62.6%) brucellosis patients, while the remaining 266 (37.4%) patients were diagnosed according to clinical and serological criteria. The overall sensitivity of the rose Bengal test was 92.9%. The specificities for groups I, II and III were 94.3%, 91.7% and 76.9%, respectively, with positive likelihood ratios of 16.5, 10.4 and 4.2, respectively. The diagnostic gain after the performance of the rose Bengal test was good or very good in patients with no previous exposure to *Brucella* or history of brucellosis, but poor in patients who were exposed repeatedly to *Brucella* or had a history of brucellosis and a low pre-test probability. Use of the rose Bengal test as the sole technique for the diagnosis of brucellosis in endemic areas should be considered very carefully in the context of patients who are exposed repeatedly to *Brucella* or have a history of brucellosis.

Keywords *Brucella*, brucellosis, diagnosis, rapid diagnosis, rose Bengal test

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INTRODUCTION

Brucellosis is a worldwide zoonosis with a high degree of morbidity in humans [1]. The disease remains endemic in many countries, particularly around the Mediterranean basin and in the Middle East, India, Mexico, and Central and South America, and thereby represents an important public health problem [2]. In humans, brucellosis behaves as a systemic infection with a very heterogeneous clinical spectrum. The disease usually presents as fever with no apparent focus, although there are focal forms in 20–40% of cases [3]. As the clinical picture of human brucellosis is fairly non-specific, a definitive diagnosis requires isolation of the causative organism, or the demonstration of high levels of specific antibodies, or seroconversion.

Febrile syndromes with no apparent focus are a cause of great concern in patients. They therefore require a fast and precise aetiological diagnosis. Results of the evaluation of a dipstick assay for rapid diagnosis of human *Brucella* infection have been reported [4–6], but these indicate that the sensitivity of the *Brucella* dipstick assay is lower than that of the rose Bengal test, a traditional serological screening test for the diagnosis of brucellosis.

The rose Bengal plate agglutination test is a rapid test which was designed originally for screening use in veterinary medicine, but is now often used for the diagnosis of human brucellosis [7–9]. Its high sensitivity, ease and speed of use,

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as well as its low cost, have made it very popular in hospital emergency departments for the diagnosis of febrile syndromes. However, few studies have evaluated its specificity with large numbers of patients with brucellosis, in comparison with representative controls, in an endemic area. Therefore, the present study assessed the diagnostic yield of the rose Bengal test to define the most suitable conditions for its efficient use in emergency departments in endemic areas.

PATIENTS AND METHODS

Between January 1983 and December 2002, 845 patients with brucellosis were diagnosed, treated and followed in the Infectious Diseases Unit of Carlos Haya University Hospital, a tertiary care centre located in an endemic brucellosis area in the south of Spain. In order to analyse the sensitivity of the rose Bengal test, the study was restricted to 711 brucellosis patients for whom results were available for two or more blood cultures and a portfolio of serological tests, comprising rose Bengal and Wright seroagglutination, indirect immunofluorescence, and Coombs or immunocapture agglutination tests. A control group consisted of 270 individuals: 176 patients with different infectious, autoimmune or neoplastic processes with a precise aetiological diagnosis, but which involved an initial differential diagnosis with brucellosis (group A); 68 asymptomatic individuals who were exposed repeatedly to *Brucella* infection during their working day (group B); and 26 asymptomatic patients with a history of brucellosis who had been treated appropriately and who had shown no evidence of relapse after 1 year (group C).

A diagnosis of brucellosis was established either by isolation of *Brucella* spp. from blood culture or other clinical samples, or the presence of a compatible clinical picture together with the demonstration of specific antibodies at significant titres or seroconversion. Significant titres were considered to be a Wright's seroagglutination titre of ≥1/160, an indirect immunofluorescence titre of ≥1/100, and an immunocapture-agglutination or Coombs anti-brucella test titre of ≥1/320.

For blood cultures, biphasic Ruiz–Castañeda medium (Materiales y Reactivos SA, Madrid, Spain) was used until 1988. After 1988, non-radiometric semi-automated BACTEC NR 730 or 9240 systems (Becton-Dickinson Diagnostic Instrument Systems, Towson, MD, USA) were used. Blood cultures were processed according to standard techniques [10], with incubation for 30 days in biphasic Ruiz–Castañeda medium or the BACTEC NR 730 system, and for 15 days in the BACTEC 9240 system. Blind subcultures were performed on chocolate agar and *Brucella* agar (Biomedics, San Sebastian de los Reyes, Madrid, Spain) after 10, 20 and 30 days, or after 7 and 15 days when the BACTEC 9240 system was used. These subcultures were incubated at 37°C in a CO_2 5–10% v/v atmosphere for 3 days. If growth appeared, the colonies were identified by colonial morphology, Gram's stain, oxidase, catalase and urease tests, and positive agglutination with specific antiserum. All isolates were sent to the National Brucellosis Reference Laboratory (Valladolid, Spain) for definitive identification and biotyping.

The rose Bengal plate agglutination test, Wright's seroagglutination, Coombs anti-brucella test and indirect immunofluorescence were performed as described previously [11–13]. The immunocapture-agglutination test (Brucellacapt; Vircell SL, Santa Fé, Spain) was performed according to the manufacturer's instructions [14].

For the epidemiological analysis, three patient groups were defined according to their level of exposure in the cohort of brucellosis patients: group I, individuals with no regular exposure to *Brucella* or history of brucellosis; group II, individuals exposed repeatedly to *Brucella* infection; and group III, individuals infected with brucellosis who had received appropriate treatment during the previous 12 months. In order to analyse the diagnostic yield of the rose Bengal test, the sensitivity, specificity, positive and negative predictive values, and the positive and negative likelihood ratios were calculated for the entire cohort of brucellosis patients, and for groups I, II and III separately.

The different pre-test probabilities of brucellosis in patients with a febrile syndrome with no apparent focus for >1 week was established as described previously [15,16], as well as from our personal experience. Patients with simply fever with no apparent focus for >1 week were assigned a pre-test probability of 10% (p 0.1); those with fever with no apparent focus for >1 week, accompanied by profuse sweating and arthromyalgias, were assigned a pre-test probability of 20% (p 0.2); and those with fever with no apparent focus for >1 week, accompanied by profuse sweating, arthromyalgias and hepatomegaly, were assigned a pre-test probability of 30% (p 0.3). In accordance with Sackett *et al.* [17], the pre-test probability was converted into pre-test odds by dividing the pre-test probability by 1 − the pre-test probability. The post-test odds were calculated by multiplying the pre-test odds by the positive likelihood ratio. The post-test odds were converted into post-test probability by dividing the post-test odds by the post-test odds + 1 [17]. The final usefulness or gain of the test was defined as the difference between the post-test probability and the pre-test probability. Statistical analysis was performed with SPSS/PC v. 11.0 software and the two-by-two analyser v. 1.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The study included 711 brucellosis patients, 487 (68.5%) males and 224 (31.5%) females. The mean age of the group was 38.2 ± 17.1 years (range, 14–91 years). The mean duration of the symptoms before diagnosis of brucellosis was 44.0 ± 84.4 days, being <2 weeks in 239 (33.6%) patients, between 2 weeks and 1 month in 256 (36%) patients, between 1 and 3 months in 152 (21.4%) patients, and >3 months in 59 (8.3%) patients. Fever with no apparent focus was present in 474 (66.7%) patients, while 237 (33.3%) had focal complications. Table 1 shows the most relevant clinical data.

In total, 445 (62.6%) patients had positive blood cultures, while diagnosis of the other 266 (37.4%)

Table 1. Clinical features of patients with brucellosis

Symptoms and signs	No of cases (%)
Fever	702 (98.7)
Chills	606 (85.2)
Sweating	597 (84)
Constitutional symptoms[a]	533 (75)
Arthralgias	353 (46.6)
Myalgias	292 (41.1)
Hepatomegaly	250 (35.2)
Splenomegaly	148 (20.8)
Focal forms	237 (33.3)
Osteoarticular	142 (20)
Genitourinary	37 (5.2)
Neurological	5 (0.7)
Cardiovascular	7 (1)
Other focal form	28 (3.9)
More than one focal form	18 (2.5)

[a]Two or more of the following: anorexia, asthenia and malaise.

patients was based on clinical and serological criteria. All the bacterial isolates obtained were identified as *Brucella melitensis*. Fourteen of the patients with brucellosis were excluded from the final analysis, as some epidemiological data were missing from the charts. Thus, the final analysis included 697 patients with brucellosis: 307 in group I; 339 in group II; and 51 in group III (Table 2).

The overall sensitivity of the rose Bengal test was 92.9%; this included 93.8% for the patients in group I, 91.7% for those in group II, and 96.1% for those in group III (Table 3). The sensitivity was 89.9% for patients whose symptoms had lasted for <2 weeks, 95.7% for those with symptoms for 2 weeks to 1 month, 94.1% for those with symptoms for 1–3 months, and 88.1% for those with symptoms for >3 months.

Table 2. Results obtained with the rose Bengal test for different groups of patients with and without brucellosis

	Rose Bengal-positive *n* (%)
Patients with brucellosis	
Group I (n = 307)	288 (93.8)
Group 2 (n = 339)	311 (91.7)
Group 3 (n = 51)	49 (96.1)
Patients without brucellosis	
Group A (n = 176)	10 (0.6)
Group B (n = 68)	6 (8.8)
Group C (n = 26)	6 (23.1)

Group I, patients with no history of brucellosis or exposure to *Brucella* spp.; group II, patients exposed repeatedly to *Brucella*; group III, patients with a previous history of brucellosis; group A, patients with different infectious, autoimmune or neoplastic diseases; group B, asymptomatic individuals with occupational exposure to *Brucella* infection; group C, asymptomatic patients with a history of brucellosis.

Table 3. Diagnostic yield of the rose Bengal test

	Sensitivity (%)	Specificity (%)	PPV	NPV
Patients with no history of or exposure to *Brucella* spp.	93.8	94.3	0.97	0.90
Patients exposed repeatedly to *Brucella* spp.	91.7	91.2	0.98	0.69
Patients with a previous history of brucellosis	96.1	76.9	0.89	0.91

PPV, positive predictive value; NPV, negative predictive value.

Table 4. Diagnostic usefulness of the rose Bengal test for different groups of patients

Brucellosis patients	Pre-test probability (%)	Positive likelihood ratio	Post-test probability (%)
Group I	10		64
	20	16.51	80
	30	(95% CI, 9.2–30.1)	87
Group II	10		53
	20	10.40	72
	30	(95% CI, 5.1–22.3)	81
Group III	10		31
	20	4.16	50
	30	(95% CI, 2.2–0.17)	64

Group I, patients with no history of brucellosis or exposure to *Brucella*; group II, patients exposed repeatedly to *Brucella*; group III, patients with a previous history of brucellosis.
95% CI, 95% confidence interval.

Of the 270 patients in the control group, 22 (8.1%) had a positive rose Bengal test result, including ten (0.6%) of the 176 patients in group A, six (8.8%) of the 68 patients in group B, and six (23.1%) of the 26 patients in group C. Therefore, the specificity of the test fell markedly from group I to group III (Table 3).

The positive likelihood ratios of the rose Bengal test were 16.5, 10.4 and 4.2 for groups I, II and III, respectively. Combination of the pre-test probability with its corresponding positive likelihood ratio showed that the diagnostic usefulness or gain was good in all patients with suspected brucellosis who had no regular exposure to or previous history of the disease, but that it was inadequate for patients who were exposed repeatedly with a low pre-test probability, and for those patients with a recent history of brucellosis whose likelihood of having the disease was not high (Table 4).

DISCUSSION

Despite the important advances made in the diagnosis of human brucellosis following the general introduction of new semi-automated methods for blood culture processing [18], diagnosis of this disease is still based mostly on the demonstration of specific antibodies by means of different serological techniques. This is mainly because the greatest incidence of brucellosis is found in under-developed countries with poor technical resources, as well as the fact that it tends to occur in rural communities.

A large number of different tests have been used for the serological diagnosis of brucellosis, thus demonstrating the lack of an ideal technique. Rose Bengal is a rapid plate agglutination test that uses a suspension of *Brucella abortus* in an acid buffer. It is able to detect agglutinating and non-agglutinating antibodies, and avoids the prozone phenomenon. This endows the rose Bengal test with a high degree of sensitivity for diagnosing infection with *Brucella* spp., irrespective of the stage of the disease. This high sensitivity, together with the fact that the technique is simple and rapid (*c.* 4 min), makes the rose Bengal test ideal for screening patients for human brucellosis.

Although many studies have confirmed the high sensitivity of the rose Bengal test, information about its specificity in endemic areas is scarce. In most studies, the information is biased by the fact that control groups comprised healthy individuals or patients with other diseases, or both. Patients who are exposed occupationally to brucellosis, or who have a recent history of the disease, have been represented poorly in control groups.

In a study in an endemic area of Spain, the specificity of the rose Bengal test was found to be only 75% [19]. Studies have shown that oligosymptomatic, or even asymptomatic and self-limiting, episodes of infection are common in endemic areas [20,21] and that IgG anti-brucella antibodies can persist for many months after the conclusion of treatment [22,23]. This accounts for the high seroprevalence of anti-brucella antibodies in endemic regions [24,25] and in individuals who are exposed repeatedly [26]. Despite this, the rose Bengal test is now used in many emergency departments as a rapid test with which to establish a diagnosis of brucellosis and initiate therapy.

The results of the present study showed that the specificity of the rose Bengal test was 91% in individuals exposed repeatedly, falling to 76.9% in patients with a history of brucellosis during the previous year. The post-test probability and the diagnostic gain, which were very high in patients with no history of brucellosis or regular exposure to *Brucella*, also fell considerably in patients who were exposed repeatedly and had a low pre-test probability, especially for patients with a history of brucellosis.

Unless accompanied by such characteristic focal forms as sacroiliitis, orchiepididymitis or sub-acute lymphocytic meningitis, the symptoms of brucellosis are non-specific, and haematological and biochemical tests are of little diagnostic value [2,3]. Establishing a diagnosis of brucellosis, and prescribing suitable therapy, based on a positive rose Bengal test in patients who are exposed repeatedly to or who have a history of the disease does not therefore seem justified. To do so would potentially subject a large number of individuals to unnecessary treatment for 45 days, involving potential drug toxicity, as well as needless additional tests during the following months. This would not only generate unjustified health and social costs, but more importantly, it might also mask or hide other potentially severe diseases. The characteristics of the *Brucella* lipopolysaccharide mean that this pathogen has a very low capacity to cause severe sepsis, shock and disseminated intravascular coagulation [27], so that a delay in therapy for a few days does not affect the prognosis. As the results of serological tests are generally available within 48 h, there is no justification for not using these serological techniques in particular patient groups.

A possible bias in the present study might derive from the method used to assign the values for the pre-test probability. However, these values were not assigned at random, but were based on data from large aetiological studies of fever of intermediate duration in the south of Spain [15,16] and from experience in studying the clinical spectrum of brucellosis for >20 years [3]. The external validity of these pre-test values therefore seems clear, at least for countries around the Mediterranean basin and in the Middle East, where brucellosis is a frequent cause of febrile syndrome with no apparent focus.

In conclusion, use of the rose Bengal test as the sole diagnostic tool to establish treatment of brucellosis in endemic areas is not a reliable practice with individuals who are exposed repeatedly to the disease and who have a low pre-test probability, and is even less so with individuals who have a recent history of brucellosis, irrespective of their likelihood of having the disease.

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