

Serological diagnosis of human brucellosis in Morocco and prospects for advanced diagnostic techniques

Aicha Qasmaoui^{1,2*}, Samira Natoubi³, Mehdi Bougharouine⁴, Farida Ohmani¹, Karima Halout¹, Jamila Hamamouchi¹, Bouchra Belkadi^{2†}, Reda Charof^{1†}

¹Department of Medical Bacteriology, Laboratory of Epidemic Diseases, National Institute of Hygiene, Rabat, Morocco

²Department of Biology, Team of Microbiology and Molecular Biology, Faculty of Sciences, Mohammed V University, Rabat, Morocco

³Department of Nursing, Higher Institute of the Nursing Professions and Technical Health of Casablanca, Laboratory of Health, Care and Sustainable Development, Casablanca, Morocco

⁴Department of Epidemiological Surveillance, Provincial Health Safety and Monitoring Unit, Provincial Delegation of the Ministry of Health, Laayoune, Morocco

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ABSTRACT

Background and Objectives: Brucellosis, a contagious infection caused by *Brucella* spp, remains the most widely reported bacterial zoonosis globally. Since the clinical manifestations are often non-specific, reliable laboratory confirmation, in accordance with World Health Organization recommendations, is essential. This study reports human brucellosis cases between 2017 and 2025 based on serological confirmation; it also discusses approaches to improve diagnostic accuracy for better surveillance, timely treatment, and support public health strategies.

Materials and Methods: A total of 95 serum samples were obtained from patients presenting with clinical manifestations suggestive of brucellosis. Initial screening was performed using the Rose Bengal test, and positive or equivocal samples were further analyzed by Enzyme-Linked Immunosorbent Assay to detect both IgG and IgM antibodies for serological confirmation.

Results: Among the 95 patients investigated, the Rose Bengal test yielded positive results in 69.5% of cases. Serological confirmation by ELISA demonstrated IgM seropositivity in 57.9% of patients and IgG seropositivity in 55.8%. The diagnostic performance of ELISA showed a sensitivity of 83.3% for IgM detection and 80.3% for IgG detection. Regarding patient demographics, the mean age was 37.9 ± 16.4 years, with a slight male predominance (54.7%).

Conclusion: The study reveals a considerable proportion of brucellosis-positive cases, confirming the value of serological testing in endemic regions such as Morocco. Nonetheless, serology should be complemented with advanced diagnostic methods, including PCR to improve both the accuracy and timeliness of diagnosis. These findings support the adoption of integrated diagnostic approaches and the reinforcement of laboratory capacity in high-risk areas.

Keywords: Diagnostic; Brucellosis; Enzyme-linked immunosorbent assay; Rose bengal; Sensitivity; Specificity

*Corresponding author: Aicha Qasmaoui, M.Sc, Department of Medical Bacteriology, Laboratory of Epidemic Diseases, National Institute of Hygiene, Rabat, Morocco; Department of Biology, Team of Microbiology and Molecular Biology, Faculty of Sciences, Mohammed V University, Rabat, Morocco. Tel: +212670564899 Fax: +212537772067 Email: qasmaouiacha@gmail.com

†These authors contributed equally to this work.

INTRODUCTION

Brucellosis is recognized as a re-emerging neglected zoonotic disease that affects humans as well as numerous domestic and wild animal species (1, 2). The causative agents are bacteria of the genus *Brucella*, which currently comprises 12 species. In humans, the most clinically relevant species are *B. abortus*, *B. melitensis*, *B. suis*, and *B. canis* (3). Transmission to humans occurs either directly, through contact with infected animals or their secretions, or indirectly, most often via consumption of contaminated animal products such as unpasteurized milk (4). Because of its persistence and wide distribution, brucellosis continues to pose a major public health problem in many developing countries, where it is associated with significant socio-economic consequences (5).

The epidemiology of human brucellosis is closely linked to infection dynamics in animals (6). According to the World Health Organization (WHO), it is the most prevalent bacterial zoonosis worldwide, with more than 500,000 human cases reported annually. However, this figure is likely underestimated due to under-reporting, diagnostic difficulties, and frequent misclassification (7). Brucellosis remains endemic in many regions, including Morocco, where it is listed as a notifiable disease (8, 9).

Clinically, brucellosis is characterized by non-specific and often misleading manifestations, which makes laboratory confirmation essential in accordance with WHO recommendations. Culture-based isolation of *Brucella* is considered the reference method, but it is time-consuming, technically demanding, and often less sensitive, particularly after antibiotic treatment (10). For these reasons, serological techniques are widely used in practice. The Rose Bengal test, an agglutination-based assay, is frequently employed because of its simplicity and accessibility (11). Nonetheless, its sensitivity and specificity are limited, particularly in chronic or atypical cases (12). To address these limitations, the WHO recommends combining different serological methods, such as IgM and IgG ELISA, in order to improve diagnostic performance and provide better insights into the stage of infection (13). This combined strategy offers a more reliable approach for early diagnosis and appropriate clinical management (6).

In this context, the present study seeks to document suspected cases of human brucellosis analyzed in the laboratory using the Rose Bengal test and IgM/

IgG ELISA assays, while also proposing recommendations to optimize diagnostic strategies in accordance with international standards.

MATERIALS AND METHODS

Description of the cases. As part of this study, a comparative descriptive research design was employed. It was conducted retrospectively over an eight-year period, from May 2017 to May 2025. A total of 95 suspected cases of brucellosis, identified based on compatible clinical signs, were recorded in the southern regions of Morocco. The corresponding serum samples were analyzed at the Medical Bacteriology Laboratory of the National Institute of Hygiene (NIH) in Rabat, Morocco.

Inclusion criteria. Patients presenting with clinical signs suggestive of brucellosis, admitted between May 2017 and May 2025, with at least 1.5 mL of serum available, and complete results for all three diagnostic tests (Rose Bengal test, and ELISA for IgM and IgG antibodies).

Exclusion criteria. Patients with incomplete clinical information, insufficient serum volume, or missing results for one or more of the three diagnostic tests.

Serological tests. For each patient, 1.5 mL of serum was collected and analyzed for the presence of *Brucella*-specific antibodies. Serological confirmation was based on two complementary methods: the Rose Bengal test and enzyme-linked immunosorbent assay (ELISA) for both IgM and IgG antibody classes.

Rose Bengal test (RBT). The Rose Bengal Test (RBT) is a qualitative slide agglutination assay used to detect antibodies against *Brucella* spp. It is particularly useful for the serological diagnosis of infections caused by *B. melitensis*, *B. abortus*, *B. suis*, or *B. bovis*, primarily through the detection of IgG immunoglobulins.

In this study, the RBT was performed in accordance with the manufacturer's instructions (Rose Bengal, Bio-Rad®, France). Briefly, 30 µL of Rose Bengal reagent was mixed with an equal volume (30 µL) of serum on a glass slide. The mixture was incubated at room temperature for 4 minutes and then examined

visually. The presence of visible agglutination was interpreted as a positive result.

According to the manufacturer, the RBT demonstrates a sensitivity of 89% and a specificity of 100%, which supports its use as a reliable screening tool, particularly in endemic settings.

Enzyme-linked immunosorbent assay (ELISA).

Detection of IgM and IgG antibodies against *Brucella* spp. was performed using commercial ELISA kits (Vircell®, Granada, Spain), strictly following the manufacturer's instructions. The assays were conducted on 96-well microtiter plates pre-coated with *Brucella*-specific antigens. Each serum sample was analyzed in duplicate for both IgM and IgG antibody classes.

For IgM detection, titers above 11 DU/mL were interpreted as positive, values between 9–11 DU/mL as doubtful, and values below 9 DU/mL as negative. According to the manufacturer, the assay demonstrates 100% sensitivity and 100% specificity. All procedures were carried out in compliance with Good Laboratory Practice (GLP) standards to reduce the risk of cross-contamination and ensure analytical reliability.

Statistical analysis. Data was first entered into Microsoft Excel and subsequently exported to JAMOWI software (version 2.3) for analysis. Continuous variables were presented as mean $\pm$ standard deviation, while categorical variables were expressed as frequencies and percentages. Associations between categorical variables were evaluated using the Chi-square (χ^2) test. A p-value < 0.05 was considered statistically significant.

The diagnostic performance of the serological tests was assessed by calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

RESULTS

Participant characteristics. A total of 95 patients were included in the study, comprising 52 males (54.7%) and 43 females (45.3%). The mean age was 37.9 ± 16.4 years (range: 9–67 years).

Serological findings. Among the 95 serum samples analyzed, 66 (69.5%) were seropositive on the Rose Bengal test, suggesting presumptive brucellosis. Of

these, 55 patients (57.9%) were IgM-positive and 53 (55.8%) were IgG-positive by ELISA, while simultaneous positivity across all three tests was observed in 47 cases (Table 1). The Rose Bengal test detected the highest proportion of seropositive cases, whereas the ELISA assays showed slightly lower positivity rates but provided complementary information by distinguishing between IgM and IgG responses. These findings indicate that although the Rose Bengal test remains a valuable screening tool in endemic regions, the combined use of IgM and IgG ELISA enhances diagnostic accuracy and supports a more precise assessment of infection status.

The Rose Bengal Test was used as a comparative reference. ELISA demonstrated high diagnostic performance with sensitivities of 83.3% for IgM and 80.3% for IgG, and a specificity of 100% for both assays (Table 2). All IgM-positive ($n = 55$) and IgG-positive ($n = 53$) cases were also detected by Rose Bengal, resulting in an NPV of 100%. However, 11 IgM-negative and 13 IgG-negative samples were classified as positive by Rose Bengal, reducing the PPV to 80.3% (Table 4).

These findings highlight the strong reliability of ELISA in ruling out false positives, while also pointing to its slightly reduced sensitivity compared with Rose Bengal, thus supporting the use of combined testing strategies for optimal case detection. With respect to gender distribution (Table 2), seropositivity was marginally higher in males across all tests, yet no statistically significant differences were observed ($p = 0.696$ for Rose Bengal; $p = 0.997$ for IgG; $p = 0.965$ for IgM), suggesting that sex was not a determinant of brucellosis seropositivity in this cohort.

Table 1. Results of serological tests used for the diagnosis of Brucellosis (Rose Bengal, ELISA IgG, IgM)

Test	Negative		Positive	
	n	%	n	%
Rose Bengal	29	30.5	66	69.5
ELISA IgG	42	44.2	53	55.8
ELISA IgM	40	42.1	55	57.9

Table 2. Performance of ELISA IgM and IgG.

Test evaluated	Sensitivity (%)	Specificity (%)
ELISA IgM	83.3	100
ELISA IgG	80.3	100

Gender distribution of seropositive cases. Among the 66 patients who tested positive with the Rose Bengal test, 37 (71.2%) were male and 29 (67.4%) were female (Table 3). This reflects a slight male predominance among brucellosis-positive cases. The difference may be explained by greater occupational exposure of men to livestock and animal products, while the proportion of female cases suggests that household-related transmission routes, particularly consumption of unpasteurized dairy products, also play a role.

When compared to the Rose Bengal test, ELISA assays demonstrated strong concordance, with all IgM- and IgG-positive cases also detected by RBT, resulting in an NPV of 100% (Table 4). However, 11 IgM-negative (11.6%) and 13 IgG-negative (13.7%) samples were identified as positive by RBT, lowering the PPV of both ELISA assays to 80.3%. These

findings indicate that while ELISA provides excellent specificity and reliability in excluding false positives, its slightly reduced sensitivity may lead to missed cases if used alone, thus reinforcing the importance of combining different serological approaches for accurate diagnosis.

From a therapeutic perspective, all patients were treated with recommended antibiotic regimens, most frequently rifampicin in combination with doxycycline or trimethoprim/sulfamethoxazole, with clear clinical improvement observed across the cohort. Epidemiological investigation revealed that the main sources of exposure were the consumption of raw milk and undercooked livers. Notably, all cases originated from urban areas, underscoring the persistence of risky dietary practices even outside rural, livestock-associated environments.

Table 3. Distribution of test results by gender

Test	Result	Gender	Effective	%	p-value
Rose Bengal	Negative	F	14	14.7	0.696
		M	15	15.8	
	Positive	F	29	30.5	
		M	37	38.9	
IgG	Negative	F	19	20.0	0.997
		M	23	24.2	
	Positive	F	24	25.3	
		M	29	30.5	
IgM	Negative	F	18	18.9	0.965
		M	22	23.2	
	Positive	F	25	26.3	
		M	30	31.6	

No statistically significant differences were observed between the sex of patients regarding brucellosis serological test results: Rose Bengal ($p = 0.696$), IgG ($p = 0.997$), and IgM ($p = 0.965$).

Table 4. Diagnostic performance of rose Bengal Test versus ELISA IgM and ELISA IgG

RBT	ELISA IgM		ELISA IgG	
	Positive (n, %)	Negative (n, %)	Positive (n, %)	Negative (n, %)
Positive	55 (57.9)	11 (11.6)	53 (55.8)	13 (13.7)
Negative	0 (0)	29 (30.5)	0 (0)	29 (30.5)
PPV %		80.3		80.3
NPV %		100		100

DISCUSSION

Human brucellosis is a neglected zoonosis, still poorly recognized by many practitioners, particularly in endemic regions such as Africa, including Morocco (14, 15). Because of its non-specific clinical manifestations such as prolonged fever, night sweats, or joint pain, laboratory confirmation remains indispensable. Although culture of *Brucella* spp. is considered the reference method, it is rarely used in routine settings due to its long incubation time, low yield, and the biological risk involved (16).

Serology is therefore the most widely used diagnostic alternative. It is accessible, cost-effective, and relatively easy to implement, even in resource-limited environments. However, its sensitivity and specificity vary according to the technique used, the stage of infection, and the epidemiological context (3, 17).

Among serological approaches, ELISA is widely employed in both human and veterinary medicine, as it can detect IgM antibodies (suggesting acute infection) and IgG antibodies (chronic or past infection) separately. Nevertheless, several limitations affect its interpretation, including cross-reactivity with other bacteria, inter-individual variability in immune response, and the absence of universally standardized cut-off values (18).

In this study, a high proportion of positive cases was identified: 69.5% by Rose Bengal, 57.9% by IgM ELISA, and 55.8% by IgG ELISA. These findings suggest significant circulation of *Brucella* spp. in the

population studied. The peak of positivity observed in 2017 corresponds to a documented outbreak in the province of Laayoune (9), which likely explains the high rates recorded in our series.

According to previous studies, the sensitivity of the Rose Bengal test in endemic areas is estimated between 88% and 100% (19). In our study, 69.5% of patients tested positive by RBT, which reflects the specific epidemiological context, as most samples were collected during the 2017 outbreak in Laayoune, where more than 61% of cases were concentrated (9).

The Rose Bengal test is valued for its rapidity, simplicity, affordability, and good sensitivity (20, 21). However, it lacks specificity and therefore requires confirmation. The WHO recommends using RBT primarily as a screening tool, systematically followed by confirmatory assays such as ELISA (22). In our data, all IgM-positive and IgG-positive cases were also detected by RBT, resulting in an NPV of 100%. Conversely, 11 IgM-negative and 13 IgG-negative samples were RBT-positive, leading to a PPV of 80.3%. These discordances may reflect early infection, residual antibodies, or false-positive RBT results. This confirms that while RBT is an excellent initial test, its interpretation must be corroborated by ELISA.

Combining RBT with ELISA therefore emerges as a relevant and effective diagnostic strategy (23). This sequential approach increases sensitivity in endemic areas and is consistent with international recommendations. When interpreted together, IgM and IgG ELISA can achieve excellent specificity, approaching 100% in compatible clinical settings (14, 24, 25).

In our study, 57.9% of patients were IgM-positive, suggesting that more than half were in the acute phase of infection. Similar results have been reported elsewhere, confirming the robustness of IgM ELISA in early diagnosis (26). However, many authors recommend complementing IgM with IgG detection or confirmatory molecular methods (PCR or culture) to cover the full spectrum of disease evolution (27).

A total of 55.8% of patients were IgG-positive, which is consistent with other reports, although the percentage was lower than IgM in our series. This may be explained by the reduced sensitivity of IgG ELISA in acute phases and the persistence of IgG antibodies for months or years after infection (28). This persistence complicates the interpretation of IgG results in endemic settings, as positivity may reflect either current or past infection (29).

Profiles combining IgM positivity with IgG negativity were observed in several patients, strongly suggesting acute infection. Nevertheless, cross-reactions with other pathogens cannot be excluded, requiring confirmation by PCR or culture to ensure diagnostic accuracy (30).

It is also important to note that both IgM and IgG detection may be affected by cross-reactions or by the presence of rheumatoid factor, which can lead to false positives. For this reason, PCR is increasingly recommended as a confirmatory method, given its high sensitivity and specificity (17, 24, 31).

In this study, 30.5% of patients tested negative on serology despite presenting clinical signs. In endemic areas, such cases should not be disregarded and require confirmation by molecular techniques, as they may represent early or atypical infections (32). Combining multiple serological assays remains useful, but their limitations highlight the need to integrate molecular tools such as real-time PCR into diagnostic routines (6, 33). This integration must be adapted to local constraints of cost, feasibility, and biosafety (34).

PCR offers the advantage of detecting infection within days of symptom onset, even before seroconversion (35). In endemic settings, symptomatic patients with negative serology should systematically undergo PCR testing to minimize false negatives and improve early detection (34).

Our results also confirm that brucellosis affects all age groups. The mean age in this study was 37.9 ± 16.4 years, corresponding to an active adult population frequently exposed to risk factors. No significant gender differences were found, with a slight male predominance but an overall balanced distribution, in line with other reports (36, 37).

Certain risk factors were identified through patient interviews, notably the consumption of raw milk and undercooked liver, which are well-established routes of transmission (4). The fact that all cases originated from urban areas underscores the persistence of risky food practices even outside rural livestock settings. This is of particular concern in southern Morocco, where animal brucellosis seroprevalence is estimated between 2.8% and 3.3% (15, 38).

This study has several limitations. Only a single serum sample was collected per patient, although paired sera would allow demonstration of seroconversion (3). Furthermore, PCR confirmation was not available at the time of data collection, although this

method has since been incorporated into our laboratory routine. Another limitation is the absence of complete clinical and epidemiological information, such as occupation, animal exposure, and treatment history, which restricted risk factor analysis. These gaps highlight the need for better data collection and improved collaboration between clinicians and laboratories.

Given the constraints of serology and the growing role of molecular tools, an integrated diagnostic strategy is required. Combining RBT with IgM/IgG ELISA and, when discordant, confirmation by PCR would improve both sensitivity and specificity. This approach should be standardized and implemented in line with international recommendations, while strengthening laboratory capacity and clinician training.

Based on these findings, we propose a diagnostic algorithm beginning with RBT as a screening tool, followed by IgM/IgG ELISA for serological characterization, and PCR in discordant cases. This step-wise strategy, complemented by dual-sample testing and improved physician training, would enhance diagnostic reliability and strengthen surveillance in endemic regions.

CONCLUSION

Brucellosis remains a major public health concern in endemic regions such as Morocco, largely linked to the consumption of unpasteurized dairy products. The high proportion of seropositive cases observed in this study highlights the urgent need for reliable diagnostic strategies. Integrating real-time PCR with conventional serological assays would improve both sensitivity and specificity, reduce diagnostic errors, and provide valuable insight into circulating *Brucella* species. Future research should focus on validating combined diagnostic algorithms in larger, multicenter cohorts, while also exploring molecular typing tools to better characterize local epidemiological patterns. In parallel, studies assessing the cost-effectiveness and feasibility of routine PCR implementation in resource-limited settings are needed. Ultimately, sustainable control of brucellosis will require a “One Health” approach, combining veterinary control, strengthened laboratory capacity, and public education to reduce transmission and achieve long-term eradication.

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