THE ROLE OF ROSE BENGAL TEST IN DETECTING HUMAN BRUCELLOSIS

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ABSTRACT

Brucellosis remains a serious public health concern, necessitating effective monitoring. This study evaluated the seroprevalence and identification of *Brucella* spp. in 300 blood specimens from patients, using phenotypic characterization and the Rose Bengal Test (RBT) for diagnosis. The percentage of positive serological tests was 9.3% (28/300), with a higher infection rate in females (10.0%) than in males (8.7%). The highest ratio was observed among patients aged 31-40 years (13.3%). Bacterial isolation confirmed *Brucella* species in 7.0% (21/300) of samples. Epidemiological analysis revealed a significantly higher prevalence among rural (10.6%) than urban (7.9%) participants and clear seasonality, with a peak incidence in September (12.0%). This study confirms the endemicity of brucellosis, establishes the RBT as a vital screening tool, and identifies key demographic and seasonal risk factors for guiding public health intervention strategies.

Keywords: Human Brucellosis, Prevalence, Risk Factors, RBT, Zoonosis.

INTRODUCTION

Human brucellosis is a bacterial infection that primarily affects humans and a variety of species, including domestic livestock, wildlife, rodents, and marine mammals. It is a highly prevalent zoonotic infection (Almashhadany *et al*., 2026; WHO, 2020; Khairullah *et al.,* 2024; Heshteli *et al*., 2025). The bacterium of the genus Brucella causes the condition. The bacteria are characterized as gram-negative intracellular coccobacilli, non-motile, and lacking spore formation. They are aerobic and urease-positive, and notably, they do not possess a capsule. Certain strains require a 5-10% CO_2 environment for optimal primary isolation (Hadad et al., 1997; Bayhan *et al*., 2020; Ötkün & Gürbilek, 2025; Shen *et al*., 2025).

The infectious disease is human brucellosis, also known as Mediterranean fever, Malta fever, and undulant fever. Recently, the worldwide spread has undergone an important transformation due to a multifaceted interplay of factors, including animals, political instability, socioeconomic conditions, and inadequate sanitary controls (Almashhadany et al., 2022; Bandara *et al*., 2026; tkn & Grbilek, 2025). Brucellosis remains a persistent issue in many regions worldwide, including the Middle East, South and Central Asia, and Africa. This is mainly due to the lack of veterinary infrastructure and the widespread use of conventional livestock husbandry methods (Menshawy *et al*., 2025). According to reports, Southern Europe has sporadic occurrences of the disease, whereas Northern and Western Europe have successfully eradicated it (European Health and Digital Executive Agency, 2022; Ram, 2025).

Several factors affect the prevalence of undulant fever, including susceptible animal hosts, social and economic conditions, cultural practices, and the implementation of public health interventions, as well as a combination of biological, environmental, social, and economic factors (Corbel, 2006; Dadar & Maurin, 2025). Cattle, sheep, goats, and swine serve as critical reservoirs, excreting *Brucella* spp. through milk, urine, excrement, and reproductive materials (Parsaeimehr *et al*., 2026; Zeb *et al*., 2025). The maintenance and dissemination of the infection to domestic populations depend on wildlife reservoirs, including elk, bison, and wild boar (Rebollada-Merino *et al*., 2024; Wang *et al*., 2025).

Consumption of unprocessed milk and milk products significantly contributes to the incidence of human brucellosis. Implementing effective control measures such as vaccination and strict food safety protocols is vital for reducing the burden of the illness (Mascolo *et al.,* 2026; Kithuka *et al*., 2025). Official data from the World Health Organization (WHO) indicate an annual incidence of approximately 500,000 cases, yet it is widely regarded as significantly underestimating the true extent of infection (Mohammed & Girma, 2026; Fernandez-Georges *et al*., 2025).

Humans can become infected through various pathways: by ingesting unprocessed dairy and milk derivatives; by inhaling contaminated aerosols in the environment, and via direct contact with infected animals and their products. The bacteria infiltrate via mucous membranes, damage skin lesions, proliferate in the lymph nodes, and cause bacteremia, thereby disseminating throughout the body. While infrequent, instances of transmission between individuals have been documented through breastfeeding, blood transfusions, bone marrow transplants, sharing of contaminated needles, and transplacental infection (Al-Abd *et al.,* 2025; Mazzeo *et al*., 2025).

Clinical symptoms appear 2 to 4 weeks following infection, with an onset that may be gradual and insidious or notably acute. The symptom profile features intermittent fever, significant malaise, headache, myalgia, arthralgia, and respiratory or gastrointestinal issues such as cough and abdominal pain. Commonly observed are constitutional symptoms like anorexia, weight loss, and night sweats (Jin et al., 2023; Ibrahim et al., 2025; Hussein et al., 2025).

The genus Brucella includes 12 known species. The first category includes six traditional species: *Brucella abortus, Brucella melitensis, Brucella suis, Brucella canis, Brucella ovis, Brucella neotomae*, and the second category includes six recently identified species: *Brucella ceti, Brucella pinnipedialis, Brucella microti, Brucella inopinata, Brucella papionis, and Brucella vulpis*. Each species exhibits a distinct preference for its host reservoir: cattle are the host for *B. abortus,* goats and sheep are considered hosts for *B. melitensis*, and *Brucella suis* is known to infect pigs. The significance of these three species lies in their global endemicity (Ferrero et al., 2020; Rossetti et al., 2022; Qureshi et al., 2024; Han et al., 2025).

Present diagnostic approaches integrate clinical assessment, which is nonspecific, with serological testing. Serological assays remain fundamental in routine diagnosis due to their affordability, ease of operation, and high sensitivity (Almashhadany et al., 2023; Pang et al., 2025). The serology test (RBT) is a quick test that depends **on** agglutination on a slide **using a colored** Brucella abortus antigen at an **acidic** pH of 3.6-3.7. Initially developed for veterinary screening, its straightforward nature, affordability, rapid results, and high precision have led to its widespread use as a preliminary test for identifying brucellosis in humans (Elbehiry et al., 2023; Akhtardanesh et al., 2025; Almuzaini et al., 2025). Consequently, this study aims to: assess the prevalence of human brucellosis in Erbil Governorate, categorized based on habitation area (urban or rural), sex, age groups, and to evaluate the effect of seasonal conditions throughout the study period on the spread of this disease

METHODS

Collection of Specimens and Serological Identification

A total of 300 human blood specimens were randomly obtained. Data on participants registered by site of habitation, age, and sex were collected in Erbil City. The study was conducted from 1/7/ 2024 to 30 /12 /2024. 150 samples were obtained from each sex, with ages ranging from 1 to over 70 years. The samples included 140 blood specimens collected from an urban area and 160 from a rural area. The blood samples of each individual were collected in ten (10) ml increments. Each sample was divided into two tubes: Five milliliters were collected in a vacutainer tube that did not include any anticoagulants, and five milliliters were collected in a vacutainer tube that did contain anticoagulants. The collected specimens were transported in a sterile container to the Medical Microbiology Laboratory, Knowledge University/College of Science. The initial blood fraction was allowed to coagulate before centrifugation, which separated serum for Brucella antibody detection. The Brucella species were isolated using the second portion of the blood. (Al-mashhadany 2014).

Detection of Brucella Antibodies

The diagnosis of specific antibodies in patients was identified depending on the RBT TEST. According to Al Mashhadany (2018), mix 0.03 ml of test blood with an equivalent amount of antigen on an enamel plate, gently agitate for 4 minutes, and record agglutination as any perceptible reaction.

Isolation of Brucella Species

For Brucella species isolation, 5–7 mL of blood was inoculated into an aerobic blood culture bottle. Blood culture was performed using the BACTEC 9240 system (Becton Dickinson, Franklin Lakes, USA), and all bottles were incubated for up to 4 weeks. When a positive signal was detected, subculture onto Brucella base blood agar was performed; colonies were identified based on colony morphology and biochemical tests (oxidase, urease, catalase, nitrate reduction), as described elsewhere (Almashhadany et al., 2022). Cultures that did not produce a positive signal by the end of the fourth week were considered negative for Brucella spp.

Identification of Brucella Species

All *Brucella* spp. isolates were identified to the species level based on H_2S production, thionine sensitivity, CO_2 requirement, and agglutination with monospecific sera A and M (Hadad et al., 1997; Almashhadany et al., 2022).

Statistical Analysis

Data were analyzed using the chi-square test in SPSS version 15 to assess differences.

RESULTS

Detection of RBT Results

The results in Table 1 showed that 28 (9.3%) brucellosis cases were recorded among the 300 blood specimens assessed. The percentage of infections among female patients was 15 (10.0%), compared with 13 (8.7%) among male patients.

Table 1: Distribution of RBT among Human Samples According to Sex

Sex	No of Samples examined	Positive N (%)		Negative N (%)		X^2	P-Value
Male	150	13	8.7	137	91.3	0.158	0.691
Female	150	15	10.0	135	90.0		
Total	300	28	9.3	272	90.7		

RBT results among Human Samples based on Age

The high prevalence of brucellosis infection was recorded in two age groups, between (31- 40) and (21–30) years, with percentages of (13.3 %) and (12.5%), respectively, as shown in Table 2.

Table 2: Results of RBT Among Human Samples According to Age

Age Group (Years)	No. Examined	Positive N (%)		Negative N (%)		X^2	P-Value
1-10	30	2	6.7	28	93.3	2.572	0.922
11-20	35	3	8.6	32	91.4		
21-30	40	5	12.5	35	87.5		
31-40	45	6	13.3	39	86.7		
41- 50	35	4	11.4	31	88.6		
51 - 60	45	3	6.7	42	93.3		
61 - 70	40	3	7.5	37	92.5		
< 71	30	2	6.7	28	93.3		
Total	300	28	9.3	272	90.7		

Habitat distribution of Human Brucellosis

The data from our study revealed that the prevalence of brucellosis infection, based on habitation, was higher in rural areas than in urban areas, at 17/160 (10.6%) and 11/140 (7.9%), respectively (Table 3).

Table 3: Seroprevalence of Human Brucellosis in Human Blood Samples According to Residency

Source of samples	No examined	Positive N (%)		Negative N(%)		X^2	P-Value
Urban area	140	11	7.9	129	92.1	0.676	0.411
Rural area	160	17	10.6	143	89.4		
Total	300	28	9.3	272	90.7		

Brucella spp Identification

The results in Table 4 showed that, according to the morphological characteristics of growth on culture media and biochemical tests, *Brucella* species were isolated from 21 (7.0%) of 300 specimens. This study revealed that *Brucella abortus* was isolated from male 6/9 (66.7%) and female 7/12 (58.3%), while *Brucella melitensis* was isolated from male and female in ratios of 3/9 (33.3%) and 5/12 (41.7%), respectively.

Table 4: Isolation rate of Brucella species from Human Blood Samples

Sex	**No. sample**	**Positive N (%)**		**Negative N (%)**		***Br. melitensis* N (%)**		**X^2**	**P-Value**
Male	150	9	6.0	6	66.7	3	33.3	0.205	0.651
Female	150	12	8.0	7	58.3	5	41.7		
Total	300	21	7.0	13	61.9	8	38.1		

Relation between seasonal conditions and seroprevalence.

The results, as shown in Table 5, illustrated differences in the seroprevalence of specific Brucella antibodies in patients' blood. High value of infection found in September 6/50 (12.0%), then in August 5/50 (10.0%), whereas the infection percentage reduction in December was 3/50(6.0%) and in July 4/50 (8.0%)

Table 5: Seasonal Variation of Brucellosis Seroprevalence (RBT)

Months	No. examined	RBT N (%)		X^2	P-Value
July	50	4	8.0	1.261	0.939
August	50	5	10.0		
September	50	6	12.0		
October	50	5	10.0		
November	50	5	10.0		
December	50	3	6.0		
Total	300	28	9.3		

DISCUSSION

Brucellosis illness is considered a persistent and important problem for human health that severely impacts global socio-economic progress, particularly in the developing world, as consistently reported by major international organizations (Franc *et al.*, 2018; Kanu, 2024), including the Food and Agriculture Organization (FAO) and the World Organization for Animal Health (OIE). World Health Organization (WHO), The serological test RBT is a widely adopted global tool for epidemiological studies that uses human sera due to its affordability, simplicity, sensitivity, and ease of use, making it exceptionally effective for the detection of specific bacterial antibodies (Di Bonaventura et al., 2021; Islam *et al.,* 2025). The 9.3% value recorded in our research is consistent with Din *et al*. (2013), who reported a reach of 9.33% according to RBT. In comparison, Salem *et al*. (2014) in Egypt reported a prevalence of 11.1%, while Shome et al. (2017) in India reported a prevalence of 6.38%. In Angola, Mufinda *et al*. (2017) mentioned a general weighted percentage of 15.6%. The study found that females were more exposed (10.0%) than males (8.7%). This was compatible with Diju (2009) in Pakistan (25.58% in females vs 10.18% in males). Conversely, Salem et al. (2014) found lower rates in females (8.9%) than in males (11.6%), and Tumwine et al. (2015) also reported higher rates in males (20.5% vs 15.3%). However, our study revealed no statistically significant difference between sexes according to the chi-square test (0.158).

Regarding Table 2, the highest percentage was in patients aged 31–40 years, followed by 21–30 and 41–50 years (13.3%, 12.5%, and 11.4%, respectively), with no statistically significant variation in age-based risk according to the chi-square test ($\chi 2 = 2.572$). This aligns with Abdul-Razag (2015) in Yemen, who noted a high prevalence (17.4%) in the same age group among males. However, Riabi et al. (2017) in Iran reported a higher incidence in patients over 50 years old (30%).

In an investigation of brucellosis seroprevalence among Indian veterinary healthcare workers, Shome *et al*. (2017) analyzed 1,050 serum samples and found a significant gender-based risk. While males constituted the vast majority of the study group (94.5%), all seropositive cases were confined to this demographic, yielding a 7.46% infection rate and a statistically significant result (p=0.029). The current results were consistent with Sharma et al. (2016), who reported an overall seroprevalence of 4.96% and test-wise seroprevalences of 9.91% by RBPT, 9.91% by mRBPT, 9.09% by STAT, and 16.52% by I-ELISA. They reported that the prevalence of brucellosis was higher in the 35–50 years age group than in the 20–35 and 50–65 years age groups, and also confirmed that the sex-wise seroprevalence was higher in males than in females. This finding contrasts with research conducted in Uganda by Tumwine *et al*. (2015), which reported a higher seroprevalence of human brucellosis in individuals over 60 years old, at 22.2%. Additionally, the percentage among all patients in the younger age groups, specifically those with ages less than thirteen years, 13 to 19 years, and 20 to 59 years, was recorded at 16.7%. Furthermore, a recent study by Riabi *et al*. (2017) in Iran indicated that the majority of brucellosis cases occurred in patients older than 50 years (30%), while the lowest incidence 12.3 % was observed in individuals aged 31–40 years.

Nielsen and Gall (2001) indicated that the specificity and sensitivity of the RBT are reported to fluctuate with the serum hydrogen ion concentration (pH) and the surrounding temperature of the antigen. The principle of RBT operation depends on detecting immunoglobulins (Ig types: A, M, G1, G2) in the test serum and on a reduction in IgM activity at a lower pH value.

Based on the participants' living conditions (Table 3), a notable seroprevalence of Brucella infection was observed: 17 out of 160 (10.6%) in rural areas and 11 out of 140 (7.9%) in urban areas. Ebrahimpour *et al*. (2012) reported in Iran that out of 337 participants diagnosed with brucellosis, 25.72% were urban dwellers and 74.28% lived in rural settings. In a study by Dieckhaus and Kyebambe (2017) conducted in Uganda, it was noted that rural patients are often involved in livestock farming; additionally, drinking unpasteurized milk may be a major route of transmission. No variation in the percentage of humans was found by habitation (0.676).

Based on the research data (Table 4), the total isolation rate was 21 (7.0%) out of 300 specimens, depending on the morphological characteristics of Brucella. This finding revealed that the isolation rate in males and females for Brucella abortus was 6/9 (66.7%) and 7/12 (58.3%), while for Brucella melitensis it was 3/9 (33.3%) and 5/12 (41.7%), which showed no statistically significant variation among the isolation rates of Brucella species from human blood samples. Our findings were consistent with those of Minas et al. (2007) and Arshi et al. (2017), which reported isolation rates of 8.49% and 6.0%, respectively, for Brucella spp. At the same time, Ogola et al. (2014) reported a higher percentage than our findings in their study assessing the prevalence of disease in animals and humans living in the same house. They reported human infection rates ranging from 2.2% to 14.1% and animal infection rates ranging from 1.2% to 3.4%.

Another aspect of this study addresses the correlation between months and the prevalence of Brucella antibodies in human samples. The results presented in Table 5 indicate that the peak antibodies occurred in September (6 out of 50; 12.0%), followed by August (5 out of 50; 10.0%), while the lowest rates were recorded in December (3 out of 50; 6.0%) and July (4 out of 50; 8.0%), respectively, and no variation was found in the ratio of different months. However, bacterial transmission may be associated with contaminated food products such as meat and milk. Minas et al. (2007) reported that human brucellosis exhibits a strong seasonal pattern, with the majority of cases diagnosed between December and May. Riabi et al. (2017) in Iran found that, among 176 patients, 94.8% resided in rural areas and 5.2% in urban areas. The highest incidence of reported cases occurred in June and July, with the lowest figures recorded in January. It appears that the disease prevalence begins in weather with optimal temperatures (in spring), peaks in high-temperature environments (in summer), and shows a low disease prevalence in autumn.

CONCLUSION

Brucellosis remains a pressing public health challenge in Iraqi Kurdistan, necessitating a coordinated, multi-sectoral strategy. The Rose Bengal Test (RBT) is an ideal surveillance tool for human cases due to its affordability, simplicity, and high sensitivity. However, effective control extends beyond diagnostics; it requires sustained public awareness campaigns across all media platforms to educate communities about transmission pathways. Special emphasis should be placed on rural populations, where promoting the consumption of pasteurized milk and dairy products is essential. Ultimately, the long-term control of this zoonotic disease hinges on eliminating brucellosis in livestock, an indispensable foundation for safeguarding human health. A unified effort involving public health authorities, veterinary services, and community engagement will be key to reducing the burden of brucellosis in the region.

Conflicts of Interest

The authors declare no conflicts of interest.

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