JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Assessing human brucellosis infection rates in high-risk occupational groups

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Abstract

Introduction: Human brucellosis is a significant zoonotic disease with a substantial public health impact, particularly among individuals in high-risk occupations such as veterinarians, farmers, and laboratory workers. Despite its global prevalence, data on the occupational risk of brucellosis, particularly in specific regions like the Kurdistan region, remains limited. This study aimed to investigate the prevalence of *Brucella* infections among different occupational groups in Erbil, Kurdistan.

Methodology: A cross-sectional study was conducted from July to December 2023, involving 350 human blood samples collected from participants with various occupations in Erbil. *Brucella* infection was assessed using the Rose Bengal Test (RBT) and bacterial culture method. The results were analyzed with a focus on the association between occupation, gender, residence, and age; with the prevalence of *Brucella* infection.

Results: The overall prevalence of *Brucella* infection was 10.9% by RBT and 8.9% by bacterial culture. Veterinarians and veterinary assistants had the highest infection rates (16.0% RBT, 14.0% culture). Gender and residence had no significant impact on infection rates, although rural residents exhibited slightly higher prevalence. The age group 31–40 years showed the highest positivity rates, but differences across age groups were not statistically significant.

Conclusions: The prevalence of brucellosis in Erbil has remained stable over the past decade. High-risk occupations, particularly veterinarians and laboratory workers, require targeted preventive measures, including the use of personal protective equipment. This study emphasizes the need for enhanced occupational health strategies to reduce the risk of brucellosis in vulnerable groups.

Key words: *Brucella abortus*; *Brucella melitensis*; Kurdistan region, Iraq.

J Infect Dev Ctries 2026; 20(1):79-86. doi:10.3855/jidc.21171

(Received 08 December 2024 – Accepted 22 May 2025)

Introduction

Zoonotic diseases, as well as emerging and re-emerging infectious diseases, are serious challenges to public health worldwide. Epidemiological studies reveal that 61% of the 1415 known human infectious pathogens are zoonotic, and 75% of emerging or re-emerging diseases are believed to be zoonotic. In terms of transmissions, 35% and 61% of emerging zoonotic pathogens are known to be transmitted through direct and indirect contact, respectively [1–3]. A portion of these zoonotic diseases are more prevalent in certain groups of the population. In this context, human brucellosis remains the predominant occupational disease throughout the world. However, the actual percentage of reported cases that are acquired from various livestock-related occupational groups is not well known [4–7].

Brucellosis, caused by bacterial species within the genus *Brucella*, is a severely weakening classical bacterial zoonotic disease that constitutes a high public health threat and poses a major threat to human health. It is a very old disease since animals are the only source of infection, and a recent indication from Egyptian ancient skeletons has revealed that brucellosis has been existing since as far back as 750 BC. *Brucella* species can be traced back to 2.8 million years by probable indication of pathologic variations in a late Pliocene hominin skeleton [8,9]. *Brucella* is an intracellular Gram-negative short rod that survives mostly in infected animal hosts and humans. The genus is currently under taxonomic expansion with 12 validated species. The most important 6 species are *B. melitensis*, *B. abortus*, *B. canis*, *B. suis*, *B. ovis*, and *B. neotomae* with a widespread global distribution in domestic livestock and wildlife [10,11].

Brucellosis is a highly contagious neglected zoonotic disease that is endemic in many low- and middle-income countries, placing a burden on healthcare systems and the livestock industry, representing a persistent global health issue [12,13]. According to the World Health Organization (WHO), Brucellosis is one of the most widespread zoonosis infections globally [14–16]. Although numerous developed countries, including Australia, Canada, Japan, and New Zealand, have eradicated the disease, brucellosis remains a public health issue in Africa, the

Middle East, parts of Asia, and Latin America as a result of its high endemicity in these regions [17–19].

Brucellosis affects various species of wild and domestic animals, particularly food-producing animals, including large and small ruminants such as cattle, buffaloes, camels, sheep, goats, pigs, and reindeer. Through the previous two decades, the infection has also been recognized in marine mammals, including beaked whales, dolphins, cetaceans, porpoises, and seals, which may present an emerging risk to individuals professionally exposed to contaminated tissues from such animals. This disease is highly contagious with an infectious dose of 10–100 cells sufficient to cause systemic infection [20,21].

Human brucellosis, also known as undulant fever, Malta fever, and Mediterranean fever, is one of the most popular zoonosis in the world. The disease is considered an occupational risk to persons dealing with animals and animal products [22,23]. The worker groups most exposed to the pathogen are veterinarians and veterinary assistants, abattoir workers, milkers, dairy workers, livestock farmers, slaughterhouse workers, hunters, and laboratory personnel; all of whom are considered to belong to the high-risk occupational group [24]. According to WHO statistics, more than 500,000 new brucellosis cases are reported worldwide annually, with about 10 per 100,000 inhabitants [25–27]. However, the correct incidence was estimated to be between 5,000,000 and 12,500,000 cases per year [13,28]. In fact, there is a need for more accurate data on the epidemiology of job-related brucellosis to allow the implementation of more effective preventive measures, which will reduce the impact of the disease in groups exposed by their work activities. The availability of such information could also be translated into health protection behaviors among susceptible professionals. Thus, the aim of this study is to investigate the prevalence of *Brucella* in different occupational high-risk groups in Erbil, Kurdistan region.

Methodology

Study design and participants

A total of 350 human blood specimens were collected between July to December 2023 from Rizgary hospital and some medical diagnostic laboratories located in Erbil Governorate, Kurdistan region, Iraq. The subjects had different indoor and outdoor occupations. The blood samples were collected from 215 males and 135 females (rural: 165; urban: 185); the age of the participants ranged from 1 to > 71 years. All the participants in the study were informed about the study goal and that they could withdraw at any stage of the study. They were also informed that their information would be handled confidentially and anonymously. A standardized questionnaire was prepared for getting information about occupation, gender, age, residence, and date of collection of the samples.

Sample collection

Diagnosis of brucellosis was carried out by the Rose Bengal Test (RBT) to detect *Brucella* antibodies in the serum and blood culture to isolate *Brucella* species. A total of 50 participants were randomly selected each month and specimens were collected from each. About 10 mL of venous blood was collected from each participant. Firstly, 3 mL were collected into a plain tube for Rose Bengal Test (RBT), and then 5–7 mL were inoculated into an aerobic blood culture bottle for isolation of *Brucella* species. Blood samples were allowed to clot and then centrifuged at 4000 rpm for 10 minutes. The resulting serum was separated and used to detect *Brucella* antibodies [29].

Rose Bengal Test

Brucella antibodies were detected by a commercially available antigen kit (Torax Biosciences, Newtownabbey, United Kingdom) according to the following procedure. Briefly, a volume of 0.03 mL of serum was mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone of approximately 2 cm in diameter. The mixture was agitated gently for 4 minutes at ambient temperature; any visible agglutination was considered a positive result [30].

Isolation of Brucella spp.

Blood culture was performed by BACTEC 9240 (Becton Dickinson, Franklin Lakes, USA). All the bottles were incubated for up to 4 weeks. Whenever the instrument gave a positive signal, a subculture on *Brucella* base blood agar was done and identification of the colonies was confirmed by colonial morphology, and biochemical tests such as oxidase, urease, catalase, and nitrate reduction, as described elsewhere [31]. The culture was considered negative for *Brucella* spp. at the end of the fourth week without a positive signal.

Identification of Brucella species

All isolates of *Brucella* spp. were classified to the species according to H_2S production, sensitivity to thionine, CO_2 requirement, and agglutination with monospecific sera A and M [31,32].

Statistical analyses

Data was analyzed using SPSS statistical software, version 25 (IBM Corp, Armonk, NY, USA). Confidence intervals were calculated based on the culture results by Wilson method and bacterial culture results since isolation of *Brucella* from blood is the gold standard and definitive diagnosis. Chi-square test was used to evaluate differences between groups and $p < 0.05$ was considered as significant.

Results

Brucella infection according to occupation

The rates of *Brucella* infection were 10.9 and 8.9% according to RBT and the isolation of *Brucella* species respectively (Table 1). Based on the results of the two detection methods, the veterinarians and veterinary assistants' group were the most infected (16.0 and 14.0%). It is alarming that the infection prevalence in laboratory workers was similar to the rate among farmers. However, no significant difference was found between the occupations ($\chi^2 = 2.407$, $p = 0.879$).

Despite the apparent differences in the prevalence of *B. abortus* and *B. melitensis* within and among groups of occupations (Figure 1), these differences were not statistically significant ($p > 0.171$).

Brucella infection according to gender and residence

Based on RBT and bacterial culture results (Table 2), males were slightly more seropositive than females; however, such differences are subtle and statistically insignificant ($p \geq 0.558$ for RBT and 0.701 for bacterial culture results). Similarly, residents of rural areas were slightly more (sero)positive for the infection with a significantly different prevalence ($p \geq 0.294$ for RBT and 0.101 for bacterial culture results). The prevalence of *B. abortus* and *B. melitensis* (Figure 2) showed no significantly different distribution between genders and residence ($p > 0.556$).

Figure 1. Prevalence of *Brucella* species in human blood samples (n = 350).

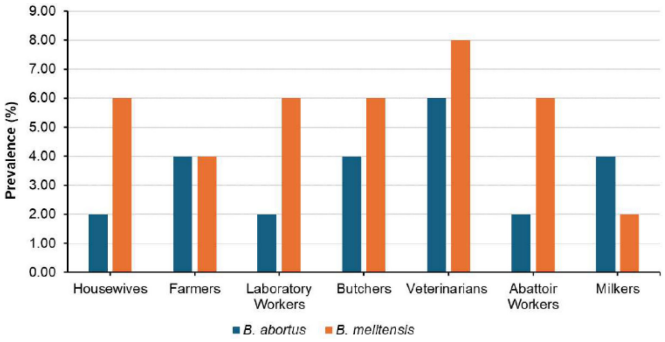

Figure 2. Gender (A)- and residence (B)-based prevalence of brucellosis in humans.

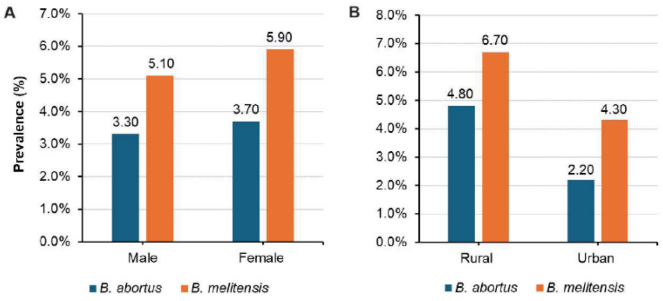

Table 1. Frequency of *Brucella* antibodies (by Rose Bengal test, RBT) according to occupations.

Occupation	Samples	RBT positive n (%)	Culture positive n (%)	95% CI
Housewives	50	6 (12.0)	4 (8.0)	3.15–18.84
Farmers	50	4 (8.0)	4 (8.0)	3.15–18.84
Laboratory workers	50	4 (8.0)	4 (8.0)	3.15–18.84
Butchers	50	5 (10.0)	5 (10.0)	4.35–21.36
Veterinarians[a]	50	8 (16.0)	7 (14.0)	6.95–26.19
Abattoir workers	50	6 (12.0)	4 (8.0)	3.15–18.84
Milkers	50	5 (10.0)	3 (6.0)	2.06–16.22
Total	350	38 (10.9)	31 (8.9)	6.31–12.30

[a] Including veterinary assistants.

Table 2. Gender- and residence-wise frequency of *Brucella* infections according to Rose Bengal test (RBT) and culture.

	No. exam	RBT positive n (%)	Culture positive n (%)	95% CI
Gender				
Male	215	25 (11.6)	18 (8.4)	5.36–12.84
Female	135	13 (9.6)	13 (9.6)	5.71–15.78
Total	350	38 (10.9)	31 (8.9)	6.31–12.30
Residence				
Rural	165	21 (12.7)	19 (11.5)	7.50–17.28
Urban	185	17 (9.2)	12 (6.5)	3.75–10.99
Total	350	38 (10.9)	31 (8.9)	6.31–12.30

Brucella infection according to age

The distribution of RBT and culture positivity across age groups is summarized in Table 3. Notably, participants aged 31–40 years exhibited the highest RBT and culture positivity rates at 14.5 and 16.4%, respectively. This age group stood out as having a higher likelihood of both tests being positive compared to other age groups, where positivity rates were generally lower. However, no age group showed a significantly higher rate of infection (as indicated by RBT or culture positivity) compared to the others ($p \geq 0.851$ for RBT and 0.219 for bacterial culture results).

Similarly, both *Brucella* species were prevalent in the age group 31–40 years in comparison to other groups. Generally, *B. melitensis* was more prevalent than *B. abortus* (Figure 3) without a significant difference between age groups ($p \geq 0.661$). The cause of not detecting *B. abortus* in the age group 61–70 years is not known.

Brucella infection according to seasonality

The temporal variation showed that the highest percentages of *Brucella* infection, according to RBT and culture isolation, were in September (15.9%) and October (13.3%). The isolation of *Brucella* species was high in September (11.5%) and October (6.5%) (Figure 4A). *B. melitensis* was more prevalent than *B. abortus* in all months of the study (Figure 4B).

Discussion

The current study aimed to detect brucellosis in different occupational groups of subjects in Erbil city. The overall prevalence of *Brucella* is consistent with previous studies in the Kurdistan region [31,33] and nearby countries in the Middle East [13]. However, different higher rates were also reported from the region [34] and other nearby countries [35,36]. Such discrepancies are likely to be multifactorial owing to the multiple factors affecting the prevalence, detection methods, and study design. For instance, inclusion of higher number of subjects with higher risks of being infected, such as slaughterhouse workers or farmers, is expected to result in higher prevalence rates [5]. Similarly, reports from endemic areas are very likely to show higher prevalence [27]. It is worth mentioning that RBT has limitations that include potential for false-positive results in endemic areas, cross-reactivity with other Gram-negative bacteria, and failure to detect early-stage infections [29]. Indeed, some studies have raised concerns on the low sensitivity of RBT in chronic cases and low specificity in endemic areas [37,38]. However, RBT managed to gain popularity due to its nature of being a rapid and cost-effective screening tool, its high sensitivity for detecting *Brucella* antibodies, and effectiveness when following standard protocols to screen large number of samples in poor-resource settings. In fact, some studies indicated that when following the standard protocol, RBT was not surpassed in effectiveness by more advanced and costly

Figure 3. Distribution of two *Brucella* species in different age groups.

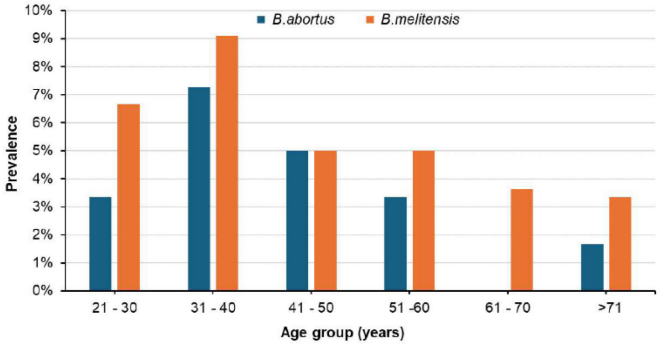

Figure 4. Monthly prevalence of *Brucella* infections according to serological and bacterial isolation approaches.

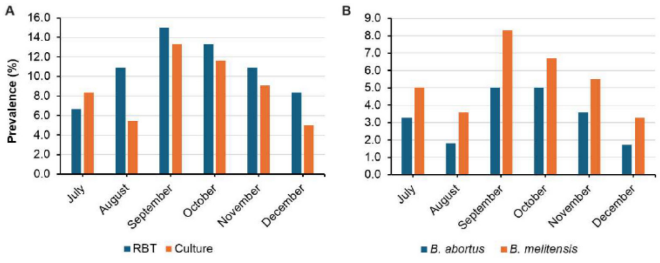

Table 3. Frequency of *Brucella* infections according to Rose Bengal test (RBT) and culture in different age groups.

Age (Years)	No. exam	RBT positive n (%)	Culture positive n (%)	95% CI
21–30	60	7 (11.7)	6 (10.0)	4.66–20.15
31– 40	55	8 (14.5)	9 (16.4)	8.86–28.26
41–50	60	7 (11.7)	6 (10.0)	4.66–20.15
51–60	60	6 (10.0)	5 (8.3)	3.61–18.07
61–70	55	6 (10.9)	2 (3.6)	1.00–12.32
> 71	60	4 (6.7)	3 (5.0)	1.71–13.70
Total	350	38 (10.9)	31 (8.9)	6.31–12.30

methods, such as serum agglutination, Coombs test, competitive enzyme-linked immunosorbent assay (ELISA), Brucellacapt, and lateral flow immunochromatography for detecting IgM and IgG in patients infected with *Brucella* [39].

The published data on the association of high prevalence of brucellosis and age is inconsistent and it is largely unknown whether they are somehow linked to each other or not. For instance, Ali and associates reported that the highest seroprevalence was found in participants older than 36 years of age, followed by the age groups of 26–35 years and younger than 26 years [6]. Higher age was also found to be associated with a higher seroprevalence in Bangladesh [40]. In contrast to this study, research conducted in Saudi Arabia and India reported seroprevalences to be higher in study participants younger than 40 years [41]. The seroprevalence (11.3%) was high in participants younger than 30 years in Potohar region, Pakistan, but most of the participants in that study were slaughterhouse workers [6].

Regarding gender, the prevalence seems not to be affected by gender but by the community lifestyle. For instance, the communities in which men are involved in livestock contact and farming, males were found more infected than females, as seen in studies from Saudi Arabia, Qatar, western parts of Iran, and Serbia [27,42–44]. On the other hand, in communities with an opposite lifestyle, such as Pakistan [45], India [46], and Iran [47], women were found to be more infected.

Regarding the identified species, it was not surprising that *B. melitensis* was more prevalent than *B. abortus*, since it has a high pathogenicity for humans while the latter has moderate capabilities [48]. Indeed, *B. melitensis* was the first species to be isolated from a human subject with Malta fever in 1886 [49]. Several studies reported similar findings [13,31,35,36,47].

The bacterial culture result is likely to reflect the actual prevalence rates of *Brucella*, especially the current infection, since bacterial isolation is the definitive diagnostic standard to confirm an infection [48,49]. However, it is not always successful or applicable due to the prolonged incubation, the requirement of CO_2 during incubation for some strains, and the availability of resources especially in basic laboratories. The recovery rate also declines if the specimen was collected from patients with a chronic, focal, and complicated stage of brucellosis [48].

The findings of this study reveal inconsistencies in the association between age and prevalence of brucellosis, which aligns with the varied results reported in previous studies [50–53]. Some research has indicated higher rates in older populations, while others have found increased prevalence among younger individuals [54]. These discrepancies may be attributed to various factors, including regional differences in exposure patterns, occupational practices, and potential biological variations in susceptibility across age groups. Additionally, socio-economic factors and healthcare access could play a role in these inconsistent age-related trends.

Human brucellosis exhibits a seasonal pattern, with prevalence typically peaking during the spring and summer months [55,56]. This trend is closely associated with the reproductive cycles of livestock, particularly sheep, goats, and cattle; which serve as primary reservoirs for *Brucella* species [57]. During these seasons, increased exposure to birthing fluids and placentas, which are highly infectious, coincides with agricultural practices such as animal handling and the consumption of unpasteurized dairy products, leading to higher transmission rates [58]. Additionally, warmer weather may enhance the environmental survival of *Brucella*, further elevating the risk of infection. However, the specific seasonal patterns of brucellosis can vary geographically, influenced by local agricultural practices, temperature, the predominant *Brucella* species, and the regional animal reservoirs [59]. Hence, a full-year monitoring is likely to provide a clearer picture of the seasonality as it will capture the trends during winter and spring.

Lastly, we acknowledge the limitations of the cross-sectional study design in establishing causality between variables. While this approach provides valuable insights into current associations, it cannot determine the direction of relationships or changes over time with a larger sample size. To address these constraints, future research employing longitudinal designs is suggested to explore how the observed prevalence of brucellosis evolves and to better establish causal links. Additionally, the findings may have limited generalizability due to the specific context and sample size of the study. Despite these limitations, the results offer important preliminary evidence and serve as a foundation for generating hypotheses for more comprehensive future investigations in this field.

Conclusions

The prevalence of brucellosis in Erbil remained roughly unchanged for nearly a decade. It is alarming that the infection prevalence in laboratory workers is similar to the rate among farmers. Gender, age, and residence seem to have no effect on prevalence of brucellosis. The prevention of brucellosis transmission

among occupations that directly deal with animals, or their products relies on effective defensive measures, such as the adoption of personal protective equipment during activities bearing risk of *Brucella* spp. infection. The study shows the need for strategies for safety at work to minimize the risk of infection. Raising awareness for the prevention and use of proper personal protection equipment during the slaughtering and treatment of animals is highly needed. Laboratory teams should also take precautions to avoid the acquisition of brucellosis. Further molecular epidemiology studies with larger sample sizes are expected to shed light on the prevalence to revise or confirm the findings.

Acknowledgements
We extend our gratitude to Knowledge University for providing the facilities necessary to conduct this research.

Authors' contributions
DAA: methodology, writing, data analysis, and supervision; HIM, BZO, and ZMT: lab experiments and writing.

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Conflict of interest
No conflict of interest is declared.

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