

Original Article

## Epidemiological study of human brucellosis among febrile patients in Erbil-Kurdistan region, Iraq

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### Abstract

**Introduction:** Human brucellosis is one of the most common zoonosis infections, with an important impact on the health and economy worldwide. This study aimed to update and provide epidemiological information on this infection and evaluate Rose Bengal Test, which is used as an essential diagnostic test for brucellosis in Erbil.

**Methodology:** A total of 325 participants seeking care and reporting fever at Rizgary Teaching Hospital were enrolled. Blood samples were tested for *Brucella* spp. antibodies using Rose Bengal Test and blood culture followed by species identification. A questionnaire was administered to detect the risk factors.

**Results:** The prevalence of probable and confirmed brucellosis was 12.3% (95% CI 9.2–16.3) and 9.5% (95% CI 6.8–13.2) respectively. The majority of cases were in the age group of 18–39 years. Brucellosis was significantly associated with raw milk consumption (OR = 10.3 95% CI 5–22.4) and contact with livestock (OR = 11.5 95% CI 5.6–23.9). *Brucella melitensis* (58.1%) and *Brucella abortus* (41.9%) are the dominant species in the area. The sensitivity, specificity, positive predictive value, and negative predictive value of the Rose Bengal Test in comparison to the blood culture were 100%, 96.9%, 77.5 %, and 100% respectively.

**Conclusions:** Brucellosis is a significant cause of fever in Erbil and could be diagnosed by the Rose Bengal Test taking into account the compatibility of clinical features with the positive result. The vaccination of livestock and boiling or pasteurization of milk are essential procedures to reduce the frequency of human brucellosis.

**Key words:** Brucellosis; epidemiology; seroprevalence; Rose Bengal Test.

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### Introduction

Brucellosis (also known as Malta fever, undulant fever, and Mediterranean fever) is among the most widespread zoonosis infection according to the World Health Organisation (WHO) [1]. Annually, there are more than 500,000 new brucellosis cases are reported worldwide, with about 10 per 100,000 inhabitants [2]. This infection is caused by gram-negative coccobacilli of the genus of *Brucella*. Most cases of human brucellosis are associated with *Brucella melitensis*, *Brucella abortus*, *Brucella suis*, and *Brucella canis* [3], all these species were isolated from ungulates, especially livestock that is the main source of human infection [4]. The transmission of *Brucella* spp. from infected animals occurs by direct contact with blood, placenta, aborted fetuses, and vaginal secretions, or indirectly by consumption of infected livestock products without proper heat treatment such as milk or meat [5–7].

Human brucellosis is presented with many clinical manifestations; the most common one is the non-

specific fever, in addition to, headache, muscles, and joint pain [8]. The minority of patients have chronic brucellosis, as some symptoms persist for more than one year. Many organs can be affected by *Brucella* spp., resulting in severe complications such as cardiovascular complications [9], neurological complications [10], and osteomyelitis [11]. In untreated or severe cases, death can occur [8].

Risk factors for human brucellosis are largely diverse because of the variety in the animal reservoir and cultural practices. For example, infection of brucellosis has been linked to slaughtering pigs in the USA [12], intake of camel milk in Yemen [13], and contact with cattle placentas in Chad [14]. The identification of risk factors is very critical for disease control in endemic areas; some interventions stop infection transmission such as vaccination programs, slaughter procedures, and improving food safety, in addition to, using a proper diagnostic test at the proper time. The poor accuracy of tests leads to misdiagnosis and inappropriate management.

The diagnosis and treatment of human brucellosis is a major challenge in those countries where this disease is endemic. The confirmation of brucellosis infection couldn't be done based on signs and symptoms, it should be accompanied by the detection of the *Brucella* spp. in blood or bone marrow which is considered the gold standard [15], or demonstration of increased titers of specific antibodies by serological tests [16–18]. Many of these tests are not available or need complicated procedures, especially in rural areas where the rate of human brucellosis is high [19], so, finding alternative tests are urgently needed.

Accurate data on cases of human brucellosis are lacking in many countries, especially developing countries, owing to a lack of active surveillance. Therefore, there is an underestimation of the burden of the disease [19,20].

In this study, we aimed to inform the status of human brucellosis in Kurdistan Iraq and contribute epidemiological information, especially risk factors associated with this disease to reduce its incidence through proper procedures. Besides, the assessment of the Rose Bengal test has been done in the diagnosis of human brucellosis in endemic areas based on blood culture results.

## Methodology

### *Study design and participants*

Our cross-sectional study is conducted in Rizgary Teaching Hospital, Erbil-Kurdistan Iraq, between July and December 2018. This hospital has 500 beds, with 12 Operating Theaters and 10 specialties, serving Erbil governorate, the capital of the Kurdistan region. The economy of Erbil depends on agriculture, industry, and oil exports, some people work in raising sheep and cows, and there are many poultry farms and feed factories. Erbil is a predominately rural area.

We enrolled adult outpatients presenting to Rizgary Teaching Hospital with an axillary temperature of more than 37.5 °C and at least one of the following symptoms: sweat, anorexia, headache, myalgia, or arthralgia. We considered the participant not eligible for the study if: 1) they had an alternate disease, 2) they had been treated for brucellosis within the past year, or 3) they were below 18 years of age.

All the participants in the study knew that they can withdraw at any stage of the study and that their information would be handled confidentially. A standardized questionnaire was prepared for getting information about socio-demographic characteristics, dietary habits, and contact with livestock.

### *Sample collection*

The diagnosis of brucellosis was carried out by blood culture and Rose Bengal Test that detect *Brucella* antibodies in the serum. About 10 mL of venous blood was collected from each participant. Firstly, 5-7 mL were inoculated into an aerobic blood culture bottle, and then 3 mL were collected into a plain tube for Rose Bengal Test (RBT).

### *Isolation of *Brucella* spp.*

Blood culture was performed by BACTEC 9240 (Becton Dickinson, Franklin Lakes, USA). All the bottles were incubated for up to four weeks, whenever the instrument gives a positive signal, 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. 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<sub>2</sub>S production, sensitivity to thionin, CO<sub>2</sub> requirement, and agglutination with monospecific sera A and M.

### *Rose Bengal Test*

We detected *Brucella* antibodies by (Torax Biosciences, United Kingdom) according to the following procedure; briefly, a volume of 0.03 mL of serum is 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 four minutes at ambient temperature, any visible agglutination was considered to be a positive result.

### *Case definition*

A patient is diagnosed with probable brucellosis based on clinical suspicion and a positive result of the Rose Bengal Test (RBT), and the case is considered confirmed brucellosis based on isolation of *Brucella* spp. from blood [21].

### *Statistical analysis*

Descriptive analysis of participant characteristics namely (participant's gender, age, residence, consumption of raw milk, and contact with livestock) was done. The prevalence of human brucellosis was calculated by dividing the number of participants with the appropriate definition of probable or confirmed

human brucellosis by the total number of participants tested for brucellosis. The statistical software program VassarStats (<http://vassarstats.net/>) was used to calculate Odds Ratio (OR); 95% Confidence Intervals (CI); Risk Ratio (RR). A Chi-square test was used to compare categorical variables and *p* values less than 0.05 indicated a statistically significant difference.

**Results**

*Participant characteristics*

A total of 325 participants were enrolled in the study. The mean age was 44 years with a range between 18 and 82 years and 42% of participants were 40-59 years old, 53% and 52% of participants were males and from urban areas, respectively. The minority of individuals reported consumption of raw milk (19%), and (41.1%) had contact with livestock. Characteristics of participants according to the presence of probable brucellosis are summarized in Table 1.

*Prevalence of human brucellosis and risk factors*

Forty participants of the 325 had a positive RBT result and 31 had a positive blood culture for *Brucella* spp., as a result, the prevalence of probable and confirmed brucellosis was 12.3% (95% CI 9.2–16.3) and 9.5 % (95% CI 6.8–13.2) respectively. All positive blood culture cases were also positive by RBT and 9/40 (22.5%) of cases with positive RBT were culture negative.

Two major risk factors of brucellosis were investigated; consumption of raw milk and history of livestock contact. Both tests were associated with an increase in the positivity rate with a statistically significant difference (Table 1).

*Identification of Brucella species*

Depending on biochemical and agglutination tests of *Brucella* isolates, we identified two species namely *Brucella abortus* and *Brucella melitensis* (Table 2).

*Diagnostic accuracy of RBT*

The sensitivity % (95% CI), specificity % (95% CI), positive predictive value % (95% CI), and negative predictive value % (95% CI) of RBT in comparison to the blood culture (gold standard) were 100 (86.2-100), 96.9 (94.1-98.5), 77.5 (61.1-88.6), and 100% (98.3-100) respectively.

**Discussion**

The current study showed the prevalence of probable brucellosis was 12.3% among suspected patients in Erbil-Kurdistan Iraq. A study conducted in the same hospital (Rizgari Hospital at Erbil) in 2012 reported a 10.7% prevalence which is close to the present study [22]. A slightly higher prevalence was found in another study conducted in Azadi general hospital in Duhok-Kurdistan, Iraq in 2017 with a seroprevalence rate of 17.8% [23]. Both studies were carried out among suspected patients of brucellosis using the RBT test.

Outside Kurdistan-Iraq, different prevalence of human brucellosis were detected worldwide: Rwanda 6.1% [24], Iran 6.59% [25], Turkey 8.8% [26], Saudi Arabia 12.8% [27], India 16.7% [28], South Sudan 23.3% [29], and in Pakistan 70% [30]. All the previous studies were conducted by using serology tests among nonspecific symptoms patients. These large differences between countries are the result of the difference in the prevalence of brucellosis among livestock, in addition

**Table 1.** The association of probable brucellosis with the characteristics of the participants.

Characteristic	Overall (N = 325)	Probable brucellosis (Positive RBT <sup>1</sup> ) N (%)	OR <sup>2</sup> (95% CI <sup>3</sup> )	RR <sup>4</sup> (95% CI <sup>3</sup> )	<i>p</i> value
<b>Gender</b>					
Male	172	19 (11.1)	0.8 (0.5- 1.4)	0.8 (0.4-1.5)	0.5
Female	153	21 (13.7)			
<b>Age</b>					
18-39	110	20 (22)	2.5 (1.2-5.6)	2.3 (1.1-4.5)	0.02
40-59	137	11 (15)			
≥ 60	78	9 (7)			
<b>Residence</b>					
Urban	170	18 (10.6)	0.7 (0.4-1.4)	0.7 (0.4-1.3)	0.3
Rural	155	22 (14.2)			
<b>Raw milk consumption</b>					
Yes	56	23 (41.1)	10.3 (5-22.4)	6.5 (3.7-11.4)	< 0.0001
No	269	17 (6.3)			
<b>Livestock contact</b>					
Yes	61	25 (41)	11.5 (5.6-23.9)	7.2 (4.1-12.8)	< 0.0001
No	264	15 (5.7)			

<sup>1</sup>Rose Bengal Test; <sup>2</sup>Odds Ratio; <sup>3</sup>Confidence Intervals; <sup>4</sup> Risk Ratio.

to the socioeconomic factors. This point highlights the importance of recognizing the major risk factors in each area to achieve good control of this infection through prevention programs.

In the present study, we identified two risk factors; raw milk consumption and contact with livestock. We found a statistically significant difference between brucellosis and consumption of raw milk ( $p < 0.0001$ ). This supports the results of other studies indicating either increased risk with consuming raw milk or protective impact of boiling it [30–32]. Therefore, a very important step to reduce the incidence is the safe preparation of milk, especially since milk and its products are essential in nutrition. We found an association between human brucellosis and livestock contact with a statistically significant difference. This finding is in agreement with another study from Erbil [22] and indicates an important role of livestock in the epidemiology of human brucellosis in this province. Further studies to determine which species of livestock are the reservoirs for *Brucella* spp. would be valuable in the protective programs. The same result was obtained in the neighboring countries, Iran [25] and Saudi Arabia [33].

The infection of brucellosis can occur at any age, but the peak is in young adults [34,35]. This fact is also supported by the result of the current study because young adults have more contact with livestock and more exposure to occupational risks. On the other hand, no statistically significant difference was observed in this study between gender and brucellosis cases. This point is controversial, for instance, a study in Saudi Arabia indicated a higher prevalence of brucellosis in males since males were more exposed in comparison with females [36], while another study in Pakistan

found the majority of brucellosis patients were females due to the women work directly with livestock and share the men fields work [30]. We think the link between gender and brucellosis depends on the lifestyle of the community. According to the participants' residence area, we noticed that a high prevalence of *Brucella* infection rate was found among residents of rural areas. This observation is similar to the findings reported in Iran [37] and Uganda [38]. Rural inhabitants are likely to be in more contact with livestock.

The transmission of human brucellosis extremely depends on the *Brucella* species. *B. abortus* infects humans via ingestion of the organisms or by direct contact between this species and mucous membrane or abraded skin, whereas *B. melitensis* transfers by ingestion of unpasteurized milk or related products [39]. In addition, the host of each species is different, sheep, goats, and buffalo are the main host of the *B. melitensis*, while *B. abortus* is hosted mainly by cattle [40]. Therefore, the identification of common species in the area enables taking preventive measures to stop the transmission such as vaccination of proper livestock as no vaccine is available for humans. We found that 58.1% of *Brucella* infections were caused by *B. melitensis*, and 41.9% were caused by *B. abortus*. There is an increase in the *B. abortus* compared with a study conducted in 2012 in Erbil where the prevalence of *B. abortus* and *B. melitensis* was (28.25%) and (71.75%) respectively [22]. It seems that *B. abortus* is responsible for an increasing number of cases in recent years, such as in Yemen, where *B. abortus* was identified in 45 cases and *B. melitensis* in 7 cases out of 330 cultures performed [41], and in Pakistan, 79.59% of human brucellosis was caused by both species followed by *B. abortus* in 16.32% of cases and *B. melitensis* in just

**Table 2.** The distribution of *Brucella* species according to the characteristics of the participants.

Characteristic	Negative <i>Brucella</i> blood culture N (%)	<i>B. abortus</i> N (%)	<i>B. melitensis</i> N (%)	Total N (%)
<b>Gender</b>				
Male	158 (91.8)	6 (3.5)	8 (4.7)	172 (100)
Female	136 (88.9)	7 (4.6)	10 (6.5)	153 (100)
<b>Age</b>				
18-39	101 (91.8)	4 (3.6)	5 (4.6)	110 (100)
40-59	125 (91.2)	5 (3.6)	7 (5.2)	137 (100)
≥ 60	68 (87.2)	4 (5.1)	6 (7.7)	78 (100)
<b>Residence</b>				
Urban	158 (92.9)	5 (2.9)	7 (4.2)	170 (100)
Rural	136 (87.7)	8 (5.2)	11 (7.1)	155 (100)
<b>Raw milk consumption</b>				
Yes	38 (67.9)	7 (12.5)	11 (19.6)	56 (100)
No	256 (95.2)	6 (2.2)	7 (2.6)	269 (100)
<b>Livestock contact</b>				
Yes	42 (68.9)	6 (9.8)	13 (21.3)	61 (100)
No	252 (95.5)	7 (2.6)	5 (1.9)	264 (100)

4.08% of the cases. On the contrary, in Iran, 60 *B. melitensis* and 8 *B. abortus* were isolated from 68 human and animal specimens [42], in Saudi Arabia, *B. melitensis* has been reported as the leading cause of brucellosis [43], and in Turkey, *B. melitensis* was detected in 98.1% of samples [44]. The difference in the species between countries is mainly because of the difference in the traditional livestock [30].

Isolation of *Brucella* spp. from clinical specimens is considered the gold standard for brucellosis diagnosis [45]. Unfortunately, this procedure cannot be often done for many reasons (time-consuming, complicated, previous antibacterial treatment). Therefore, finding an alternative test is very important, especially in developing countries that lack laboratory capabilities. Based on culture results, RBT showed high sensitivity, specificity, and negative predictive value, while the positive predictive value was low (77.5%) which increases false-positive cases. We recommend in endemic areas where the isolation of *Brucella* or ELISA are difficult to apply, that RBT could be an accepted test for brucellosis diagnosis, taking into consideration the compatibility of clinical features when the clinician interprets the positive results, this suggestion is in agreement with recent studies [46,47].

There were few limitations in this study; the number of risk factors of human brucellosis that was investigated is low, limiting our ability to making comprehensively assess the causes and applying proper preventive procedures, especially in urban areas, in addition, all the data depends on the participants reporting, so there will be prone to recall bias. However, this study despite its limitations provided a valuable update of epidemiological information on this endemic infection. To our knowledge, this is the first study in Erbil-Kurdistan Iraq that used the blood culture to identify the predominant *Brucella* species in the area.

## Conclusions

Human brucellosis is a considered cause of febrile patients in Erbil-Kurdistan Iraq, especially among young adults. *B. melitensis* and *B. abortus* are the main species responsible for this infection. Consumption of raw milk and contact with livestock are the main risk factors associated with brucellosis. These findings highlight the need for the vaccination of livestock and creating public awareness of the importance of pasteurizing or boiling the milk before consumption to reduce the prevalence of this infection and its consequences.

RBT had a good agreement with blood culture results so that it could be used for brucellosis diagnosis,

as it is easy, fast, and cost-effectivity, with caution in interpreting the positive results when the clinical features are inconsistent.

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