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Original Article

Epidemiology of human brucellosis in military hospitals in Jordan: A five-year study

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Abstract

Introduction: Brucellosis is a zoonotic disease with significant impacts on livestock and human health. It is a severe community health burden in the Middle East with an estimated 2000 times higher prevalence than in North America and Western Europe. To date, there are limited studies on human brucellosis and risk factors for infection in Jordan. Our study aimed to analyze documented brucellosis cases in Jordan and use geographic and socio-demographic data to better understand its prevalence and transmission.

Methodology: This retrospective study examined electronic medical records describing 1,497 cases of febrile illness that were tested for brucellosis at Royal Medical Services hospitals between 2016 and 2020. A total of 465 confirmed brucellosis cases, aged 0 to 80 years were included. Serum samples were screened for anti-Brucella antibodies, and positive samples were additionally tested for antibody titer using the Wright tube agglutination test.

Results: Our results showed that 31.1% (456/1497) of the febrile diseases were brucellosis. We found that young adults and working age, northern governorates, rural location of residency, occupations involving regular contact with livestock, and Spring/Summer season were highly significant as risk factors. Seropositivity was highest among dairy factory workers with 64.4%.

Conclusions: This study adds to our understanding of human brucellosis in Jordan and its impact on public health. These data will be useful in the prevention of brucellosis and will inform reliable disease control policies.

Key words: Seroprevalence; infection; zoonoses; Middle East; developing countries.

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Introduction

Human brucellosis is caused by four *Brucella* species; *B. melitensis*, *B. abortus*, *B. suis,* and *B. canis* [1,2]. *Brucella* is a Gram-negative coccobacillus that causes disease in animals and humans [3]. Transmission occurs primarily from ruminants to humans through the consumption of raw dairy products, direct contact with products of aborted animals, inhalation of infected droplets, or inoculation into the conjunctiva or damaged skin [3-5]. Consequently, brucellosis is an occupational hazard for farmers, veterinarians, and abattoir workers [6]. Vaccination programs for some ruminants (particularly cattle) help to control infection by *B. abortus* and *B. melitensis* [7]. Human brucellosis manifests clinically with prolonged fever, excessive sweating, joint pain, and generalized weakness [8]. As the clinical presentation is similar to other acute febrile illnesses, brucellosis cases are often misdiagnosed and thus the human incidence is greatly underestimated [9].

Although brucellosis has low mortality in humans, it may cause serious disability with irreversible sequela such as arthritis, osteomyelitis, spondylitis, sacroiliitis, endocarditis, meningoencephalitis, epididymal-orchitis, and pneumonia [5,10]. Brucellosis can present with similar symptoms to various multisystem diseases, and complications can affect the musculoskeletal, nervous, hematological, digestive, and urogenital systems. This large clinical interference with other diseases commonly leads to misdiagnosis and treatment postponements [8]. Long-term complications from human brucellosis affect almost 15% of patients, with osteoarticular involvement recorded in 10-25% of patients with arthritis, and many other organs can be affected [11]. Despite well-established treatment protocols (such as the World Health Organization's antibiotic regimen), treatment failure and relapse occur in 5-15% of cases [12,13].

Human brucellosis is endemic in many countries around the world [14-16] and is widespread in more than 170 countries, primarily in the Mediterranean, Asia, and Central and South America. There are an estimated five to six million human cases of brucellosis

globally, with 500,000 cases reported by the World Health Organization (WHO) annually [17-19]. Although brucellosis is a significant public and animal health problem in developing countries, it is now well-controlled in most developed countries [20]. Brucellosis incidence varies from low in Western Europe and North America (≤ 0.1 cases per 100,000 population), to moderate in Central and Southern Latin America and parts of Southeastern Europe (3.5–35 cases per 100,000 population) to high in the Middle East and Asia (> 250 cases per 100,000 population) [21]. In some high-income countries, it has been reported that brucellosis is eliminated or transmitted at a relatively low level. For instance, the 28 European Union countries in 2017 reported an overall rate of 0.09 cases per 100,000 person-years [22].

Working with ruminants, contact with manure, milking animals, and consumption of milk and its products are highly associated with brucellosis in the univariate analysis. In multivariate logistic regression analysis, risk factors that were significantly associated with brucellosis include milking animals and the intake of raw cheese, while the consumption of cows' milk and boiled feta cheese decreased the risk of brucellosis [23].

Due to its negative impacts on livestock and human health, brucellosis is a severe community health burden [16]. Despite the threat posed to human and animal health, to date there are limited studies on human brucellosis and risk factors for infection in Jordan. This study was carried out to address this gap in our understanding of brucellosis cases from around Jordan; using electronic records from Jordanian Royal Medical Services hospitals along with geographic and socio-demographic data to better understand current prevalence and risk factors. The results of this study can inform public health efforts to control and prevent brucellosis in Jordan.

Methodology

Study design and samples information

This retrospective study was conducted at Princess Iman Research and Laboratory Science Center, Royal Medical Services between January 2016 and December 2020. A total of 465 laboratory-confirmed brucellosis cases, aged 0 to 80 years were included. Available demographic information included age, gender, occupation, geographic location, and medical data. Data were collected using electronic medical records from the Hakeem program, an electronic health program used in Military and Health Ministry hospitals in Jordan. Hakeem records contain medical history, radiological and laboratory investigations, medications, and admission and discharge notes. Serum samples were collected from patients complaining of brucellosis-like symptoms (prolonged fever with no focus on infection, excessive sweating, joint pain, and generalized weakness) with a history of consumption of raw dairy products or direct contact with products of aborted animals. Samples were collected from pediatric and medical clinics at Military Hospitals in North Jordan (Mafraq, Irbid, and Ajloun), Central Jordan (Amman, Zarqa, Madaba, and Balqaa), and in South Jordan (Karak, Tafila, Ma'an, and Aqaba). This study included only patients who are covered by military insurance and did not include all patients in each governorate.

Serological analysis

Whole blood samples were drawn from each patient in yellow-topped ACD vacutainer tubes and sent to the lab associated with each hospital for centrifugation and immediate testing. Samples not tested immediately were stored at -20 °C for up to 7 days and thawed at room temperature (15-25 °C) prior to testing. Exclusion criteria included hemolyzed, icteric, and/or lipemic samples. Serum samples were screened for anti-brucella antibodies using the Cromatest Rose Bengal agglutination test kit (Linear, Chemicals S L U; Barcelona, Spain.), which is a rapid slide agglutination test to detect IgG and IgM antibodies. The assay is performed by testing a suspension of *B. abortus* strain colored with Rose Bengal against unknown sera. The kit contains negative and positive controls for quality control. The presence or absence of visible agglutination indicates the presence or absence of antibodies in the samples tested; the minimum detectable unit (analytical sensitivity) is approximately 25 IU/mL and the diagnostic specificity is 100%, with no prozone effect detected up to 1,000 IU/mL.

Positive samples were additionally tested for antibody titer (allowing serological diagnosis of acute brucellosis), using the Wright tube agglutination test kit (Bio-Rad; France), per manufacturer's instructions. The quantitative test detects IgM, thus allowing serological diagnosis of active brucellosis. A titer equal to or greater than 1/80 (120 IU/mL) indicates active brucellosis [24,25].

Statistical analysis

Brucellosis infection status, demographic data, and risk factor information for each patient were entered into a Microsoft Excel sheet (Microsoft, Redmond, WA), which was then imported into SPSS 20 (SPSS Corp., IBM, Armonk, NY) for analysis. Within this

study, "seropositivity" is defined as the percentage of febrile cases that are identified as brucellosis. Univariate analyses were conducted to analyze *Brucella* seropositivity associations with demographic and environmental variables including age, gender, occupation, season and year, place of residency, and governorate. The multivariate model for *Brucella* seropositivity was constructed by manual stepwise forward logistic regression analysis. A final logistic regression was run to include variables found to be significant at a p value < 0.05 in the multivariate analysis and variables reported to be risk factors for *Brucella* seropositivity in the literature. The model fitness was evaluated with the Hosmer–Lemeshow goodness-of-fit test.

Ethical statement

This study was approved by the Jordanian Royal Medical Services, an authorized national public health authority. Samples were collected as part of routine

Table 1. Descriptive statistics for demographic variables associated with Brucellosis in Jordan, 2016-2020 (n = 1,497).

Variable	% seropositive (# seropositive / # tested)	Chi-Square	*p*-value
Seropositivity*	31.1% (465/1497)		
Age		62.83	0.000
4-14	20.6% (83/403)		
15-29	43.6% (133/305)		
30-49	41.3% (118/286)		
50-80	26.0% (131/503)		
Gender		1.02	0.169
Male	32.1% (269/837)		
Female	29.7% (196/660)		
Governorate		79.97	0.000
Mafraq	46.1% (106/230)		
Karak	35.4% (68/192)		
Irbid	35.0% (78/223)		
Amman	33.8% (79/234)		
Madaba	33.3% (36/78)		
Balqa	31.7% (38/120)		
Ajloun	26.5% (26/98)		
Aqaba	23.1% (6/26)		
Tafila	21.2% (11/52)		
Ma'an	20.0% (5/25)		
Zarqa,	11.6% (19/164)		
Jarash	5.5% (3/55)		
Place of Residence		213.81	0.000
Urban	13.7% (103/753)		
Rural	48.7% (362/744)		
Season		152.07	0.000
Summer	53.5% (166/310)		
Spring	36.6% (131/358)		
Autumn	29.9% (110/368)		
Winter	12.6% (58/461)		
Occupation		174.98	0.000
Dairy Factory	64.4% (112/174)		
Shepherd	48.9% (46/94)		
Farmer	40.3% (58/144)		
Veterinarian	26.9% (35/130)		
Preschool and School Student	25.1% (140/558)		
House wife and Retired	12.7% (20/158)		
Other Occupations	35.1% (33/94)		
Not Known	14.5% (21/154)		
Year		12.89	0.012
2016	26.6% (64/173)		
2017	25.0% (55/220)		
2018	38.0% (123/324)		
2019	31.7% (132/416)		
2020	29.9% (109/364)		

*Seropositivity is the percentage of brucellosis cases out of the total number of febrile cases for each variable (i.e., the percent of febrile cases that were determined to be caused by *Brucella sp.*).

public health surveillance activities and data were de-identified before analysis by the research team. All data collected related to the samples were kept confidential and were shared only with the referring clinician.

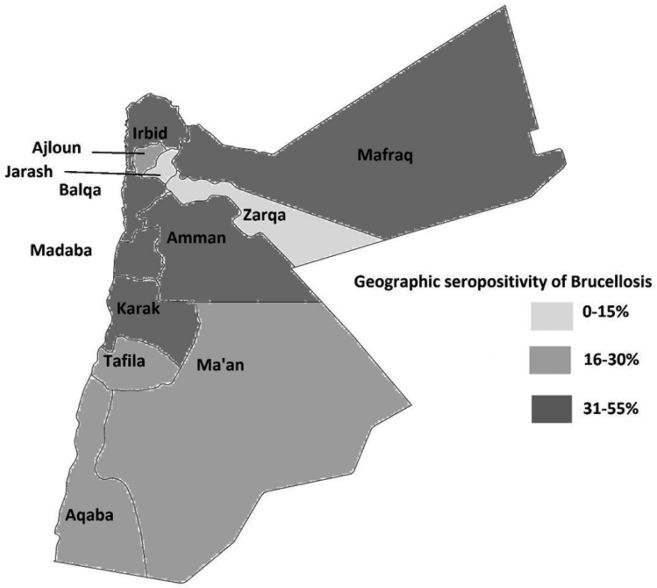
Results

A total of 1,497 cases of febrile illness were tested for brucellosis. Among these, 465 cases (31.1%) were diagnosed with brucellosis. The age of seropositive individuals ranged from 0-80 years (mean age = 35.5). Descriptive statistics for demographic variables associated with Brucellosis in Jordan, 2016-2020 are listed in Table 1.

Of the 465 positive brucellosis cases, 17.8% were in the pediatric age (0-14 years), 28.6% were in young adult age (15-29 years), 25.4% were middle age (30-49 years), and 2.8% were in the elderly age group (50-80 years). There was no significant gender difference in the percentage of positive brucellosis cases. Males made up 57.8% (269/465) of brucellosis cases while females made up 42.2% (196/465). According to our data, 39% (181/465) of brucellosis cases in the age groups (15-29 and 30-49) and 54.0% (251/465) of brucellosis cases among all age groups have occupations where direct contact with animals (sheep and cattle) occur regularly. Cases were distributed as follows: most commonly in dairy factory workers, 24.1% (112/465), followed by farmers 12.5% (58/465), shepherds 9.9% (46/465), and lastly veterinarians 7.5% (35/465).

The summer and spring had the most cases, accounting for 63.9% (297/465) of total cases, with the summer alone making up over a third of the cases (35.7%; 166/465).

Figure 1. The geographic distribution of brucellosis seropositivity in Jordan from 2016-2020.



The 465 cases varied by geographic location, Mafraq governorate accounted for 22.8% of cases. This was followed by Amman 17.0%, Irbid 16.8%, Karak 14.6%, AL Balqa 8.2%, then Madaba 7.7%, Ajloun 5.6%, Tafila 2.4%, Aqaba 1.3%, and Ma'an 1.1%

Overall, we found the highest number of cases and seropositivity in younger and working-age adults, with fewer cases in children and older adults. There was a mean case age of 34.9 years. The seropositivity was highest in ages 15-29, with 43.6% (133/305) of febrile cases testing positive for brucellosis. This was followed by those aged 30-49 (41.3%; 118/286) and 0-14 (20.6%; 83/403). We found the lowest seropositivity in ages 50-80 years (2.6%; 13/503). The differences in seropositivity between groups was statistically significant $\chi^2 = 62.83$, ($p < 0.001$).

There was no significant association between gender and brucella seropositivity, $\chi^2 = 1.02$, ($p = 0.169$), with 32.1% (269/837) of males and 29.7% (196/660) of females seropositive from all febrile cases. Occupational exposure to ruminants or cattle is a significant risk factor for Brucella infection. Occupation information was recorded for 1,352 patients. According to occupation, the chances of a febrile case testing positive for brucellosis is also high in these groups, dairy factory workers have the highest seropositivity, 64.4% (112/174), followed by shepherds 48.9% (46/94), farmers 40.3% (58/144), and veterinarians 26.9% (35/130), $\chi^2 = 174.98$, ($p < 0.001$).

Season impacted both the number of cases and seropositivity. The seropositivity was found to vary by season, with significantly higher seropositivity in summer and spring, 53.5% (166/310) and 36.6% (131/358), respectively, $\chi^2 = 152.07$, ($p < 0.001$).

Our study also identified the residence location as a significant risk factor for Brucella infection, with 77.8% of cases found in rural areas of each governorate and 22.2% in urban areas ($p < 0.001$). Geographic distribution was found to be a highly significant risk factor in seropositivity in brucellosis. Patients with febrile illness seen at clinics in the Mafraq governorate were seropositive at the highest rate, 46.1% (106/230). This was followed by Karak 35.4% (68/192), then Irbid 35.0% (78/223), Amman 33.8% (79/234), Madaba 33.3% (36/78), AL Balqa 31.7%, (38/120), Ajloun 26.5% (26/98), Aqaba 23.1% (6/26), Tafila 21.2% (11/52), and Ma'an 20.0% (5/25). The lowest seropositivity was found in Zarqa 11.6% (19//164) and Jarash with 5.5% (3/55), $\chi^2 = 79.97$, ($p < 0.001$). The

geographic distribution of brucellosis seropositivity in Jordan from 2016-2020 is shown in Figure 1.

Using multivariate logistic regression analysis, we found that governorate, location of residency, occupation, and season were highly significant as risk factors. Factors positively associated with brucellosis seropositivity in the multivariate logistic regression model (2016–2020) are listed in Table 2.

Seropositivity also varied by year: in 2016 (26.6%), in 2017 (25.0%), in 2018 (38.0%), in 2019 (31.7%), and in 2020 (29.9%), $\chi^2 = 12.89$, ($p = 0.012$).

Table 2. Factors positively associated with Brucellosis seropositivity in the multivariate logistic regression model (2016–2020) (n = 1,497).

Variable	Adjusted odds ratio (aOR)	95% CI	*p* value
Place of Residency	4.86	3.72-6.36	< 0.001
Governorate	0.96	0.92-0.99	0.029
Occupation	0.88	0.84-0.92	< 0.001
Season	0.64	0.58-0.72	< 0.001

Hosmer and Lemeshow test: Chi-square 10.12; df 8; *p* value 0.256.

Discussion

Understanding the epidemiological distribution of human brucellosis is valuable for controlling its spread and implementing disease surveillance programs. We reported that 465 individuals were diagnosed with brucellosis from 1,497 febrile cases over a five-year period, yielding a seropositivity of 31.1%. A review article from 2020 reported that the annual average number of total human brucellosis cases in Jordan in recent years was 441 cases [1]. A likely explanation for the divergence is the difference in data sources (Jordanian Royal Medical Service vs. World Animal Health Information Database).

Studies in Saudi Arabia and China have reported that human brucellosis occurs at higher rates in males than females, with males making up 74.5% of patients [26,8]. Our study revealed a similar, although less pronounced, trend with 57.8% of cases being male. The slight difference could be explained by behavioral differences (i.e., females being more involved in cattle/ruminant associated work in Jordan) between the countries, as well as the fact that 403 febrile patients of the 1,497 total were from the pediatric age group and that most female patients in our cohort had occupations related to contact with ruminants and cattle and their products.

In the past, brucellosis was assumed to be rare in childhood, but it is now documented that individuals of all age groups are vulnerable. Among the 465 brucellosis cases, children (0-14 years) made up 17.8%. A study done in Turkey reported that among the 1,028 cases, 3.6% were aged 3–12 years [27]. We observed that children (aged 0-14 years) made up a much higher percentage of cases in our population. In our study, young adults (aged 15-29 years) had the highest seropositivity for brucellosis, followed by the 30-49 age group. According to our data, 39% (181/465) of brucellosis cases in the age groups (15-29 and 30-49) have occupations with regular contact with animals (sheep and cattle). These two age groups accounted for 54% of all brucellosis cases. Similarly, a study conducted in China showed that middle-aged men between the ages of 41 to 65 were the main group of brucellosis cases [8].

During our five-year study, occurrences of brucellosis in Jordan have been relatively high. The number of cases of human brucellosis is variable from 2016 to 2020 but trending upward. A recent study reported that the incidence of brucellosis in mainland China has been moderately high and rising. Incidences in 2014 are about 2.8 times of that in 2007 [19]. It is unclear as to whether this rise represents a global trend or random variation.

Additionally, brucellosis has noticeable seasonal differences. Seropositivity and total case counts were higher in summer and spring and reached the highest in the summer months every year. This is likely because cattle and sheep commonly give birth and consumption of their milk, and its products, increases in these seasons. Our data are consistent with Jiang *et al.* who reported that (67.8%) of brucellosis cases occur during the spring and summer seasons [8]. Another study conducted in China found that incidences of brucellosis were greater in the spring and summer seasons with the highest incidence in May annually [19]. Likewise, a study conducted in Saudi Arabia found that maximum cases of brucellosis were encountered during the summer season [26].

We have reported in our study that Mafraq and Karak had the highest seropositivity among all governorates in Jordan, and this may be explained by the fact that individuals living in those two governorates are in more direct contact with *Brucella* species because of regular interactions with infected cattle, goat, and sheep. The number of livestock in Mafraq was 977,000 and in Karak was 572,000 (data sourced from the Jordan Department of Statistics; number of livestock by governorate in 2019)[28]. Our study found that Mafraq accounted for the highest percentage (23%) of brucellosis cases. A study in Jordan conducted by Al-Majali *et al.* in 2009 showed the seroprevalence of pediatric brucellosis to be higher in Mafraq and Ma'an

governorates and the authors suggested a similar explanation [20].

Residents of rural areas have a higher seropositivity and accounted for nearly 80% of cases, as compared with urban area residents. This agrees with reports from around the world that show higher rates of brucellosis in rural populations. Similar findings have been previously reported in Jordan [20]. A study conducted in Turkey found that 57.8% of their patients were from rural areas and 42.2% were from urban areas of Turkey [29]. In Pakistan, a study found that rural areas had a greater prevalence (23%) of brucellosis compared to urban areas (10%) [30]. A study conducted in Iran found that about 78% of the patients were rural residents [31]. Our results agree with global trends that are reported in other developing nations.

Our study indicated that over half (54%) of the patients have occupations that put them in regular, direct contact with cattle and ruminants as well as their products (dairy factory workers, shepherds, farmers, veterinarians). A Jordanian study from 1996 found similarly that veterinarians had a prevalence of 24.5%, sheep farmers 12.5% and meat handlers 4.9% which were significantly higher than other occupations [32]. Also, a recent study in Pakistan revealed that farmers have the highest prevalence as compared to other occupations [30], and in Egypt, it was determined that occupations involving contact with animals had significantly elevated risk, with a 2.4-fold higher risk than those in occupations not involving contact with animals [6].

Conclusions

Brucellosis remains a significant public health issue in the Middle East. This study aimed to improve our understanding of human brucellosis in Jordan including risk factors for infection. We conclude that rural settings, occupations with frequent contact with livestock, and Spring and Summer seasons were highly significant risk factors for infection. The higher seropositivity from febrile cases among these groups should alert local physicians to the importance of considering brucellosis with febrile patients with one or more of the identified risk factors. This study adds to our understanding of human brucellosis in Jordan. These data will inform future studies of brucellosis in Jordan and can be drawn upon to design targeted public health policies that prevent brucellosis in humans and animals.

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