

Seroprevalence of bovine brucellosis and its incidence in human in Thi-Qar province

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Abstract

Brucellosis is a contagious bacterial disease that causes public health problems and financial losses in animal productions. This study aims to describe the incidence of human brucellosis, seroprevalence of brucellosis in cattle, and risk factors of disease spreading. The total incidence cases of human brucellosis in Thi-Qar province are estimated at 5.4% per 100,000 people for eight years, and the highest number of 43 patients are observed in 2019. The number of the positive samples using the Rose Bengal test (RBT) are 32 (21.8%) from a total of 147 blood samples assembled randomly from cattle populations. The seroprevalence of brucellosis is estimated as 3.4% (95%CI:1.1-7.8) through employing the Enzyme-Linked Immunosorbent Assay (ELISA). The questionnaire interview with the animal owners revealed that the large proportion neither handle aborted animals properly nor dispose of the waste materials of abortions. Besides, the majority of the owners prefer lending bulls for mating or breeding purposes rather than artificial insemination. The study concludes that brucellosis is an endemic disease in Thi-Qar Province and that it is important to abide by the One Health Approach for the management of disease control in animal population and minimize human incidence rates.

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Introduction

Brucellosis is recognized as a re-emerging and neglected zoonosis in middle- and low-income countries. The disease is caused by proteobacteria (gram-negative bacteria) belonging to the genus *Brucella*. A wide range of domesticated and wild animals are susceptible to this bacterium, with *B. melitensis* mostly infecting sheep and goats, *B. abortus* cattle, *B. suis* pigs, and *B. canis* dogs (1-3). Brucellosis remains a highly prevalent disease in different parts of the Mediterranean regions, Africa, Eastern Asia, Mexico, and Central and South America (4-6). Although the disease was eradicated from most European countries (7), Australia, New Zealand, and Canada, it has lately been re-emerging in Southwestern Europe, with 0.09 incidence cases per 100,000 people reported, annually (8). The epidemiological trends of brucellosis in animals and humans has considerably shifted because of specific

attributable factors including the hygienic condition, socioeconomic status, geopolitical disruptions, and international-trade movement patterns (4). Humans mainly acquire the infection with brucellosis through direct contact with sick animals or due to eating contaminated milk products. Farmers, veterinarians, and laboratory technicians are a vulnerable group to the *Brucella* infection as the disease is an occupational hazard (9). The patients diagnosed with brucellosis manifest a range of common symptoms including fever, weakness, malaise, and weight loss, in addition to that it is responsible for long-term infertility and inducing abortion or preterm birth. The median financial losses due to the disability-adjusted life years (DALYs) of human brucellosis was estimated over US\$ 10 million per year (10). Treatment regimens of human brucellosis may be taken between six to eight weeks and require administration of more than one broad-spectrum antibiotic, such as doxycycline and rifampicin (11). Taking

care of food safety measures, raising population awareness and maintaining environmental hygiene are key elements for the preventative approach. The disease can transmit from animal to another through sexual contact or contaminated pasture and drinking water with fluids of aborted animals. Purchasing or introducing a new animal into herds is a potential factor for increasing the prevalence of brucellosis (12). Brucellosis has visible impacts on animal welfare ranging from lower milk quantity, causing abortion, and reducing body weight (7). The annual economic losses due to bovine brucellosis was estimated at US\$ 448 million (13) and US\$ 6.8 per infected cattle, which resulted from reducing fecundity, productions, and mortality (14). Treatment of brucellosis in animals is unfeasible due to intracellular tropism of the pathogen that requires a long course to administer chemotherapy (15). The prevention strategies involve livestock vaccination, maintaining sanitation, and removal strategies (test and remove infected animals).

Brucellosis remains an endemic disease in Iraq with a large number of infected livestock documented. During the last decades, the proportion of infected ruminates has spiked from 5 to 50% in different Iraqi regions (16-18). The epidemiological features of brucellosis are poorly understood in Thi-Qar Province. Therefore, the current study aims to estimate the incidence of human brucellosis, assess the prevalence of brucellosis in cattle, and determine the risk factors in Thi-Qar Province, Iraq.

Materials and methods

Ethics approval and consent to participate

Ethical approval was obtained from the scientific committee in the College of Veterinary Medicine, University of Basrah (UB.VET.2021.106) on Aug 11, 2021. Written and verbal consent was obtained from cattle owners prior to administering the questionnaire form.

Area of study

The study was completely performed in Thi-Qar Province, which lies in the south of Iraq. This province has a tropical climate condition especially during summer, with mean high temperatures exceeding 40°C, while in winters remain slightly temperate. Rainfall is limited to November - April, with an annual average of precipitation 100 mm (19). The population in Thi-Qar was estimated as 2152155, according to the census conducted in 2017 (20).

Hospital data record

The information on human brucellosis was collected retrospectively from Thi-Qar Department of Public Health throughout an eight-year period 2012 - 2019. The important information included the number of patients diagnosed with brucellosis distributed by month and year. Age, sex, and place address of the patients were also accessible. The data

were entered into Excel spreadsheets and thereafter were subjected to statistical analysis.

Animal samples

The sampling period started from September to December, 2021. Based on a participatory approach with the help of recruited veterinarians from the Veterinary Hospital in Thi-Qar, who are knowledgeable with the addresses and names of owners. Overall sample size of 147 was required, based on the estimated prevalence 10% and the absolute precision of 5% for a 95% confidence level (21). A systematic interval (every-two-thirds or fourth animal) was adopted for selecting animal samples. All cattle flocks were divided into three flock-size strata based on the number of animals sampled: small flock (<50=3-5 cattle samples), medium flock (50-100=5-10 cattle samples), and large flock (>150 =15 cattle samples).

Administered questionnaire

The questionnaire was administered to the cattle owners to collect information about potential risk factors that might be influenced by the spreading of *Brucella*. The questionnaire is briefly outlined as follows: demographic characteristics of owners, ownership of dogs, tie up dogs, flock grazing pattern, abortion occurrence, isolation of aborting cattle, dispose of aborted foetus and placenta, purchase new animals, sources of water supply for herds, immunization against bovine brucellosis, hygiene conditions of animal housing, and the owners prefer to borrow a bull or to use artificial insemination for fertilizing mature cows. Most of these questions are close-ended questions with different forms of questions, including dichotomous, multiple choices, and ranking. The questionnaires were pre-tested on four owners prior to the actual survey setting to determine content validity. A total of 24 owners were interviewed in this study face-to-face by the veterinarians from different selected areas. The questionnaire was written in English language and then translated into Arabic, specifically the native language of the people in the middle and south of Iraq. Prior to the allocation of the questionnaire form, the owners were asked for verbal consent if he/she will participate in this survey.

Collection and testing of the blood samples

Blood samples were obtained from the animals of different ages and sexes randomly. The owners were exhorted to tie up their animals in order to collect the blood samples. Animal ages were determined by the dentition method. The blood samples (amount 5-10 ml) were directly taken from the jugular vein and placed carefully in the labelled vacutainer tubes. The samples were subsequently transferred via a cooling box to the microbiological lab at the Veterinary Hospital of Thi-Qar. The samples were centrifuged to separate serum. After that, the separated sera were decanted into sterile Eppendorf tubes. A serological

test for screening of sera was carried out to identify the positive samples with *Brucella* infection through using the Rose Bengal Test (RBT) and an enzyme-linked immunosorbent assay (ELISA). The ELISA test has a sensitivity of 99.6% and a specificity 98.6% (22), while the RBT test has a sensitivity of 79.12% and a specificity of 39.58% (23). The ELISA test was performed in the Central Veterinary Laboratory (CVL) in Baghdad Province.

The Rose Bengal Test (RBT) for determination of anti-brucella antibodies

The protocol procedure for using the RBT was given by the manufacturer instruction (Linear Chemical-Cromatest-France). The assay was performed by testing the buffered suspension (pH 3.6) of *Brucella abortus* coloured with Rose Bengal against unknown sera. The presence or absence of a visible agglutination indicates the presence or absence of antibodies in the samples collected.

The indirect ELISA-IgG (anti-brucella antibodies)

The ELISA test was used for the detection of antibodies (IgG) against *Brucella abortus* and *Brucella melitensis* in bovine serum. The test was performed according to the manufacturer instruction (ID. Vet Innovative Diagnostic-France).

Data analysis

The data were analyzed by the statistics software (STATA Corp. 14.2). The hospital records about patients infected with brucellosis were illustrated as figures and tables. The incidence cases were described as numbers and percentages. The seroprevalence of animal brucellosis combined with 95% confidence interval was assessed. The risk factors were described in numbers and percentages in the tables. The Odds Ratios (OR) and P value (considered significant ≤ 0.05) were also calculated.

Results

Hospital records

Collectively, the total number of human brucellosis was reported in 116 cases for eight years (2012 - 2019), with estimated total incidence cases of 5.4 per 100,000/ persons. The highest number of these cases (43 patients) were observed in 2019 (Table 1). Figure 1 shows the monthly distribution of the human brucellosis cases within 2012 - 2019, with the highest number of human cases that dramatically increased between July and September in 2019, then rapidly declined in October. The highest incidence of human brucellosis (1.96/10000) was observed in Al-Rafia District, while the lowest incidence (0.08/10000) was observed in Nassiryia District (Table 2).

The highest percentage of human brucellosis was reported in the age group 40-49 (41.7%) in 2018, and in 2019 (25.58%) of the cases was found among the age group

30-39 years old (Table 3). From 2018 to 2019, the incidence risk of brucellosis in female patients increased from 0.07 to 0.23, while it increased in male patients from 0.05 to 0.17 from 2018 to 2019 (Figure 2).

Table 1: Annual incidence cases of human brucellosis per 100,000 people from 2012 to 2019

Year	Patients (n)	Incidence per 100,000
2012	19	0.88
2013	10	0.46
2014	4	0.19
2015	7	0.33
2016	10	0.46
2017	11	0.51
2018	12	0.56
2019	43	2.00

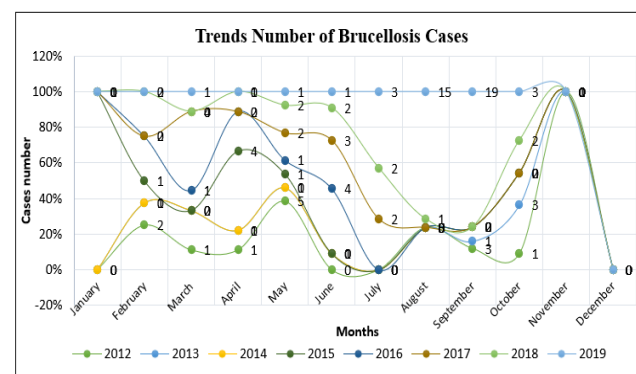


Figure 1: Monthly distribution human brucellosis cases in Thi-Qar from 2012 to 2019.

Table 2: Incidence of human brucellosis per 10000 people from 2012 to 2019

Districts	Number of cases	Population (census, 2017)	Incidence per 10000 people
Nassiriyah	7	793472	0.08
Suq Al-Shoyokh	3	329909	0.09
Al-Chibayish	2	105147	0.19
AL-Shatra	16	475145	0.33
Al-Rifai	88	448482	1.96
Total	116	2152155	0.54

Socio-demographic characteristics of the cattle owners

The total number of the animal owners participating in the questionnaire was 24 owners. The age distribution of these owners ranged from 25 to 66 years (mean=42.5, SD=10.64) and the majority of them were males (96%) (Table 4). The majority of the owners hold a low educational background.

Table 3: The numbers and percentages of human brucellosis linked to age and gender

Category	Number/percentage (2018)	Number/percentage (2019)
Age group		
<19	3 (25.0)	10 (23.26)
20-29	2 (16.7)	9 (20.93)
30-39	1 (8.3)	11 (25.58)
40-49	5 (41.7)	9 (20.93)
>50	1 (8.3)	4 (9.30)
Gender		
Male	5 (41.7)	18 (41.9)
Female	7 (58.3)	25 (58.1)

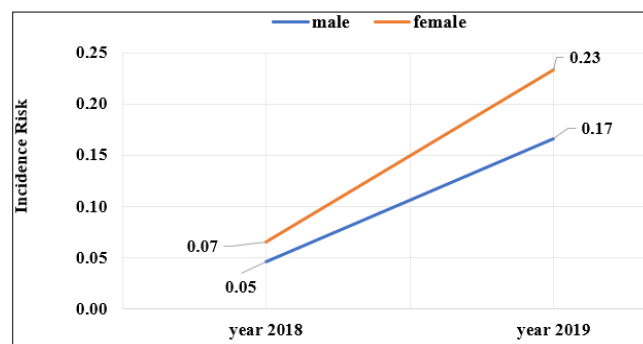


Figure 2: Incidence risk for gender infected with brucellosis per 10,000 people.

Table 4: Descriptive analysis of the socio-demographic characteristics of owners

Factor	Category	Frequency	Percentage (95% CI)
Owner gender	Female	1	4.17 (0.1-21.1)
	Males	23	95.83(78.9-99.9)
Education background of owners	Never been in school	5	20.83 (7.1-42.2)
	Primary school	9	37.50 (18.8-59.4)
	High school	10	41.67 (22.1-63.4)

Seroprevalences of brucellosis in cattle

The number of positive samples tested by Rose Bengal were 32 (21.8%). The seroprevalence of brucellosis (apparent prevalence) in cattle was estimated to be 3.4% (95% CI:1.1-7.8) using the ELISA test. There is no significant difference between sex groups (Table 5). The odd ratio increased in cattle with the age of more than one

year (OR:1.74, 95% CI: 0.19-16.00). In addition, there is no significant difference observed in the seroprevalences estimated among the districts although Al-Chibayish District had the highest proportion rate 8.7% (Table 6). The true prevalence was calculated as 3.5% (95%CI: 1.2-7.9) after adjusting the sensitivity and specificity of the test.

Table 5: The number and proportion of the cattle (sex & age) were tested by RBT and ELISA

group category	Test result		Odd ratios (95%CI)	P value
	Positive (%)	Negative (%)		
Sex				
Male	1 (14.3)	6 (85.7)	1.0	0.1
Female	4 (2.6)	136 (97.4)	0.18 (0.02-1.83)	
Age group				
<1 year	1 (2.3)	43 (97.7)	1.0	0.6
>1 year	4 (3.9)	99 (96.1)	1.74 (0.19-16.00)	

Table 6: Estimation of seroprevalence of brucellosis and comparisons between different districts

District	Number positive	Number negative	Percentage (95%CI)	P .value
Al-Chibayish	2	21	8.7 (1.1-28.0)	0.2
Nassiriyah	0	8	0 (0.0-36.9)	
AL-Shatra	0	42	0 (0.0-8.4)	
Suq Al-Shoyokh	0	17	0 (0.0-19.5)	
Al-Rifai	3	54	5.3 (1.1-14.6)	
Total	5	142	3.4 (1.1-7.8)	

The outcomes of questionnaire associated with animal management

Figure 3 summarizes the results of the questionnaire allocated with the cattle owners. Although the majority of the owners have dogs, only 12% admitted they tie their dogs. More than 80% of the owners permit their animals to graze outside farms with other flocks. Around 40% of the owners indicated occurrence of abortion in their herds, with 70% of the abortions stage occurring between 6 to 7 months of the gestation period. The majority of the owners did not isolate aborted animals from the herd; meanwhile 40% of them did not dispose of aborted fetuses and placentas properly. The owners also indicated that the veterinary authorities had not provided any *Brucella* vaccines during the last 12 preceding months. Almost a third of the owners said they would prefer to borrow a bull other than artificial insemination for breeding purposes. The owners confirmed cleaning and disinfecting the animal housing despite the fact that more than half of them do it once every month.

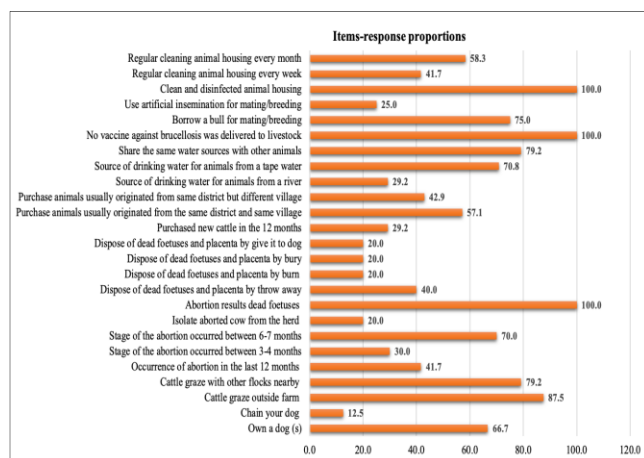


Figure 3: Questions themes related to animal management and practices.

Discussion

This research was designed to capture an episcopic image of the epidemiological situation of brucellosis in Thi-Qar in humans and cattle. Brucellosis is classified among the most important economic diseases in animals and has detrimental effects on human health. The disease continues to disperse broadly over several parts of Iraq, where there is no surveillance and ongoing control programmes to halt its spreading (24,25). The study reported three main components involving the assessment rate of brucellosis in humans using ex-post-facto hospital records, the estimation proportions of the disease in cattle populations using serological tests, and the determination of potential risk factors of the disease using the questionnaire survey. The animal blood samples were subjected to RBT and

subsequently to ELISA tests to validate true negatives and exempt false positives. The information in this research would help the veterinary authorities in collaboration with other disciplines to mitigate the disease infection and to develop an effective control programme.

According to our study, the incidence cases of brucellosis in humans for eight years estimated as 5.4% per 100,000 of people. The occurrence of human brucellosis differs across continents. For instance, human brucellosis incidence in Egypt and Saudi Arabia was documented as 0.28-70.00/100,000 and 6.00-149.54/100,000, respectively (5). The estimated incidence in the study originated from the data reported over a period of time unlike a prevalence measured in a specific period of time. In Erbil (a province to the north of Iraq), the prevalence of human brucellosis was reported as 12% from a total of 325 participants, where *B. melitensis* and *B. abortus* are often identified species (26). Whereas the seroprevalence of human brucellosis in Tanzania and North India was assessed as 11.37 and 15.4%, respectively (27,28). A large-scale survey needs to be set out to determine a true prevalence of brucellosis among humans using state-of-the-art scientific tools including ELISA for confirming new cases and molecular techniques (PCR) for the identification of *Brucella* species.

The highest incidence of brucellosis was recorded in Al-Rafia District (1.96 person/10000). Other scholars have noted that the pathogen of this disease poses a greater risk of infection in rural or pastoral areas than in urban areas (29,30). The handling of livestock, lack of hygienic conditions, and consumption of unpasteurized milk products are widely recognized risk factors that coexist in rural areas where livestock is farmed extensively (5,30). In addition, the greatest risk for infection with brucellosis was found in men compared to women. Similar results were found from studies in Ethiopia and Uganda even though other studies were carried out by Ghugey *et al.* (29), who refuted this hypothesis and ascertained the odds risk between genders is due to milking or preparing cheese or because feeding animals. Hence, health education is a keystone for protection from *Brucella* infection.

The estimation of the prevalence of cattle populations was 3.4%. Brucellosis prevalence in cattle varies by Iraqi regions. The prevalence of brucellosis was reported to be 16.7% in Mosul Province while 5.81% in Najaf Province (31,32). Bovine brucellosis is still endemic in many parts of Asia and Africa. For instance, the prevalence of brucellosis in India and Bangladesh were found to be 15% & 21%, respectively (33,34). Obviously, variation in prevalence of brucellosis between geographical sites is influenced by many factors including farm management and ecological factors.

Moreover, the study also showed an association between age and infection, with those older than one year having a higher chance of being *Brucella* seropositive. This is consistent with previous studies that showed the disease

occurrence is more frequent in adult animals than in young animals (33,35). Susceptibility of acquiring *Brucella* increases after sexual maturity or throughout the mating period. Hence, early boosting animals with vaccines conceivably prevent the spread of brucellosis.

The owners also indicated the occurrence of abortion in their herds and reported 70% of these abortions occurred between 6-7 months during pregnancy. In their study, Lindahl *et al.* (36), stated that the seropositivity of *Brucella* increased in the herds having a history of abortion. The placenta and reproductive organs contain large amounts of erythritol, which is a favourite substrate for *Brucella* for growth and replication, and subsequently inflammation of placenta tissues (37). In a cross-sectional study executed in West Bandung District of Indonesia, it is found that brucellosis causes a significant number of abortions in dairy cattle during the third trimester of pregnancy (38). In addition, the majority of the owners did not isolate the aborting cows or discarded the wasting materials from abortions. This would pose a great threat to spread the infection to other healthy animals from contaminated environments if this is not thoroughly buried or burned.

Other risk factors reported by owners included that a vaccine against brucellosis was not delivered to their animals. The vaccine is a useful tool to prevent the spread of brucellosis, particularly in areas where the disease is widespread. S19 and RB51, two attenuated vaccines for *B. abortus* are available for controlling brucellosis (39), and thus can ameliorate the economic losses resulting from abortions (25). The survey also showed that many owners preferred to borrow a bull than to use artificial insemination for mating or breeding purposes, which is a possible source of brucellosis spread and driving factor to increase a seropositivity of brucellosis in cattle through exchanging bulls for natural breeding. According to Cárdenas *et al.* (40), utilizing bulls from uncertified herds for natural breeding carries twice the risk compared to artificial insemination. Therefore, establishing a health education campaign is an important element to raising owners' awareness of brucellosis.

Conclusions

This research is an attempt to evaluate the rates of brucellosis in cattle and to describe the factors that may have contributed to the spreading of the disease, as well as to coherently delineate human cases over an eight-year period. It is recommended that the control program should be operated immediately to reduce the number of new brucellosis cases in both humans and animals. Young animals must be immunized against brucellosis, and an annual vaccination program is crucial to preventing epidemics. In addition, animal owners should be educated about the risk of the disease, and how to maintain sanitation and improve the management of animal location.

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Conflicts of interest

All the authors in this paper declare that there is no conflict of interest.

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الانتشار المصلي لداء البروسيلة البقري ونسبة حدوثه في الإنسان في محافظة ذي قار

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الخلاصة

تعد البروسيلة (الحمى المالطية) من الأمراض البكتيرية المعدية التي تسبب مشاكل صحية عامة وخسائر مالية في الإنتاج الحيواني. تهدف هذه الدراسة الى وصف الإصابة بمرض البروسيلة في الإنسان والانتشار المصلي للمرض في الأبقار وتحديد عوامل خطر انتشار هذا المرض. يقدر إجمالي حالات الإصابة بداء البروسيلة البشرية في محافظة ذي قار بنسبة ٥,٤٪ لكل ١٠٠,٠٠٠ شخص لثماني سنوات ويلاحظ أن أعلى عدد حالات إصابة ٤٣ مريض كان في عام ٢٠١٩. ويقدر عدد العينات الموجبة باستخدام اختبار الـروزبنكال بحوالي ٣٢ (٢١,٨٪) من إجمالي ١٤٧ عينة دم تم جمعها بشكل عشوائي من

استعارة الثيران لأغراض التزاوج أو التكاثر على التلقيح الاصطناعي. استنتجت الدراسة الى أن داء البروسيللا من الأمراض المستوطنة في محافظة ذي قار وانه من الأهمية بمكان مراعاة منهج الصحة العامة الواحدة للسيطرة على المرض في حيوانات المزرعة وخفض معدلات الإصابة البشرية.

الماشية. كما وقد الانتشار المصلي لداء البروسيللا بنسبة ٣,٤٪ (٩٥٪ س ل: ١,١ - ٧,٨) باستخدام اختبار الاليزا. ويكشف الاستبيان المجرى مع أصحاب الحيوانات أن النسبة الكبيرة منهم لا تتعامل مع الحيوانات المجهضة بشكل صحيح ولا تتخلص من النفايات الناتجة عن عمليات الإجهاض. الى جانب ذلك، يفضل غالبية أصحاب تلك الحيوانات