



## A cross sectional study on the seroprevalence of bovine brucellosis in Al-Najaf province in Iraq

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### Article information

#### Article history:

Received May 19, 2020

Accepted May 4, 2021

Available online October 1, 2021

#### Keywords:

Brucellosis

Cattle

Rose Bengal

c-ELISA

Al-Najaf

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### Abstract

Brucellosis is an endemic disease in many countries of the middle east including Iraq, despite the high attempts to control the disease in animals using vaccination programs. The study was conducted from January to March 2012, to determine the prevalence of bovine brucellosis in the Al-Najaf province in Iraq, which represent risk factors associated with brucellosis in cattle. Blood samples were collected from 172 cows and 15 bulls of 20 dual-purpose cattle herds. Serological examinations are the most useful and widely used tool for the diagnosis of brucellosis in man and animals. All animal serum samples were tested by the Rose Bengal plate test (RBT), and the positive cases were confirmed by competitive enzyme-linked immunosorbent assay (c-ELISA). The overall prevalence of brucellosis was 5.81% divided between 5.81% in females and 0% in males, and 40% as herd numbers level. Females showed a higher seropositive reaction to the disease than males. In conclusion, this study demonstrated the presence of the disease and detected the seropositive reactions in animal samples in different farms of the province, with a difference in infection rate regarding the risk factor. Sanitary measures must be taken to avoid the occurrence and spread of the disease in humans and animals.

DOI: [10.33899/ijvs.2021.127143.1471](https://doi.org/10.33899/ijvs.2021.127143.1471), ©Authors, 2021, College of Veterinary Medicine, University of Mosul.

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### Introduction

Brucellosis is a bacterial infectious disease caused by the gram-negative *Brucella* species, that can affect domestic, wild animals as well as humans (1). Brucellosis can cause considerable economic losses in livestock production which have been estimated at 7-12 million dollars per each year (2). In Iraq, several studies have been carried out on brucellosis seroprevalence (3). In Mosul city, seroprevalence rate was 18.25% and 5.8% in cattle (4), and 8.50 % in buffalo (5). In the camel, the prevalence rate was 16.29% in Wasit province (6), and 8.6% in Erbil province (7). In Mosul province, the infection rate of ovine brucellosis was 15.9 % using the ELISA test on different samples of sheep herds (8) and 25.6% using Rose Bengal test and 2- mercaptoethanol test in goats (9). In cattle, abortion is presented as the main clinical manifestation of the disease with *B. abortus* strain (10). Potential predisposing and risk factors include the climatic

conditions, geographical region, animal species, sex, age, compliance with sanitary measures, such as milk and dairy products pasteurization, disposal of an aborted fetus (11). In Mosul city, Al-Iraqi reported a seroprevalence of *Brucella* antibodies in female buffalo 50.8% and 28.8% by using c-ELISA and RBT respectively (5). Another study showed that the seroprevalence of *Brucella* antibodies in cattle was 16.7% and 18.3% by using c-ELISA and RBT respectively (12).

The objective of this study was to determine the prevalence of bovine brucellosis in Al-Najaf province in Iraq.

### Materials and methods

#### Study area and population

The seroprevalence of Brucellosis was performed in clinically healthy 198 animals 172 cows, 15 bulls aged more

than 36 months, on 20 dual purpose cattle herds in Al-Najaf province during the period January - March 2012 was used in this study. The number of examined animals represents 1.01% of the cattle population in the province.

### Sample collection and study design

Blood samples were collected from the coccygeal vein of 198 (172 cow, 15 bulls) clinically healthy animals. Serum was separated by centrifugation of blood sample at 2000 rpm/5m and kept at -20°C. All samples were labeled with sampling date, animal age, and sex.

### Serological test

Antibodies against *Brucella* spp. were detected by the Rose Bengal test using a commercially available test kit (RBT; VIRCELL, Granada, Spain). Competitive ELISA test was used to confirm the positive results of Rose Bengal (Synbiotics®) (13). The kits were used as directed by the manufacturer and all tests were performed by using (Bio-Tek Instruments, Inc. ELX-800). The dye of Rose Bengal is buffered at  $\text{PH } 3.65 \pm 0.05$ , because the low pH reduces the

nonspecific agglutination of smooth *Brucella* antigens and favors the activity of antibodies especially the IgG1 isotype.

### Statistical analysis

Data obtained from the study were expressed as mean  $\pm$  standard error of the mean (mean  $\pm$  SEM) and analyzed using the SPSS software package (Version 16). One-way ANOVA was used to compare the differences in the mean values between the male and female animals. Values of  $P < 0.05$  were considered significant.

### Results

The results showed that the overall prevalence in cattle would be 5.05%, and the prevalence rate of the disease in screened bulls and cows was 0.0 and 5.81% respectively (Table 1). Our study showed that the positive cases of brucellosis were found to be present in eight herds from a total of twenty herds on the basis of Rose Bengal test and cELISA test, in which the general prevalence rate was 40%.

Table 1: Seroprevalence rate of bovine brucellosis in cattle older than 36 months in Al-Najaf province

	Total number	Negative result No.	Positive result No.	Seroprevalence (%)	P-value
Female	172	162	10	5.81 %	0.0254
Male	15	15	0	0%	

### Discussion

The results of the current study agree with the results of different studies of bovine brucellosis seroprevalence. The different condition of animal's husbandry included environmental factors, husbandry system, and management practices reveal the different percentage of infection with the disease (14).

There is a great difference in the infection rate of the disease in various Iraqi cities, and this runs in the same direction with the results gained by other researchers in the world.

It is mentioned that the disease prevalence differs markedly between countries and within the one country; this great difference belongs to many factors such as occupational, socioeconomic, and demographic factors. In the same city there are also changes in the rate of seroprevalence in animals in various areas (15).

In the studied area in Al-Najaf, the main factor for the prevalence rate is the contamination with uterine discharge, vaginal discharge, milk, aborted fetus, and semen of infected bulls. Feces of infected animals also considered as source of infection.

According to the sex of the animal, the prevalence rate of the disease in this study was 0% in males and 5.81% in females. There are significant differences between males and females in susceptibility towards the disease.

Anyway, there are different data regarding the disease prevalence in both animal sexes, and our results are consistent with the results of previous researchers, who recorded that the females are more susceptible to infection than males, whereas some scientists found that there is no apparent relation between sex of animal and susceptibility to *Brucella* infection (6). Other researchers suspected that ruminant's males are more affected by brucellosis than females (16).

As reported by different organizations such as OIE, FAO, and WHO, brucellosis is seemed to be one of the significant zoonotic in the world through ingesting of unpasteurized milk or dairy products of diseased animals (10).

Al-Hamdani and Al-Zawadi (17) mentioned that there is no difference between RBT, tube agglutination test, and 2-mercaptoethanol, while other studies mentioned that the tube agglutination, rose Bengal and 2- Mercapto-ethanol tests performed by (18) was less accurate than the Indirect ELISA test.

On the other hand, more confirmatory tests in some epidemiological studies were used besides to confirmation of *Brucella* spp in culture (19).

In our study, all the serum samples were examined by the Rose Bengal test, then the positive samples were confirmed using the competitive ELISA test.

The c-ELISA test was used to overcome problems that came from residual antibodies, and cross-antigenicity

between these bacteria and many Gram-negative bacteria (20).

Serological tests are safe, rapid, and somewhat inexpensive diagnostic tools; Rose Bengal test, enzyme-linked immunosorbent assays, complement fixation test is advisable tests for large-scale eradication objectives (21,22).

ELISA test used in this study as suggested by (23) to confirm and avoid many problems of other serological tests (24).

Also by using different serological tests, the variation between the results are depends on the serological test specificity and sensitivity. ELISA, low coast, quantitative, sensitive, but requires standardization of the antigen used. c-ELISA test has high sensitivity and specificity as a substitute to classical tests such as the Rose Bengal test, which is cannot differentiate between some strains like *B. abortus* strain 19 (25).

## Conclusions

The result of this work provides another screening information for Brucellosis in cattle and gives a better epidemiological saw that could be used for better control of such an essential disease in Iraq.

## Acknowledgments

The authors would like to thank the staff of College of Veterinary Medicine at Kufa University for helping during research.

## Conflict of interest

No conflicts of interest regarding the publication of this manuscript.

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لتحديد مدى انتشار مرض الإجهاض الساري في الأبقار في محافظة النجف في العراق لأن هذا الانتشار الواسع يشكل أحد عوامل الخطورة التي تؤثر على الصحة العامة في المجتمع. تم جمع عينات الدم من ١٧٢ بقرة و ١٥ ثور من ٢٠ مزرعة ماشية تربي لغرض اللحم والحليب للفترة من شهر كانون الثاني إلى آذار عام ٢٠١٢. الفحوصات المصلية هي الأداة الأكثر استخدامًا لتشخيص مرض الإجهاض الساري في الإنسان والحيوان. تم استخدام اختبار لوحة الروز بنغال وتأكيد الحالات الإيجابية بواسطة اختبار الاليزا المناعي التنافسي. بلغ معدل الانتشار الكلي لمرض الإجهاض الساري ٥,٨١٪ وكان بنسبة ٥,٨١٪ عند الإناث، و ٠,٠٪ عند الذكور و ٤,٠٪ كمستوى أعداد القطعان. أظهرت الإناث استجابة مناعية مصلية للمرض أكثر من الذكور. أكدت هذه الدراسة وجود المرض وتم تشخيص الاستجابة المناعية المصلية الموجبة في عينات الحيوانات في المزارع المختلفة في محافظة النجف-العراق، مع اختلاف في معدل الإصابة فيما يتعلق بجنس الحيوان. يجب اتخاذ التدابير الصحية لتجنب حدوث وانتشار المرض في الإنسان والحيوان.

## دراسة مقطعية-عرضية مسحية للانتشار المصلي لمرض الإجهاض الساري البقري في محافظة النجف في العراق

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### الخلاصة

مرض الإجهاض الساري هو مرض مستوطن في العديد من دول الشرق الأوسط بما في ذلك العراق، على الرغم من المحاولات العديدة للسيطرة عليه في الحيوانات عن طريق برامج التلقيح. أجريت الدراسة