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Serological and Bacteriological Screening of Brucellosis in Blood and Milk of Cows and Buffaloes

Dhary Alewy Almashhadany [1*]

[1]Department of Medical Lab Science (DMLS), College of Science (CSCN), Knowledge University (KNU) Erbil, Kurdistan, Iraq.

Abstract

Brucellosis remains a serious infection to human and animal populations in developing countries with detrimental effects on public health. The study aimed to evaluate the occurrence of brucellosis in cow and buffaloes at Erbil Governorate, Iraq by detection anti-Brucella antibodies and isolation of *Brucella* species. A total of 265 blood samples (140 from cows and 125 from buffaloes) were collected from villages around Erbil city, and 320 raw milk samples (170 from cow and 150 from buffaloes) were randomly collected from dairy farms during the period from July to December 2019. The results showed an overall seroprevalence of 11.7% according to rose Bengal test (RBT). The isolation of Brucella abortus was 7/19 (36.8%) and 12 / 19 (63.2%), while 9/24 (37.5%) and 15 /24 (62.5%) were *Brucella melitensis* from cattle and buffaloes milk samples, respectively. Noticeable increase in occurrence was found in November (20.8%), while the lowest rate was seen in July (5.9%). In conclusion, brucellosis is still a significant public health hazard in Erbil Governorate. Based on the test performance, the study recommends MRT as a rapid screening test for detecting brucellosis in milk in farms, centers, and dairy factories. Consumers are also recommended to adequately pasteurize the milk in order to kill this milk-borne pathogen before consumption.

Keywords:

Brucella, Buffaloes Milk, Cow Milk, Milk Ring Test, Rose Bengal Test.

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Introduction

Brucellosis is a very old zoonotic disease since animals are the only source of infection (Hull and Schumaker, 2018; Almashhadany, 2019). Brucellosis affects humans and various species of the wild and domestic animals, particularly food-producing animals, including large and small ruminants (Dahl,2020).The infection has also been recognized in marine mammals, including beaked whales, dolphins, cetaceans, porpoises, and seals, which may present an emerging risk to consumers and individuals professionally exposed to contaminated tissues from such animals (CFSPH,2018). Microbiologically, *Brucella* species are facultative intracellular, non-motile non-sporing, gram-negative coccobacilli. To date, twelve *Brucella* species have been reported with a preference to different hosts (Scholz et al., 2016; Sabrina et al., 2018). *Brucella* is highly infectious with a contagious dose of 10–100 cells are adequate to cause systemic infection (Geresu and Kassa, 2016; Almashhadany, 2019)

Transmission of *Brucella* to human and large ruminants occurs via respiratory, oral, and venereal mechanisms. Body fluids or tissues and milk are associated with lateral and vertical transmissions, respectively. Indeed, after entry to host, *Brucella* use phagocytes to reach blood and finally the uterus where immune system is restricted during pregnancy (González-Espinoza et al., 2021). Reproductive organs in cattle and buffaloes are also reached and play key roles in transmission of *Brucella* during breeding seasons. As a result of heavy colonization ($\approx 10^9$ CFU/gm), placenta and fetal fluids are the most important route for transmission of the highly virulent species; B. melitensis and B. abortus during abortion events in cattle (Hull and Schumaker, 2018).

Human brucellosis is a multisystemic disease that may present with a broad spectrum of clinical manifestations. The incubation phase is not easily detected, and normally takes two to four weeks on average. The acute phase, however, is characterized by the onset of symptoms and signs such as fatigue, fever, sweats, splenomegaly, and hepatomegaly. After acute brucellosis infection, symptoms persist in a minority of patients for more than one year. Since no objective laboratory methods exist to confirm the presence of chronic disease, these patients suffer delays in diagnosis (Jiang et al., 2019; Liu et al., 2020).

Different tests have been developed for screening purposes and confirmatory diagnosis each of which has its advantages and drawbacks. This test can be bacteriological (isolation and phage typing for epidemiological studies), serological (detecting antigens and animals' antibodies), or molecular tests that rely on gene detection (Godfroid et al., 2010). The diagnosis of brucellosis is definitive only by isolation of bacteria from animals or by detection of bacterial DNA in animal-derived specimens.

In Kurdistan region, Iraq people consume milk of various animals including; cows, buffaloes, ewes, nanny goats, and camels which had been reported as a source of infection. Recently, it was reported that the occurrence of human brucellosis in Kurdistan region, Iraq is still higher than that recorded from bordering countries (Jaff, 2016). Therefore, the objectives of this study were to: (1) confirm the current incidence of *Brucella* antibodies and *Brucella* species among large ruminants' milk at Erbil Governorate (2) calculate the sensitivity and specificity of MRT in comparison to traditional bacterial culture approach, and (3) to assess the association between months and frequency of *Brucella* antibodies in milk.

Materials and methods

Study Design and Sampling:

Two hundred and sixty-five blood samples (140 from cow and 125 from buffaloes) were collected from villages around Erbil city during the period from July to December 2019. 10 ml of blood were allowed to flow freely from jugular vein through a sterile blood cannula in a sterile McCartney bottle. The samples were left to clot at room temperature for one hour and transferred to the laboratory as soon as possible. Similarly, 320 milk samples (170 from cow and 150 from buffaloes) were obtained from the animals and were kept refrigerated at 4°C overnight before examination by milk ring test (MRT). In laboratory, collected blood samples were centrifuged and sera were stored at −20°C before being tested by rose Bengal test (RBT) according to method described elsewhere (Abdel-Haleem et al., 2015).

Detection of antibodies

Anti-*Brucella* antibodies were detected by Rose Bengal Test (RBT) as previously published (Al-mashhadany, 2009). Briefly, 30 microliters of rose Bengal solution (Pourquier, rose Bengal antigen IIDEXX, Montpellier, France) were added to 50 µl of serum on a white glossy ceramic tile. The tile was then rocked at room temperature for 3 min. Any granulation formation was considered positive. For detection of *Brucella* antibodies in milk, milk ring test (MRT) was performed by adding one drop (0.03 ml) of hematoxylin-stained antigen to 1 mL of milk in a narrow test tube (11 x 100 mm). The mixture was incubated at 37˚C for 1 – 3 hours. Antibodies presence was inferred by formation of a blue ring at the top of the column of milk (Al-mashhadany, 2009).

Isolation and identification of *Brucella* spp.

The isolation of *Brucella* from blood and milk samples was done under sterile conditions (Al-mashhadany, 2018[a]). The identification of *B. abortus* and *B. melitensis* was carried out by biochemical tests performed as described previously (Corbel, 2006; Al-mashhadany, 2018[b]).

Statistical analysis

Data were analyzed using SPSS software version 25 (IBM Chicago, USA). Confidence intervals were estimated using normal distribution approximation at 5% level of probability. Chi square test was applied to test the different between groups. The sensitivity and specificity of the MRT were calculated according to standard equations, using the bacterial isolation diagnostic method as a gold standard.

Results

Prevalence of *Brucella* antibodies

The overall seroprevalence of *Brucella* antibodies in cow and buffaloes according to RBT was (11.7%) (Table 1). Similarly, the overall rate of *Brucella* antibodies in raw milk samples was 11.6%. No significant difference was detected between the tests in terms of the detected proportion of screened samples. Statistically, it is estimated that 8.09% to 16.19% (95% confidence interval) of cow and buffaloes would be seropositive for *Brucella* in Erbil Governorate if screened by RBT assay.

Table 1: Prevalence of *Brucella* antibodies in cow and buffaloes

	No. Examined	Positive samples n (%)	P value
Blood samples			
Cow	140	17 (12.1)	0.708
Buffaloes	125	14 (11.2)	
Milk samples			
Cow	170	21 (12.4)	0.636
Buffaloes	150	16 (10.7)	

Isolation of *Brucella* species

Isolation rate of *Brucella* species from blood samples and milk samples was roughly similar (Table 2). Based on phenotypic criteria, *Brucella abortus* and *Brucella melitensis* were the only isolated species from both blood and milk samples. The isolation of *Brucella abortus* was 7/19 (36.8%) and 12 / 19(63.2%), while 9/24 (37.5%) and 15 /24 (62.5%) were *Brucella melitensis* from cow and buffaloes milk samples, respectively (Fig. 1).

Table 2: Isolation of *Brucella* spp. from cow and buffaloes.

	No. Examined	Positive samples n (%)	P value
Blood			
Cow	140	14 (10.0)	0.739
Buffaloes	125	11 (8.8)	
Milk			
Cow	170	17 (10)	0.535
Buffaloes	150	12 (8)	

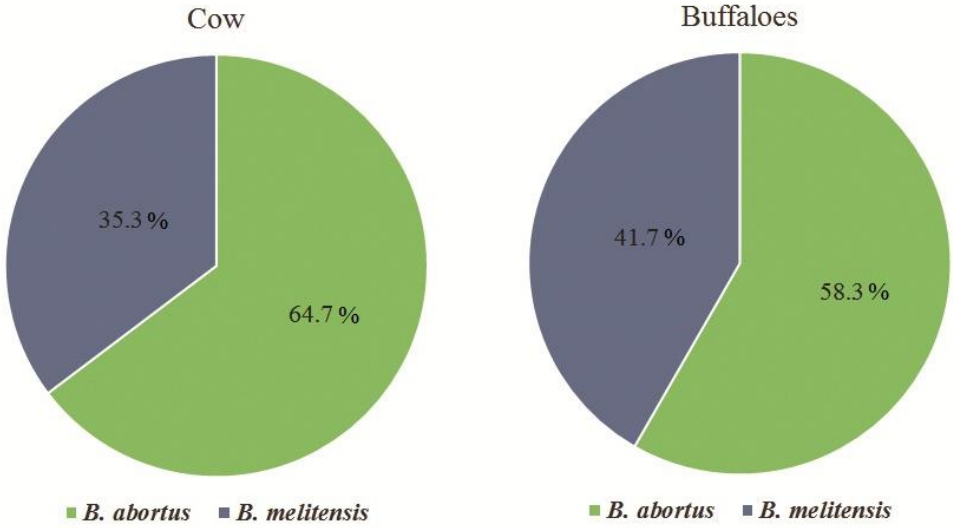

Fig. 1. Frequencies of isolated *Brucella* spp. in raw milk and blood samples.

Sensitivity and specificity of RBT and MRT

The MRT showed a sensitivity level higher than RBT method in milk samples of both populations of cow and buffaloes. However, both tests were excellent in ruling out the infection, rather than confirming it. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of MRT are given in Table 3. The efficiency (accuracy) of MRT in detecting bovine brucellosis is 96.1% compared to the culture method, which candidates the MRT is a good alternative screening/diagnostic method.

Table 3: Diagnostic evaluation of RBT and MRT for detection of brucellosis in cattle and buffalo

	RBT % (95% CI)	MRT % (95% CI)
Sensitivity	78.12 (60.03 – 90.72)	82.86 (66.35 – 93.44)
Specificity	97.96 (95.30 – 99.33)	97.65 (95.22 – 99.05)
PPV	83.33 (67.32 – 92.39)	80.56 (66.24 – 89.74)
NPV	97.17 (94.68 – 98.51)	97.98 (95.90 – 99.01)
Accuracy	95.67 (92.55 – 97.74)	96.10 (93.42 – 97.91)

Seasonality of brucellosis

Variations of *Brucella* antibodies prevalence in raw milk samples of cows and buffaloes during period of study have been addressed (Fig. 2). The highest rate of occurrence of *Brucella* antibodies noticed by MRT was found in November (20.8%), while the lowest rate was recognized in July (5.9%). No significant difference was detected between summer and autumn in terms of the detected positive cases by both tests (p = 0.156, p= 0.147, for MRT and RBT, respectively).

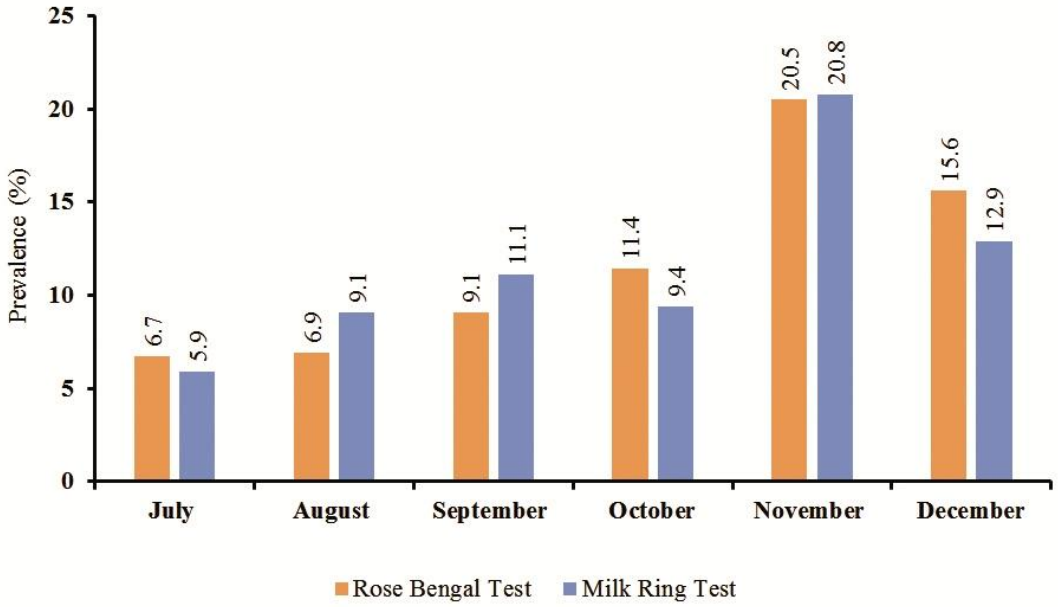

Fig. 2. Seasonal variations of positive samples in cattle and buffaloes.

Discussion

Brucellosis is primarily a disease of food producing animals (sheep, goats, cattle, buffalo, camels, and pigs) but it is transmitted to humans occurs in several ways, commonly through consumption of contaminated food, mainly raw milk or meat (Almashhadany, 2014, 2019; Nyerere et al., 2020). Office International des Epizooties (OIE) declares brucellosis as multiple species disease, infection and infestation (OIE, 2019). It is considered one of the most prevalent zoonosis by Food and Agriculture Organization and World Health Organization (Godfroid, 2017; Khurana et al., 2021).

In this study, the overall frequency of brucellosis in cow and buffaloes was 12.1% and 11.2%, respectively. These findings are consistent with other reports from Iraq and other countries where the overall prevalence range from 10% to 15% according to different serological tests (MRT, RBT, and ELISA) (Cadmus et al., 2008; Mustafa, 2010; Gholizadeh et al., 2013; Gogoi et al., 2015; Dahl, 2020; Hassan et al., 2020). However, higher rates were reported from Sudan (13.9%)(Mustafa,2010),Ethiopia (15%) (Ibrahim et al., 2010), Turkey (32%-39%) (Şahin et al., 2008), Punjab (27.95%) (Zadon and Sharma, 2015), Egypt (23.8%) (El-Diasty et al., 2016), India (38.4%) (Panda et al., 2019), and Albania (55%) (Fero et al., 2020).

In contrast, lower rates were documented in Turkey (2.67%) (Apan et al., 2007), Pakistan (3% to 8.5% in cattle and buffaloes) (Shafee et al., 2011), Libya (4.7%)

(Al-Griw et al., 2017), Egypt (<5%) (Samaha et al., 2008), Yemen (7.7%) (Al-mashhadany, 2009), Argentina (6.4%) (Konrad et al., 2013), and Pakistan (3.9%) (Jamil et al., 2020). Such variations of prevalence between countries and even cities within the same country may be attributed to difference in epidemiology, veterinary care, co-rearing practice, vaccination program, and diagnostic tests (Franc et al., 2018).

In developing countries, it is a good strategy to employ traditional and rapid assessment tests such as RBT and MRT for monitoring of animal brucellosis. MRT has been effectively used in several regions such as Chile, Sudan, India, Iraq, and Pakistan (Rivera et al., 2002; Abdalla and Hamid, 2012; Ali et al., 2013; Mohamand et al., 2014; Almashhadany, 2019). Furthermore, performance of MRT was found to be better than for other serological tests (Al-Mariri, 2015; Al-mashhadany, 2018[b]). The accuracy of MRT in ruling out the infection has also been reported previously (Al-mashhadany, 2018[b]; Al-Shemmari, 2018; Almashhadany, 2019).

In populations of cattle and buffalo, *B. abortus* and *B. melitensis* are the most commonly recovered species while their biovars are distributed differently between countries (Refai, 2002; Abbas and Talei, 2010; Nofal et al., 2017; Deka et al., 2018; Abera et al., 2019; Almashhadany, 2019). It is recognized that *B. abortus* has a preference for cattle over other ruminants, while *B. melitensis* is the common causal agent of brucellosis in sheep and goat. When cattle and buffalo are reared and co-housed with groups of sheep and goats, *B. melitensis* infects and establishes the infection in the population of cattle with prevalence much similar to *B. abortus* (Godfroid and Käsbohrer, 2002; Khurana et al., 2021). Of note, lactating females play an essential responsibility in the epidemiology of human brucellosis, because the *Brucella* species concentrate in the supra mammary lymph nodes and mammary glands in more than 80% of infected females, which persist to excrete *Brucella* in their milk during their lives (Njuguna et al., 2017; Raghunandan et al., 2018).

The seasonality of brucellosis in Kurdistani cattle and buffalo's population is still unclear. Wet season was found to be a risk factor for seropositive brucellosis in camel and goat populations (Megersa et al., 2012). This is consistent with the observed increase in positive cases during November and December when average rainfall ranges from 56 to 80 mm in Erbil governorate. However, larger sample size of milk and blood samples for a complete year may reveal a clearer picture. To the best of author's knowledge, no study has monitored bovine brucellosis in a full-year time span in Iraq or nearby countries. Moreover, there is scarcity of published data on the changing seroprevalence of bovine brucellosis among seasons. Consequently, comparing and contrasting the finding of time-related seropositive rates is not currently possible.

Conclusion

Brucellosis is a great health concern and economically significant in different areas including the Kurdistan region with potential increases during wet seasons. MRT can be used for fast routine monitoring of cow and buffaloes since this test is a simple procedure for day-to-day screening practice. Higher priority should be given to pasteurization which kills these harmful bacteria to render the milk safe to consume. People who handle animal tissues (such as hunters and animal herdsman) should also protect themselves by gloves, goggles, gowns or aprons. Application of simple and rapid screening tests for detection of Brucella in milk will aid in

control of brucellosis spread within ruminants and human.

Conflict of interest statement

The author declares that there are no conflicts of interest regarding publication of this article.

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Ethical Approval

The experimental protocols were approved by the Scientific Committee of the Knowledge University (KNU) Erbil, Kurdistan, Iraq.

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