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PREVALENCE OF BRUCELLOSIS IN BUFFALOES FROM IRAQ

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Abstract

Brucellosis, also known as Malta fever or undulant fever, is a bacterial infection caused by the genus Brucella. It primarily affects animals but can be transmitted to humans through direct contact with infected animals or their products, consumption of contaminated food or unpasteurized dairy products, or inhalation of infected aerosols. Brucellosis in buffalo milk in Iraq represents a significant public health concern. Buffalo brucellosis caused by Brucella abortus can be transmitted to humans through the consumption of unpasteurized or contaminated buffalo milk and dairy products. To diminish the effects of brucellosis in buffalo milk in Iraq, it is crucial to implement strict measures to ensure the safety of dairy products. This includes promoting pasteurization of milk to eliminate the bacteria, enforcing hygiene practices in milk production and processing, and conducting regular testing and monitoring of buffalo herds for brucellosis. Additionally, educating the public about the risks associated with consuming unpasteurized dairy products and promoting safe milk practices can help reduce the incidence of brucellosis transmission.

Key words*: brucellosis, buffalo, Brucella sp.*

INTRODUCTION

Throughout history, brucellosis has significantly influenced human communities, impacting cattle production, trade and public health.

The serious zoonotic disease known as brucellosis, sometimes called Malta fever, Mediterranean fever or undulant fever, is caused by bacteria of the genus *Brucella* (Sayer, 2016). Many species of animals are affected by this infectious disease, but domestic animals including cattle, goats and pigs are the main victims. Other mammals such as dogs, lambs and wild animals such as deer and bison can also contract the disease (Yon et al., 2019).

There are many species of *Brucella* that can infect both humans and animals (Corbel, 2020). The four primary *Brucella* species that pose the greatest threat to human health are as follows (Hull & Schumaker, 2018):

- *Brucella melitensis*. Although it can infect other animals, it mainly affects sheep and goats. It is considered the most virulent species and is responsible for most brucellosis infections in humans worldwide (Pisarenko et al., 2018).
- *Brucella abortus*. Cattle are the major source, but zebras and other animals are also susceptible. The most common ways people get sick are direct contact with sick animals or by eating contaminated dairy products (Jamil et al., 2017).
- *Brucella suis*. This species primarily affects pigs, although it can infect other animals such as dogs, rodents and wild boar. In humans, it is usually associated with occupational exposure to pig farmers, veterinarians and slaughterhouse workers (James et al., 2017).
- *Brucella canis*. This species primarily infects dogs. Although relatively rare, human infections can occur through direct contact with infected dogs, especially during the birthing process or through close contact with reproductive fluids (Hensel et al., 2018).

All these *Brucella* species have similar characteristics, including the ability to survive and replicate in host cells, especially macrophages. They have a tropism for reproductive organs, leading to abortion (Figure 1) or stillbirths in animals, which is a significant factor in transmission (Sarma & Singh, 2022).

Each species of *Brucella* has specific animal reservoirs, but they can also infect humans through different routes, including direct contact with infected animals, consumption of

contaminated animal products (especially raw or unpasteurised dairy products), inhalation of infectious aerosols or laboratory accidents (Figure 2). Understanding the causative agents of brucellosis is crucial for implementing effective control and prevention measures (Berhanu & Pal, 2020).

Figure 1. Aborted fetus due to *Brucella abortus* biovar infection (Megid et al., 2010)

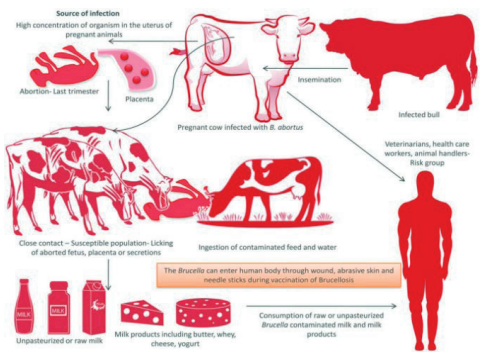

Figure 2. Transmission of brucellosis (Khurana et al., 2021)

Brucellosis can manifest as an acute or chronic disease in humans and its clinical presentation can vary greatly. Symptoms and severity of the disease may differ depending on different factors, such as the species, the dose of bacteria and the individual's immune response (Baldwin & Goenka, 2006).

The clinical presentation of brucellosis can be diverse and may mimic other infectious or inflammatory diseases, making diagnosis difficult. If brucellosis is suspected on the basis of symptoms and risk factors, laboratory tests, including blood cultures, serological tests (detection of antibodies against *Brucella*) and molecular techniques (Polymerase chain reaction - PCR) are used for confirmation (Al-Shemmari, 2018).

Brucellosis-causing bacteria can persist in the environment under specific circumstances, although they are vulnerable to a variety of environmental variables. Other diagnostic methods, such as bone marrow culture, synovial fluid culture or biopsy of affected tissues, may be used in specific cases, particularly when there is suspicion of localised infection (Pal et al., 2020).

It is important to interpret the laboratory results in the context of the clinical findings and patient history. Positive blood cultures or serological tests, together with compatible clinical symptoms, lead to the diagnosis of brucellosis (Barbuddhe et al., 2020).

Prompt diagnosis and treatment are crucial to prevent complications and ensure a favourable outcome. If brucellosis is suspected, healthcare professionals should consult with infectious disease specialists or microbiologists to guide appropriate testing and management (Pal et al., 2020).

MATERIALS AND METHODS

Several areas in Iraq were targeted in this study. Milk and blood samples were collected from buffaloes of different ages and sexes as shown in Table 1. The study was conducted during the year 2022.

Table 1. Areas in Iraq from which samples were collected

No. crt.	Area	No and type of samples
1.	Erbil area	80 samples of raw milk
2.	Governorate Basra	250 blood samples
3.	Governorate Mosul	400 blood samples
4.	Governorate Salahaldeen	205 blood samples

Raw milk samples were tested by the Milk Ring Test (MRT) method for the identification of *Brucella* antibodies (Mohamand et al., 2014). Thus 100 ml of milk were collected under sterile and hygienic conditions and placed in sterile plastic containers with screw caps. Samples were tested by culturing in Petri dishes at 37^0C, 24-48 hours, in the presence of a specific *Brucella* antigen. The formation of a ring or clusters around the antigen drop indicates the presence of *Brucella* antibodies in the milk sample, suggesting infection. Another method of testing is to add a drop of antigen to

1 ml of raw milk in a test tube. Specific antibodies attach to the antigen and rise to the surface, forming a blue ring. In the absence of antibodies, the mixture remains uniformly blue-white throughout the test tube.

The tests also aimed to isolate and identify *Brucella* strains present in raw milk by culturing on specific solid media at 37^0C for 7 days, followed by biochemical testing of the isolates.

The following tests were used to detect *Brucella* presence in milk and blood samples: a) Milk Ring Test; b) Microbiological tests, by cultivating the samples on specific culture media; c) Elisa test; d) Rose Bengal Test.

Blood samples were taken from male and female buffaloes aged 1-5 years. Using sterile syringes, 5 ml of blood was drawn from the jugular vein and placed into glass tubes. The plasma was then separated by centrifuging the blood samples at 3000 rpm/min for 5 minutes, after which it was stored in sterile plastic tubes at -20^0C until serological tests were performed.

The Rose Bengal test was performed on *Brucella abortus* antigens. Positive plasma samples were tested by a method using a phenol solution, which was prepared by dissolving 5 g phenol and 8.5 g sodium chloride in 1000 ml distilled water. *Brucella* antigen was used to test tubular agglutination, while it was diluted 1:10 using a mercaptan solution.

The tests in the Salahaldeen Governorate region involved 205 mature male and female buffaloes from which blood samples were taken. All the characteristics of the samples collected from buffaloes in this region (sex, physiological and pathological characteristics) are listed in Table 2.

Table 2. Characteristics of the samples collected from buffaloes from Salahaldeen area

Status of buffalo	Number of samples
Adult males	63
Adult females	84
Female abortion	21
Females pregnancy	37

RESULTS AND DISCUSSIONS

Bovine brucellosis is of great economic importance because the losses it causes can be very high, through miscarriages, prenatal morbidity, reduced milk and meat production, uneconomical use of production and slaughter and surveillance costs.

But the health importance of this disease is also particularly great, as it is one of the most feared zoonoses and at the same time one of the most feared professional diseases, and the toll paid by the veterinary profession over the years has been enormous.

Tests carried out on raw buffalo milk collected from the Erbil area showed a brucellosis incidence of 7.5% out of 80 samples tested (Table 3).

Table 3. Occurrence of *Brucella* antibodies among buffaloes raw milk from Erbil area according to Milk Ring Test method

Type of Milk	Number of samples	Positive samples (%)	Negative evidence (%)
Buffalo	80	7.5	92.5

Brucella species were identified in 6.3% (respectively 5 positive samples out of 80) of raw buffalo milk samples. Of the 5 *Brucella* strains found in raw milk samples, 3 strains of *Brucella abortus* (60%) and 2 strains of *Brucella melitensis* (40%) were identified. The data are presented in Table 4.

Table 4. Results obtained after the isolation of *Brucella* species from buffaloes raw milk

Type of milk	Buffaloes
Number	80
Positive (%)	6.3
Negative (%)	93.7
B. abortus (%)	60
B. melitensis (%)	40

Results obtained by the MRT method were compared with the microbiological results using milk samples. The MRT method identified 7.5% more cases of brucellosis in buffaloes.

Table 5 gives the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the MRT method. Compared to the classical microbiological method, the accuracy of the MRT method in identifying bovine brucellosis is 97%, making it a viable alternative screening/diagnostic method (Table 5, Figure 3).

Table 5. Comparison between result of MRT and isolation of *Brucella* species from buffalo milk

Type of Milk	Buffalo
Number	80
MRT positive (n/%)	6/7.5
Culture positive (n/%)	5/6.3
Sensitivity (%)	83.3
Specificity (%)	98.7
Predictive value positive (%)	83
Predictive value negative (%)	98.5
Efficiency (%)	97

Figure 3. Milk ring test (MRT) result

The Rose Bengal Test results showed that of the 250 samples collected from the Basra area, 27 of them, representing 10.8%, were positive (Table 6).

Table 6. Brucellosis in buffaloes of Basra by Rose Bengal Test

Number of samples	Rose Bengal Test		
	Seropositive (%)	Seronegative (%)	Suspicious (%)
250	10.8	64.8	24.4

The Elisa test was performed on 88 plasma samples collected from buffaloes in Basra area, which included the 27 positive and 61 suspect plasma previously detected using the Rose Bengal Test.

In Figure 4, sample 3 represent positive control, sample 5 represent negative control, while samples 1, 2, 4, represent the positive cases detected.

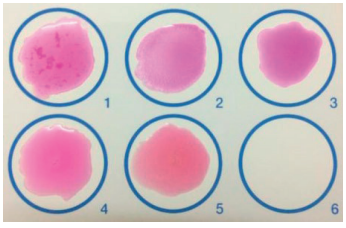

Figure 4. Positive results of infected animals using Rose Bengal Test

The Elisa test showed that of the 88 samples tested, 21, representing 23.8% were positive (Table 7). Table 7 also contains information on the regions from which samples were collected.

Table 7. Result of Elisa for *Brucella abortus* in buffaloes from Basra Guvernorate

Region	Number of probes	Number positive Elisa Test (%)
Al Hartha	30	6.8
Al Qurna	15	5.7
Al Dear	18	4.5
Al Zubier	10	3.4
Al Medaiana	7	2.3
Al Tanooma	8	1.1
Total	88	23.9
Significance		P < 0.05%

From Table 8 it can be observed that a significant impact on the severity of infection was the age of the animals tested. Of the 88 samples from animals aged between 1.5-8.5 years, the category > 5-8.5 years was strongly affected, with 12 positive results out of the 40 samples tested, representing 13.6% of the total number of samples tested (88). Although the proportion of young animals, of 1.5-5 years, was higher than that of older animals, a lower infection with *Brucella abortus* was recorded for them, only ~10% of the total number of samples tested (9 positive tests out of 48 animals tested). Out of the total of 88 samples, 67 tested negative, 66% of the total (Table 8).

Thus, *Brucella abortus* infection was detected in 24% of the buffalo tested, which indicates a serious health problem that requires prompt intervention.

Table 8. Infection rate of *Brucella abortus* according to age groups

Age (years)	Number of animals	Elisa test results	
		Positive (%)	Negative (%)
1.5 - 5	48	10.2	44.3
> 5 - 8.5	40	13.6	31.9
Total	88	23.8	76.2

The same Rose Bengal Test was also carried out on 400 buffalo in the Mosul area. For 52 tests the results were positive, representing 13% of the total number of tests (Table 9).

Table 9. Test result of Rose Bengal Test

Number of probes	Positive results (%)	Negative results (%)
400	13	87

According to the Rose Bengal Test, the highest incidence of infection was reported in females, with 8.29% positive tests, compared to 2.44% in males.

Positive Elisa results were obtained in 5.37% of females, while for males, 2.44% were positive tests (Table 10).

Table 10. Results of RBT and Elisa test of buffalo in Salahaldeen area

Buffalo status	Number of sample	Positive results		Negative results (%)
		Rose Bengal Test (%)	Elisa Test (%)	
Adults male	63	2.44	2.44	25.85
Adults female	84	3.41	2.93	34.63
Aborted female	21	2.93	0.98	6.30
Pregnant female	37	1.95	1.46	14.63
Total	205	10.73	7.81	81.46

CONCLUSIONS

- Brucellosis in buffalo milk in Iraq represents a significant public health concern.
- To diminish the effects of brucellosis in buffalo milk in Iraq, it is crucial to implement strict measures to ensure the safety of dairy products. This includes promoting pasteurization of milk to eliminate the bacteria, enforcing hygiene practices in milk production and processing, and conducting regular testing and monitoring of buffalo herds for brucellosis.
- Educating the public about the risks associated with consuming unpasteurized dairy products and promoting safe milk practices can help reduce the incidence of brucellosis transmission.

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