

Surveillance of *Brucella* in Red Meat Sold at Retail Outlets

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Abstract

Brucellosis in the Middle East is endemic and is associated with health burdens and economic losses for animals and humans. Transmission of *Brucella* from animal hosts to humans is prevalent in endemic areas, especially developing countries. This study aimed at screening for the brucellae in different fresh red meat sold in retail markets in Erbil city, Iraq. A total of 410 samples were collected between July and December 2019 and analyzed by serological and bacteriological tests for *Brucella* spp. by Rose Bengal Test (RBT), ELISA, and traditional bacterial culture. The prevalence of *Brucella* was 9.3, 7.8 and 7.1 % by RBT, ELISA, and bacteriological analysis, respectively. Both *B. abortus* and *B. melitensis* were detected in 3.17 % and 3.90 % of collected samples, respectively. In terms of seasonal variation, autumn was found to be associated with a decrease in seroprevalence. RBT was found to be suitable for ruling out the disease, but its positive results should be confirmed. The overall prevalence of *Brucella* in meat or the source livestock is alarming and requires considerable actions to prevent the transmission of brucellae to humans.

Keywords: Beef, Mutton, Goats, ELISA, *B. melitensis*

Introduction

Brucella is an intracellular gram-negative short rod that survives mostly in infected animal hosts and humans. The genus is currently under taxonomic expansion with 12 validated species. The most important 6 species are; *B. melitensis*, *B. abortus*, *B. canis*, *B. suis*, *B. ovis* and *B. neotomae* with a widespread global distribution in domestic livestock and wildlife [1]. Brucellae can survive freezing and thawing, but repetitive cycles of freezing and thawing reduce cells viability [2].

The environmental persistence of *Brucella* spp. occurs under moist and cool conditions away from direct sunlight but its epidemiological importance is controversial [2,3]. Different species of *Brucella* have different preferred hosts. For instance, *B. abortus* usually infects cattle, but other animals such as bison, camels, yaks, and African buffalo may also be infected naturally. On the other hand, *B. melitensis* prefers sheep and goats and considered to be endemic in the Middle East and the Mediterranean region. The other species are generally infecting swine, dogs, horses, foxes, and other animals [4]. Nonetheless, cattle and sheep can also be infected by *B. suis* and *B. canis*, respectively [5].

Transmission of *Brucella* to hosts occurs via respiratory, oral and venereal mechanisms. Additionally, body fluids or tissues and milk are associated with lateral and vertical transmissions respectively [6]. Indeed, after entry to host tissue, *Brucella* uses phagocytes to reach bloodstream and finally to the uterus, where immune system is restricted during pregnancy [7]. Reproductive organs are also colonized and play a key role in transmission of *Brucella* during breeding seasons [7,8]. As a result of heavy colonization ($\approx 10^9$ CFU/gm) of the placenta and fetal fluids, these materials are the most important vehicles for transmission during abortion events in cattle. In contrast, sheep and goats shed the bacteria for long periods through milk, urine and mucosal secretions [7,9]. Transmission to human occurs by close contact with infected animals and unpasteurized dairy products. Human brucellosis is widespread globally, but higher incident rates are seen in the Middle East, Mediterranean basin, central Asia and sub-Saharan Africa [6]. Although human brucellosis is generally associated with low mortality, death could result from cardiac or neurological complications [9,10].

Different tests have been developed for screening purposes and confirmatory diagnosis, each of which has its advantages and drawbacks. These tests can be bacteriological (isolation and typing by phages for epidemiological studies), serological (detecting antigens and animals' antibodies), or molecular tests that rely on gene detection [11]. The diagnosis of brucellosis is definitive only by isolation

of bacteria from animals or by the detection of bacterial DNA in animal-derived specimens [11,12]. RBT is an agglutination-based test that detects anti-*Brucella* antibodies using commercially available *Brucella* antigen kits. The ELISA approach is directed toward the detection of IgG and its subclass with high sensitivity. However, false-positive results may occur owing to cross-reactions with serotype O:9 of *Yersinia enterocolitica* infection [11-13]. These tests can also be performed on milk samples but their sensitivity is lower than of serum [11]. In Iraqi Kurdistan, the status of brucellosis among meat-producing animals is still unknown. This study aimed to survey the presence of *Brucella* in animals' red meat consumption in Erbil city, Kurdistan Region.

Materials and methods

Study design and sampling

A total of 410 fresh red meat samples (from cattle, goats and sheep) were collected randomly from retail markets in Erbil city (Iraq) between June and December 2019. Beef samples comprised 32.92 %, mutton 35.36 %, and goat 31.71 %. Each sample's weight ranged from 50 - 100 g collected aseptically in sterile polyethylene bags and transported to the Laboratories of Pathological Analysis Department, Knowledge University, in ice box with a minimum delay. Upon reception, the samples were frozen upon reception at -20 °C for maximally 7 days before analysis.

Preparation of meat juice

Meat juice was prepared according to a previously published method [14]. Briefly, after 1 week of deep freeze, samples were thawed at 20 - 25 °C in sterile containers. Meat juice (2 - 5 mL) was collected and centrifuged. The supernatant was used for RBT and ELISA tests.

Detection of *Brucella* antibodies

Rose Bengal Test (RBT)

A commercially available RBT kit (L1-M1110, Linear Chemicals SL, Spain) was used to screen for animal antibodies to *Brucella* antigen. The test was performed according to the instructions provided by the manufacturer. Briefly, equal quantities of antigen and meat juice were mixed on a clean slide by a stirring stick. The slide was tilted gently back and forth for 3 - 4 min before the inspection of agglutination that indicates a positive result.

ELISA

Competitive ELISA was performed using the SVANOVIR *Brucella* Ab c-ELISA kit (Svanova, Sweden) using *Brucella* lipopolysaccharide (s-LPS) coated wells on a microtiter plate. The procedure was done according to the manufacturing company.

Isolation and identification of *Brucella*

Brucella agar (HiMedia, India) was employed as a primary isolation medium according to a published standard procedure [15]. Briefly, meat samples were cut into small slices and smeared the cut surface on the agar using sterile containers, scissors and forceps. Plates were incubated in 5 - 10 % CO₂ conditions at 36 ± 1 °C for up to 10 days before discarded as culture-negative once colonies were not developed.

Based on growth characteristics, suspected colonies were selected for further characterization by Gram smear evaluation and traditional biochemical tests commonly used for the identification of *Brucella* spp. This included a positive reaction to the tests of; catalase, oxidase, urease, non-motility and strict aerobic growth [16].

Sensitivity and specificity of RBT and ELISA

The sensitivity and specificity of RBT and ELISA were calculated according to standard equations using the bacterial isolation diagnostic method as the gold standard [17].

Statistical analysis

Data were analyzed for descriptive statistics using Microsoft Excel 2016 (Version: 16.0.6769.2017). Confidence intervals of prevalence were estimated using normal distribution approximation at alpha level of 0.05. Confidence intervals for sensitivity, specificity and accuracy are "exact" Clopper-Pearson confidence intervals.

Results and discussion

Seroprevalence of *Brucella* spp.

From 410 samples of fresh red meat, 38 samples (9.3 %) showed a positive reaction to *Brucella* antigen detected by RBT. Yet, a slightly lower proportion was found by ELISA test (7.8 %). Based on both tests, goats' meat is the most contaminated type with *Brucella* (**Table 1**). It is estimated that 5.83 - 11.24 % (95 % CI) of red meat in Erbil markets are expected to be contaminated with *Brucella* or derived from infected animals. This prevalence is consistent with studies conducted in Mediterranean countries where brucellosis is endemic; Italy (9.3 %) and Egypt (11.1 %) [18,19]. On the other hand, low prevalence (0.4 - 2.1 %) was reported in a surveillance program in Egypt [19]. Additionally, other similar data were also reported from other countries around the globe. For instance, an Indian study found 6.8 % of goats were positive for RBT, while 10.99 % were positive when screened by ELISA [20]. However, higher rates were reported from sub-Saharan region in Africa (16.2 %) and from different Indian farms (ranged from 13.5 to 75.75 %) [20]. In contrast, lower prevalence was reported Pakistani Punjab districts where vaccination is offered to livestock animals [21]. Geographical locations where vaccination programs are scarce, history of abortion is a well-known consequence of *Brucella* spread in herds of animals [22].

The difference in geographical location, testing assays and herd vaccination are mostly standing behind such variations. Despite the fact that these tests are performed on serum samples, meat juice has been reported to be an alternative when screening of brucellosis is desired [23,24]. However, caution should be taken when generalizing these findings due to test-inherited limitations, and differences in the animal immune response to different bacterial species [14].

Table 1 Surveillance of *Brucella* antibodies among red meat screened by RBT and ELISA.

Type of meats	No. examined	Positive samples n (%)	
		RBT	ELISA
Beef	135	12 (8.9)	10 (7.4)
Mutton	145	9 (6.2)	8 (5.5)
Goats	130	17 (13.1)	14 (10.8)
Total	410	38 (9.3)	32 (7.8)

Bacteriological detection of *Brucella* spp.

The culture-based detection of *Brucella* spp. showed a similar proportion to that of ELISA. Up to 4.59 - 9.55 % (95 % CI) of red meat samples in Erbil markets are estimated to harbor *Brucella* spp. The highest recovery rate of brucellae was in goats' meat samples (10.8 %). The detailed distribution of *Brucella* spp. in screened meat types is summarized in **Table 2**. The observed host patterns for the detected species of *Brucella* are in good agreement with the expected host preference documented in the literature [1,2]. The culture-based prevalence reported in this study is slightly higher than reported in some earlier studies. For instance, a prevalence of 5.5 % was found in blood and tissue samples collected from slaughtered cattle in abattoirs of Gauteng (South Africa) [25]. In contrast, high recovery rate of *Brucella* from meat samples was reported in an Italian study (44 %) that even outperformed PCR assay [18]. Similarly, 32 % of goats' meat samples screened in Thailand were positive for *B. melitensis* [26].

Table 2 Isolation of *Brucella* species from several kinds of red meat.

Meat type	No. examined	Positive samples n (%)	Isolates of <i>Brucella</i> species n (%)	
			<i>B. abortus</i>	<i>B. melitensis</i>
Beef	135	8 (5.9)	6 (75.0)	2 (25)
Mutton	145	7 (4.8)	4 (57.1)	3 (42.9)
Goats meat	130	14 (10.8)	3 (21.4)	11 (78.6)
Total	410	29 (7.1)	13 (44.8)	16 (55.2)

Assessment of RBT and ELISA tests

Owing to its high specificity (97.69 %) and cost-effectiveness, the RBT is only useful in ruling out the disease in screening practice, while its positive results should be confirmed by ELISA or the traditional culture diagnosis. Nonetheless, both screening tests showed excellent accuracy (**Table 3**). The present findings are in good agreement with a recent study conducted in Erbil city, especially for specificity and overall accuracy (efficiency) [27]. Indeed, several studies reported similar findings regarding ELISA assay in which the specificity ranged from 90 to 99 % [28-30]. The RBT assay is known to produce false-positive results due to cross-reactions with antibodies mounted against other bacterial pathogens such *Yersinia enterocolitica*, and *Leptospira* [31,32]. On the other hand, other studies found RBT to has high sensitivity and is an excellent screening test in animal and human brucellosis [33,34].

Table 3 Contrast between RBT and ELISA proficiencies in identifying brucellosis in red meat.

Test criteria	RBT (95 % CI)	ELISA (95 % CI)
Sensitivity	76.32 % (59.76 - 88.56)	90.62 % (74.98 - 98.02)
Specificity	97.69 % (95.66 - 98.94)	99.22 % (97.73 - 99.84)
Positive Predictive Value	76.32 % (62.26 - 86.29)	90.62 % (75.69 - 96.77)
Negative Predictive Value	97.69 % (95.99 - 98.68)	99.22 % (97.74 - 99.73)
Accuracy	95.79 % (93.43 - 97.49)	98.56 % (96.89 - 99.47)

Temporal changes in *Brucella* seroprevalence

For six-month surveillance, the highest prevalence of brucellae was observed during the late of the dry season in August and July (16.4 and 11.3 %, respectively) (**Table 4**). In general, a weak association ($r^2 = 0.42$) between autumn and decrease in the prevalence of brucellae antibodies was observed. However, the actual drop in infections may be started during late summer (July) due to the fact that humoral immune response takes weeks to produce high antibody titer [7]. The observed decrease in prevalence with autumn progress contradicts the observation of earlier studies that found wet season to be associated with increase in *Brucella* prevalence [35,36]. In contrast, other studies also noted an increase in *Brucella* prevalence during dry months [37-40]. This increase in prevalence may be attributed to close animal contact at watering points and sharing of limited pasture fields during the dry season.

Table 4 Relation between months and surveillance of *Brucella* antibodies during period of study.

Month	Total examined			No. of positive						Total positive n (%)	
				Rose Bengal Test			ELISA				
	Beef	Mutton	Goat	Beef	Mutton	Goat	Beef	Mutton	Goat	RBT	ELISA
July	25	24	22	3	2	3	2	1	2	8 (11.3)	5 (7.1)
August	23	23	21	4	2	5	4	2	4	11 (16.4)	10 (14.9)
September	23	24	22	1	1	2	1	2	2	4 (5.8)	5 (7.2)
October	21	25	21	2	1	2	2	0	1	5 (7.5)	3 (4.5)
November	22	25	23	1	2	3	1	2	3	6 (8.6)	6 (8.6)
December	21	24	21	1	1	2	0	1	2	4 (6.1)	3 (4.6)
Total	135	145	130	12	9	17	10	8	14	38 (9.3)	32 (7.8)

Conclusions

Brucella prevalence in livestock animals slaughtered for meat is high in Erbil Governorate. This level poses a serious threat to farmers, abattoir workers, and consumers. Screening practice on a regular basis of livestock animals is recommended to avoid the transmission of *Brucella* to humans. RBT is suitable for the primary screening and ruling out the disease in animals but its positive results should be confirmed by other accurate tests such as ELISA. Special care by consumers during preparation and cooking and sufficient temperature are recommended to markedly reduce the probability of acquiring brucellosis. Moreover, in-charge official authorities are highly recommended to take action and set a wide surveillance program and countermeasures to prevent the zoonosis.

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