Prevalence of *Brucella* spp. in milk from aborted and non-aborted animals in Dhamar governorate, Yemen

Ayman H. Al-Afifi,[1] Dhary Alewy Almashhadany,[1,2] Aziz S.H. Al-Azazi,[3] Ahmed M. Khalaf,[3,4] Mohammed Naji Ahmed Odhah,[1] Naif A. Al-Gabri[5,6]

[1]Department of Veterinary Public Health, Faculty of Veterinary Medicine, Thamar University, Dhamar, Yemen; [2]Department of Medical Laboratory Science, College of Science, Knowledge University, Erbil, Iraq; [3]Department of Internal Medicine, Faculty of Veterinary Medicine, Thamar University, Dhamar, Yemen; [4]Faculty of Allied Medical Sciences, Al-Ahliyya Amman University, Amman, Jordan; [5]Department of Veterinary Pathology, Faculty of Veterinary Medicine, Thamar University, Dhamar, Yemen; [6]Laboratory of Salam Veterinary Group, Burayda, Al-Qassim, Saudi Arabia

Abstract

Brucella infection in animals is considered a great problem in most countries of the world. Our study designed to determine the prevalence of brucella in field animal's milk in Dhamar governorate, Yemen. Total of 808 raw milk samples from non-aborted field animals, 120 milk samples from aborted animals, and 30 pasteurized milk samples were teste by Milk-Ring Test (MRT), milk-ELISA test, isolation and identification of brucella species, and antibiotic susceptibility. The prevalence of brucella in milk samples from field animals was 0.8%, 2.6%, and 2% in cows, sheep, and goat milk samples respectively with MRT, and 0.8%, 1.3% and 1.6% in cows, sheep and goat milk samples respectively with the milk-ELISA test. The prevalence rate in milk samples from aborted animals was 33%, 64% and 41.2% with the MRT and 39%, 49%, and 41.2% in cows, sheep and goats respectively with the milk-ELISA test. All pasteurized milk samples were negative for the milk-ELISA test. The result of isolation showed 0.1% of Brucella in milk samples from field animals while 9.2% from aborted animals. All isolates of Brucella species were sensitivities to rifampicin, doxycycline, kanamycin, gentamicin, streptomycin, tetracycline, and ciprofloxacin, while resistant to ampicillin, erythromycin, and novobiocin. In conclusion, the high prevalence of milk brucella especially in aborted animals needs focusing and build controlling strategies plans to decrease the losses to the economy and avoid transferred to humans with unpasteurized milk consumption.

Introduction

Brucellosis is a bacterial disease caused by various *Brucella* species and among classical *Brucella* spp., *B. melitensis* and *B. abortus* are of paramount zoonotic importance worldwide, which mainly infect small ruminants and cattle, respectively (Poester *et al.,* 2013). Humans generally acquire the disease through direct contact with infected animals, by eating or drinking contaminated animal products or by inhaling airborne agents. Most cases are cause by ingesting unpasteurized milk or cheese from infected goats or sheep (Wainaina *et al*., 2020: Negrón *et al*., 2019: WHO, 2005; Corbel, 2006). The disease has been recorded in Middle Eastern countries such as Jordan, Algeria, Iraq, and Egypt with different rates of infection (Aggad & Boukraa, 2006; Refai, 2002; Hamdy and Amin, 2002; Ali, 1998; Hadad *et al.,* 1997; Aldomy *et al.,* 1992). Brucella infection in farm animals is considered a great problem in most countries of the world. Thus, the early detection of *Brucella* infection in a herd or flock is a pre-request for the successful control and elimination of the disease (FAO/WHO, 1986. Wasseif, 1992).Serological tests have been used extensively throughout the world for the diagnosis of brucella in animals. The tube agglutination test using standard *brucella abortus* antigen is associated with two supplemental tests, the Rose Bengal Plate Test and the Milk-Ring Tests are used in the diagnosis of brucella to minimize the risk of error (Kumar *et al*., 2017: Alton *et al*., 1988). Investagating the *Brucella* spp. in 200 raw milk, ricotta, and artisan fresh cheese samples, collected from individual marketing points in four districts in Tunisia was done by (Béjaoui *et al*., 2022) who found 31.3%, and *B. melitensis* was detected in 5.3% of positive samples. A percentage of 49.3% of samples co-harbored both species, while 14% of the Brucella spp. positive samples were not identified as either *B. abortus* or *B. melitensis*. High contamination rates were found in ricotta (86.2%), cheese (69.6%), and raw milk (72.5%) samples. Yemen is well-known for its rural culture and traditional lifestyle, where different livestock species are kept together, and people live in close to their livestock. Brucellosis is the likely cause of health impact and economic losses to owners and their animals and in addition to considering the importance of milk as an important food source for many Yemeni families and at the same time a source for the transmission of brucella to humans and its relationship to public health. Therefore, this study aimed to determine the prevalence of brucella in both aborted and non-aborted milk of cows, sheep, and goats in the Dhamar governorate using the Milk-Ring Test (MRT) and milk ELISA test and to isolate and identify *Brucella species* from milk. Moreover, the study was design to evaluate the efficiency of the MRT and milk ELISA test, in the detection of truly positive Brucella infected animals.

Correspondence: Naif Ahmed Al-Gabri, Department of Veterinary Pathology, Faculty of Veterinary Medicine, Thamar University, 87246 Dhamar, Yemen; Laboratory of Salam Veterinary Group, Burayda, Al-Qassim, Saudi Arabia
Tel.: +966547171089.
E-mail: naifaljabry@yahoo.com

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Materials and Methods

Materials

Media and Broth

Brucella Agar, Blood Agar Base, Brucella Broth, Tryptone water, Urea Broth Base, Brain Heart Infusion Agar, MacConkey Agar, Nitrate Broth, Triple Sugar Iron Agar, Simmons Citrate Agar, Miller Hinton Agar and Trypticase soy broth were used for isolation, identification and typing of Brucella isolates. The media were obtained from Oxoid Limited, Hampshire, England, obtained from Himedia laboratories Pvt. Limited, India.

Stains

Gram staining was performed following the procedures described by the manufacturer (Al-helal company, KSA).

Dyes

Thionine (1:100000; 1:50000; 1:25000); Basic Fuchsin (1:50000 and 100000), supplied by BDH company, and performed according to the procedures described by Alton *et al*. 1975 and Alton *et al*. 1988.

Reagent

Oxidase test reagent, Catalase test reagent, and Kovacs reagent (for indole test), supplied from Himedia laboratories Pvt. Limited, India, and carried out as described by (Alton *et al*., 1975; Alton *et al* .1988). In addition, H_2S production reagent and Nitrate reduction reagent was perpetrated and carried out as described by (Cowan 1993).

Antisera

Mono-specific Brucella abortus anti-sera (Anti A) and Mono-specific Brucella melitensis anti-sera (Anti M) were supplied by Murex Biotech Ltd, Dartford, England, and carried out as described by (Alton *et al*.1988).

Antigens

Antigens for the standard Milk-Ring Test (MRT) were obtain from CVL, Weybridge, UK and the ELISA antigen and reagents were obtained from Svanova Biotech AB, Uppsala, Sweden.

Methods

Study design and samples collection

A survey study was conducted during the period from 2007 April to 2008 Marsh in four districts in Dhamar governorate (Jahran, Dhoran, Al-Hada and Anss) in Yemen. Eighty hundred and eight raw milk samples from field animals (244 cows, 310 sheep, and 254 goats) were collect randomly through several stages (FAO, 2003; Pfeiffer, 2002; Nichols, 1991). The study also included an examination of 120 milk samples collected from aborted animals (18 cows, 68 sheep, and 34 goats) and 30 pasteurized milk samples from local markets.

Raw milk samples were collected under aseptic conditions in 25-50 ml sterilized tubes and transported in an icebox to the laboratory. Pasteurized milk samples belonging to the local dairy farms were collected from local markets. Each milk sample was divided into two parts, one for bacterial isolation, which was carried out in the veterinary laboratory of the public health department/faculty of agriculture and veterinary medicine/Thamar University. The other part was sent to the central veterinary laboratory in Sana'a governorate for serological analysis, including the Milk-Ring Test (MRT) and milk-ELISA test.

Serological tests

The serological tests used in testing samples were the milk-ELISA test, which perform following the procedures described by the manufacturer, and the Milk-Ring Test (MRT). Interpretation of results of MRT depending on color, according to Commonwealth Department of Health National Biological Standards Laboratory, 1984 and Alton *et al.* 1975).

Bacteriological Examination

The culture of milk was carried out under aseptic conditions. Milk samples were centrifuge for 15 minutes at 6000rrpm. The sediment cream mixture of each sample was inoculated in two plates of *brucella* agar media containing 5% serum and antibiotic supplement. One plate was incubated aerobically and the other anaerobically with 5-10% carbon dioxide and kept at 37°C. Cultured plates were examined for *brucella* growth on the 3rd day, and daily for 10 days. Suspected colonies were furtherly identified and subculture on *brucella* agar slopes. Identification of *Brucella* isolates was according to morphological characters, microscopically examination, biochemical tests, and reaction with positive sera, according to the procedures described by Alton *et al.* (1988). Typing of *brucella* isolates was done on the base of CO_2 requirement, H_2S production, growth in the presence of dyes (thionin and basic fuchsin), in addition to reaction with mono-specific sera (A & M), which was done according to Alton *et al.* (1988).

Antibiotic sensitivity testing

The *brucells* isolates were tested for their susceptibility to 11 antibiotics (Rifampicin; Ciprofloxacin; Ampicillin; Erythromycin; Novobiocin; Kanamycin; Gentamicin; Streptomycin; Tetracycline; Doxycycline and Carbenicillin), obtained from Himedia Laboratories. Testing was performed on Mueller-Hinton Agar plates using the Kirby-Bauer disk diffusion technique (Bauer & Kirby, 1966). The antibiotic resistance of each *brucella* isolate was determined based on the breakpoints of the inhibition zone diameters for individual antibiotic agents and as recommended by the disk manufacturer.

Statistical analysis

The results were analyzed by using Genestat 5 Release 3.2 (pc/windows NT). The seroprevalence is reported as percentages (%) with 95 per cent confidence inter-

Table 1. Seroprevalence of brucella in milk specimens of aborted and non-aborted animals according to MRT and milk-ELISA tests.

Milk source	No. tested	MRT Positive				milk-ELISA Positive			
		No. (%)	95%CI	X^2	p-value	No. (%)	95%CI	X^2	p-value
Non aborted animals									
Cow	244	2 (0.8)	0.3-1.9	2.53	≥0.05	2 (0.8)	0.3-1.9	0.59	≥0.05
Sheep	310	8 (2.6)	0.8-4.4			4 (1.3)	0.0-2.6		
Goat	254	5 (2.0)	0.3-3.7			4 (1.6)	0.1-3.1		
Total	808	15 (1.9)	1-2.8			10 (1.2)	0.4-2		
Aborted animals									
Cow	18	6 (33)	55.1-11.5	0.91	≥0.05	7 (39)	61.5-16.5	0.81	≥0.05
Sheep	63	31 (64)	57.8-34.2			33 (49)	61- 37		
Goat	34	14 (41.2)	67-15.4			14 (41.2)	67-15.4		
Total	120	51 (42.5)	51.3-33.7			54 (45)	54 - 36		

vals. Chi-square (X^2) was uses to measure the differences in the prevalence between animal types, and between the two serological tests.

Results

The results of the brucella survey of milk samples collected from field animals are show in Table 1. Of 808 milk samples, examined 1.9% were positive for MRT and 1.2% were positive for the milk-ELISA test. the prevalence of *Brucella* in milk samples of cow, sheep, and goats was 0.8%, 2.6%, and 2% respectively with the Milk-Ring Test (MRT), and was 0.8%, 1.3%, and 1.6% in cow, sheep, and goats respectively with milk-ELISA test. No significant difference was observed in the prevalence rate of brucella between the animal by using MRT or by using the milk ELISA test.

Regarding milk samples that were collect from aborted animals, the prevalence rate of brucella was 33%, 64% and 41.2% with the MRT and was 39%, 49%, and 41.2% in cows, sheep and goats respectively using a milk-ELISA test. The overall prevalence was 42.5% using MRT and 45% using the milk-ELISA test (Table 1). For the pasteurized milk samples, all samples were negative for milk-ELISA test.

Concerning the bacteriological examination, from all 808 milk samples of field animals, one sample (0.1%) was giving a positive result for isolation of *Brucella* species, and that was in the sheep milk sample (0.3%of sheep milk). No isolation was found in milk samples of cows and goats (Table 2). On the other hand, of 120 milk samples collected from aborted animals, 11(9.2%) gave in a positive result for isolation of *Brucella* species, representing 5.6%, 10.3%, and 8.8% of cows, sheep, and goats respectively (Table 2). No isolation was found in pasteurized milk samples. For identification and determination of the type and biotype of *Brucella*, 12 isolates of *Brucella* were isolated. All isolates displayed characteristic smooth, transparent, and prominent colonies with full convex and rounded edges with a smooth and shiny surface and were pale yellow (honey color) under transmitted light and bright gray to bluish color in the reflected light. In gram staining, the isolates appeared as gram-negative coccobacilli arranged in single, paired or chains. all isolates showed agglutination with the specific sera of *Brucella* spp. The results of biochemical tests are not shown. The result of determining the type and biotype of the isolates is not shown. From the 12 subspecies of *Brucella* were isolated, two were *B. abortus biovar 1* isolated from cow and sheep, three (3) isolates were *B. abortus biovar 3* isolated from goats and sheep, two (2) isolates were *B. melitenesis biovar 2*, isolated from goats and sheep, and *five* (5) isolates *were B. melitenesis biovar3*, isolated from cow, goats, and sheep (Table 3). Comparing the results of the MRT and milk ELISA test in association with bacterial isolation, no significant difference was observed in the prevalence of brucella between the two tests, while the results of bacterial isolation were positive for the two tests. A similar agreement was show between the results of MRT and milk ELISA in milk samples collected from aborted animals.

The sensitivity of the Milk-Ring Test was 88%, 82%, and 84%, and Specificity were 99%, 98%, 99%, in milk samples of cow, sheep, and goat respectively, using the

Table 2. Prevalence of Brucella in milk specimens of aborted and non-aborted animals according to isolation.

Milk source		Number tested	Positive isolation				
			No.	(%)	95%CI	X^2	p-value
Non aborted animals	Cow	244	0	0.0	0	0.61	≥0.05
	Sheep	310	1	0.3	0.3-0.9		
	Goat	254	0	0.0	0		
	total	808	1	0.1	0.1-0.3		
Aborted animals	Cow	18	1	5.6	16.2- 5.1	0.39	≥0.05
	Sheep	63	7	10.3	17.5- 3.1		
	Goat	34	3	8.8	18.3- 0.7		
	total	120	11	9.2	14.4 - 4		

Table 3. Results of determine the type and biotype of the *Brucella* isolates.

Milk source		*Coagulation*		*Bacteriostatic days*					*Co_2 need*	*H_2S production*
				Basic fuchsin		Thionin				
		M	A	c	b	c	b	a		
Cow	Br.abortus biovar 1	-	+	+	+	-	-	-	+	+
Sheep	Br.abortus biovar 1	-	+	+	+	-	-	-	+	+
Sheep	Br.abortus biovar 3	-	+	+	+	+	+	+	+	+
Sheep	Br.abortus biovar 3	-	+	+	+	+	+	+	+	+
Goat	Br.abortus biovar 3	-	+	+	+	+	+	+	+	+
Sheep	Br.melitensis biovar 2	-	+	+	+	+	+	-	-	-
Goat	Br.melitensis biovar 2	-	+	+	+	+	+	-	-	-
Cow	Br.melitensis biovar 3	+	+	+	+	+	+	-	-	-
Sheep	Br.melitensis biovar 3	+	+	+	+	+	+	-	-	-
Sheep	Br.melitensis biovar 3	+	+	+	+	+	+	-	-	-
Goat	Br.melitensis biovar 3	+	+	+	+	+	+	-	-	-
Goat	Br.melitensis biovar 3	+	+	+	+	+	+	-	-	-

M=specific anti-sera *Br.Melitensis*; A= specific anti-sera *Br.abortus*; a=1:25000; b=1:50000; c= 1:100000

Table 4. Sensitivity and Specificity of MRT using the results of the milk ELISA.

Milk source	Milk ELISA	No.	MRT			
			Positive	Negative	Sensitivity, %	Specificity, %
Cow	Positive	9	7	2	88	99
	Negative	253	1	252		
Sheep	Positive	38	32	6	82	98
	Negative	340	7	333		
Goat	Positive	18	16	2	84	99

results of the milk ELISA test as a reference scale (Table 4).

Regarding antibiotic susceptibility, all isolates of *Brucella* were sensitive to Rifampicin, doxycycline, kanamycin, gentamicin, streptomycin, tetracycline, and ciprofloxacin and resistant to ampicillin, erythromycin, and novobiocin. The isolate of *B. abortus* came to be resistant to the carbenicillin, while the *B. melitenesis* were sensitive to it (Table 5).

Table 5. Antibiotic susceptibility patterns of Brucella spp. Isolated.

Antibiotic Agent	*Br.abortus*	*Br.melitensis*
Rifampicin	S	S
Doxycicline	S	S
Ampicillin	R	R
Erythromycin	R	R
Novobiocin	R	R
Kanamycin	S	S
Gentamicin	S	S
Streptomycin	S	S
Tetracycline	S	S
Ciprofloxacin	S	S
Carbenicillin	R	S

R=resistant and S=sensitive

Discussion

This is the first study in Yemen that used serological milk tests the determination the prevalence of brucella. Our results were close to the results recorded by the General Administration of Livestock Resources for the period 1992-1994 for cow (0.6%) while was higher than that recorded for sheep and goat 1.002%, 1.026% respectively, by using the Rose-bengal test (RBT) (GALR reports, 2007). Our results were higher than that recorded by Hosie *et al.* (1985) in blood serum samples of sheep and goats (0.6%, 0.4% respectively) by using RBTP, CFT, and SATs in Yemen. These tests are considered more sensitive but less specific than MRT (Aggad & Boukraa, 2006), also these tests are less sensitive than the ELISA test, as the sensitivity of direct milk-ELISA is 95% - 96.5% and specificity of 99% - 100% according to (Jalali *et al.,* 2003; Kerby *et al.,* 1997; Nielsen *et al.,* 1996; Kerkhofs *et al.,* 1990; Sutherhand *et al.,* 1986). In contrast, our results lower than that mentioned by (Kang'ethe *et al*., 2000) in raw milk which ranged from 3.4% to 3.9% with MRT, and 2.4-4.9% with a milk-ELISA test in Kenya, and also lower than that recorded cow's milk with MRT test (4.0%) by in Azerbaijan (Aliyev *et al*., 2022), Tunisia (Béjaoui *et al*., 2022), Egypt (Nofal *et al*., 2017), and in Algeria (Aggad & Boukraa, 2006). No significant difference was observed in the prevalence rate of brucella among the animal by using the Milk-Ring test or by using the Milk-ELISA test. In addition, no significant difference was observed in the prevalence of brucella between the two tests. Our result came in agreement with results reported by other authors (Aggad and Boukraa, 2006; Hunter and Allen, 1972; Nicoletti, 1969). These suggest that MRT test is reliable in diagnosis of brucella, from milk samples of cows, or milk samples of sheep and goats. The sensitivity and specificity of the milk-Ring Test were determined by using the results of the milk ELISA test as a reference scale, and this is in agreement with Aggad and Boukraa, 2006; Chand *et al.,* 2005.

In general, the Milk-Ring Test is one of the simpler and quicker tests that give an initial idea of the spread of the disease and considered low cost. It is characterized by rapid performance in identifying Brucella antibodies, despite the false positive results toward colostrum, especially or with cattle vaccinated against Brucella, as well as those suffering from mastitis (OIE, 2004; Bercovich and Moerman, 1979). The indirect ELISA test may sometimes give false positive results, especially in animals vaccinated with the live Brucella vaccine. To differentiate between vaccinated or naturally infected animals, a comparative competitive ELISA test (OIE, 2004) is used. However, vaccines against Brucella are lacking in Yemen. The Milk-Ring Test and ELISA test are more commonly uses in the diagnosis of brucella in animal flocks or in individual animals (OIE, 2002; 2004; USDA & APHIS, 1998; Alton, 1990). Moreover, the Milk-Ring Test (MRT) also used in the detection and determination of Brucella antibodies in milk from sheep and goats (Hamdy and Amin, 2002; Biancifiori *et al.,* 1996; Bercovich and Moerman, 1979). The milk-ELISA test is also uses in the detection of Brucella antibodies from sheep and goat milk (Sting and Ortmann, 2000; Biancifiori *et al.,* 1996). Milk tests are used in field surveys and in programs to control brucella because of their low costs, easiness, and the result ca be obtained in a shorter period (Ning *et al*., 2013: Corbel 2006; Alton, 1975; 1990).

Regarding aborted animal's milk samples, the results were higher than that recorded in several governorates in Yemen by the veterinary laboratory of the General Administration of Livestock for the year 1990 (12.2%, 13%, 28%) in aborted cows, sheep and goats respectively, and higher than that recorded by the General Administration in Sana'a, Dhamar, and Amran governorates for the year 1998 in cows and sheep (0%, 12.19% respectively) and lower than that in goats (57.14%) also closely agreement with molecular detection of brucella in Bangladesh milk from cows (Islam *et al*., 2018). The higher prevalence may be attributed to the fact that the preventive measures are not fully applied and the introduction of animals from infected areas from inside and outside the country is not strictly controlled. Aborted animals often excrete the *Brucella* in their secretions for two to three months (Corbel, 2006; OIE, 2004). The udder is a very important

predilection site for *Brucella*, and the persistent infection of the udder is accompanied by a constant or intermittent shedding of the organisms in the milk (Corbel, 2006). It provides an important source of infection for humans and young animals. as revealed in a survey study conducted in the Human Public Health Laboratory in Sana'a by Al-Shamahy *et al.* (2000). The prevalence of the *Brucella* in milk of aborted cow, sheep and goats in our study was lower than that recorded in Egypt by Milk-Ring Test in the milk of aborted cows, sheep, and goats (67%, 76%, 83% respectively) (Hamdy and Amin, 2002), and less than that recorded in aborted goats (59%) in India (Gupta *et al*. 2006), while it agrees with that reported in Turkey in aborted cow, (33-34.6%) by Otlu *et al.* (2008). The prevalence rate obtained in this study as well as that recorded by other studies did not reach 100% in the aborted animals, this may be attributed to some viral and bacterial and protozoa diseases that may cause abortion similar to *Brucella* (Al Mubarak, 1996), in addition to physical or chemical factors.

Regarding rate of isolation (9.2%) in milk samples collected from aborted animals the results were lower than that recorded in Egypt by Hamdy and Amin (2002) in the milk sample of cow, sheep and goats infected with brucella (46.2%, 57% and 61% respectively), and this may be attributed to the fact that they collected their samples from recently aborted cases, where the isolation are high, especially in the first three weeks after the abortion. Our results were less than that recorded in Jordan (16.5%) for total cow, sheep and goats by Aldomy *et al.* (1992). The results on this study showed that the Milk-Ring Test and the ELISA test were equal in the determining the isolation rate. All positive isolate samples gave positive results by the Milk-Ring Test and did not give false identification results for the isolate. Our findings came in agreement with Hamdy and Amin (2002) who indicated that the MRT test is the best in the determination of isolate from milk compared to the serological tests for blood samples (RBT& SAT). They also attributed the fact that the MRT test is characterized by its high quality in identifying IgM antibodies more than the previous tests. It is well known that Brucella is slow-growing organisms, and therefore special culture media was used, and blood serum was added to it at a rate of (5%) to improve the isolation of Brucella and also to ensure the success of isolation and this in agree with (Ferreira, 2003), Also biochemical tests were used to ensure the typing of Brucella and to differentiate it from some Gram-negative bacteria that may be similar to them in some growth properties (Alton *et al*., 1988). The *Brucella* have not been, previously isolated nor has the determination of their types from field animals in Yemen. In this study, the isolation of *Brucella* from raw milk was *Brucella abortus biovar 1*, which was isolated from cow and sheep, and *Brucella abortus biovar 3*, from sheep and goats, and *Brucella melitenesis biovar 2*, from sheep and goats. Brucella *melitenesis biovar 3* was isolated from cattle, sheep and goats. These results confirmed that sheep and goats were infected with B.abortus, where cattle is considered the main source. This was in agreement with Corbel, (2006) who attributed it to their contact with cows previously infected with *Br.abortus* . By comparing our results with some Arab countries, the *Brucella abortus biovar 1* was isolated in Egypt (Refi, 2002), and recorded in cow's milk by Hamdy and Amin (2002). Also, *Brucella abortus biovar 1* and 3 were recorded in Iraq from milk products of field animals and camels (Ali, 1998; Hadad *et al.,* 1997) and in Algeria from cow's milk (Aggad & Boukraa 2006).

In this study, *Brucella melitenesis* isolates were isolated in high percentage from sheep and goats, as well as from cow, and this is in agreement with (Corbel. 2006), Our results agree with the results recorded in Saudi Arabia for *Brucella melitenesis biovar 2* and 3, from camel milk by Radwan *et al.* (1995) who attributed the infection of the camel to the grazing and mixing with infected sheep and goats.

Regarding in vitro antibiotic sensitivity test the study showed the sensitivity of all isolates of Brucella to the antibiotics Rifampicin, doxycycline, kanamycin, gentamicin, streptomycin, tetracycline, and ciprofloxacin, and this is in agreement with (Al-Dahouk *et al.,* 2005; Bodur *et al.,* 2003; Hadad *et al.,* 1997). On the other hand, the results showed that brucella isolates were resistant to ampicillin, erythromycin, and novobiocin, and this is in agreement with the results reported by Hadad *et al.,* 1997; Corbel, 1989). Our results also showed that *Brucella abortus* isolates are resistant to the antibiotic carbenicillin, while the *B.melitenesis* were sensitivity to it, and this is consistent with Corbel (1989), and agreed with Hadad *et al.* (1997) who reported that *Brucella melitenesis* isolates are sensitive to carbenicillin. These antibiotics have been used in many studies to differential between the types and strains of *Brucella*, and the effect of these antibiotics can be different on types as well as between strains within the type, and this is consistent with that reported by European Commission (2001) and Corbel, (1989). The negative results of erythromycin against the isolates in this study may be attributed to its concentrations. Turkmani *et al.* (2006) and Yamazhan *et al.* (2005), or due to their selective effect on some *Brucella* strains (European Commission, 2001).

Conclusions

The high prevalence of milk brucella especially in aborted animals needs focusing and build controlling strategies plans to decrease losses to the economy and avoid transferred to humans with unpasteurized milk consumption.

References

Aggad H, Boukraa L, 2006. Prevalence of bovine and human brucella in western Algeria: comparison of screening tests. East Mediterr Health J 12:119–128.

Al Dahouk S, Nöckler K, Tomaso H, Splettstoesser WD, Jungersen G, Riber U, Petry T, Hoffmann D, Scholz HC, Hensel A, Neubauer H, 2005. Seroprevalence of brucella, tularemia, and yersiniosis in wild boars (Sus scrofa) from North-Eastern Germany. J Vet Med B 52:444–455.

Aldomy FMM, Jahans KL, Altarazi YH, 1992. Isolation of Brucella melitensis from aborting ruminants in Jordan. J Comp Pathol 107:239–242.

Ali SAA, 1998. Bacterial and serological study on brucella in camels in Iraq - M.sc. Thesis, Faculty of Veterinary Medicine - Baghdad University.

Aliyev J, Alakbarova M, Garayusifova A, Omarov A, Aliyeva S, Fretin D, Godfroid J., 2022. Identification and molecular characterization of Brucella abortus and Brucella melitensis isolated from milk in cattle in Azerbaijan. BMC Vet Res 18:1-9.

Al-Shamahy HA, Whitty CJ, Wright SG, 2000. Risk factors for human brucella in Yemen: a case control study. Epidemiol Infect 125:309–313.

Alton G., 1990. Brucella melitensis. "Animal brucella". (K. Nielsen and J.R.Duncan, eds.). CRC Press Inc. Boston: 383-409.

Alton GG, Jones LM, Perrz DF, 1975. Laboratory techniques in brucellosis 2nd.ed. World Health Organization, Monograph Series 55, Geneva, Switzerland.

Alton GG, Jones LM, Angus RD, Verger JM, 1988. Techniques for the brucellosis laboratory. INRA, Paris.

Bauer AW, Kirby WM, Sherris JC, Turk M, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Path 45: 493-496.

Béjaoui A, Ben Abdallah I, Maaroufi A,

2022. Brucella spp. Contamination in Artisanal Unpasteurized Dairy Products: An Emerging Foodborne Threat in Tunisia. Foods 11:2269.

Bercovich Z, Moerman A, 1979. Non-specific positive Milk-Ring Test (s) in tank milk and Estrumater in the treatment of cattle. Tijdschrift voor Diergeneeskunde 104:713-716.

Biancifiori F, Nannini D, Di Matteo A, Belfiore P, 1996. Assessment of an indirect ELISA in milk for the diagnosis of ovine brucellosis. Comp Iramun Microbiol Infect Dis 19: 17-24.

Bodur H, Erbay A, Akinci E, Aylin C, Mustafa AC, Balaban N, 2003. Neurobrucellosis in an endemic area of brucellosis. Scand J Infect Dis. 35:94–97.

Chand P, Rajpurohit AK, Malhotra AK, Poonia JS, 2005. Comparison of milk-ELISA and serum-ELISA for the diagnosis of Brucella melitensis infection in sheep milk. Vet Microbiol 108:305–311.

Commonwealth Department of Health National Biological Standard Laboratory, 1984. Standard for brucella abortus Milk-Ring Test antigen, Therapeutic Goods Act 1966, Order No. 10. Canberra. Australian.

Corbel M. J., 2006. Food and Agriculture Organization of the United Nations, World Health organization, World Organization for Animal Health. Brucellosis in humans and animals. Geneva: World Health Organization.

Corbel M.J. 1989. Microbiology of the genus of the brucella. (eds. E. Young and M. Corbel). In Brucellosis: Clinical and laboratory aspects (eds. E. Young and M. Corbel). CRC Press, Boca Raton, Fl:53-67.

Corbel M.J. 2006. Joint WHO -FAO-OIE. Brucellosis in humans and animals. WHO/CDS/EPR/2006.7.

Cowan S.T. 1993. Cowan and Steel's Manual for the Identification of Medical bacteria G I Barrow and R K A Feltham, (des). 4th ed. Cambridge University press, Cambridge. USA, pp. 136-137.

European Commission. 2001. Brucellosis in sheep and goats (*Brucella melitensis*). Report of the Scientific Committee on Animal Health and Welfare of the European Commission.

FAO, 2003. Guidelines for coordinated human and animal brucellosis surveillance. Rome, Italy, FAO. (Animal Production and Health Paper No 156).

FAO/WHO, 1986. Joint FAO/Who expert committee on brucellosis. 6th report, World Health Organization Tech. Rep., No. 740, WHO, Geneva, Switzerland

Ferreira Ana Cristina, Cardoso Regina, Dias Travassos Isabel, Mariano Isabel, Belo Ana, Preto Isabel Rolão, Manteigas António, Fonseca António Pina, Corrêa Maria Inácia De Sá. 2003. Evaluation of a modified Rose Bengal test and an indirect Enzyme-Linked Immunosorbent Assay for the diagnosis of Brucella melitensis infection in sheep. Vet. Res. 34, 297–305.

General Administration of Livestock (GALR). 2007. Annual reports of the General Administration of Livestock during the period from 1974 – 2006 for the disease of endemic abortion. Ministry of Agriculture and Water Resources - Republic of Yemen.

Gupta VK, Verma DK, Rout PK, Singh SV, Vihan VS, 2006. Polymerase chain reaction (PCR) for detection of Brucella melitensis in goat milk. Small Rum Res 65 :79–84.

Hadad JJ, Hammed DA, Alaboudi AR, 1997. Isolation of brucella strains from dairy products in Ninevah province, Iraq. Iraqi J Vet Sci 10:39-44.

Hamdy MER, Amin AS, 2002. Detection of Brucella species in the milk of infected cattle, sheep, goats and camels by PCR. Vet J 163:299-305.

Hobbs IF, 1985. Comparison of the indirect enzyme Linked Immuno-sorbent Assays (ELISA) with the Complement fixation test (CFT) for the serodiagnosis of bovine brucellosis. NZ Vet J 33:112-116.

Hosie BD, Al-Bakri OM, Futter RJ, 1985. Survey of Brucellosis in goats and sheep in the Yemen Arab Republic: Comparison of tests for Brucella melitensis infection in sheep. Trop Anim Health Prod 17:93-9.

Hunter D, Allen J, 1972. An evaluation of milk and blood tests used to diagnose brucellosis. Vet. Rec. 91: 310.

Islam M, Khatun M, Saha S, Basir MD, Hasan MM, 2018. Molecular detection of Brucella spp. from milk of seronegative cows from some selected area in Bangladesh. J Pathogens, 2018.

Jalali A, Hemmatzadeh F, Momtaz H, Nilsson E, 2003. Elisa as an alternative method for the diagnosis of brucellosis in sheep. the 56th Brucellosis Research Conference. Sept. 15-17. University of Navarra, Pamplona (Spain).

Kang'ethe EK, Arimi SM, Omore AO, McDermott JJ, Nduhiu JG, Macharia JK, Githua A, 2000. The Prevalence of antibo-dies to brucella abortus in marketed milk in Kenya and its public health implications. Paper prepared for oral presentation at the 3P[rdP] all Africa Conference on Animal Agriculture. 6 – 9 november 2000.

Kerby PJ, Quiroga JL, McGrane JJ, Stagg DD, 1997. Field evaluation of an indirect ELISA for detection of brucellosis in lowland Bolivia. Trop Anim Hlth Prod 29:65-72.

Kerkhofs P, Botton Y, Thiange P, Dekeyser P, Limetb JN, 1990. Diagnosis of bovine brucellosis by Enzyme immunoassay of milk. Vet Microbiol 24:73-80.

Kumar VN, Bharathi MV, Porteen K, 2017. Risk factors associated with prevalence of bovine brucellosis in milk from Tamil Nadu, India. Int J Curr Microbiol Appl Sci 6:2604-9.

Mubarak A, 1996. Animal diseases and prevention and control measures - Omar Al-Mukhtar University - Libya.

Negrón ME, Kharod GA, Bower WA, Walke H, 2019. Notes from the field: human Brucella abortus RB51 infections caused by consumption of unpasteurized domestic dairy products - United States, 2017–2019. Morbidity and Mortality Weekly Report 68:185.

Neilsen K, Smith P, Gall D, Perez B, Cosma C, Muller P, Trottier J, Cote G, Boag L, Bosse J, 1996. Development and validation of an indirect enzyme immunoassay for detection of antibody to Brucela arbotus in milk. Vet Microbiol 52:165-73.

Nichols P, 1991. Social survey methods: a field guide for development workers.No.6. series editor Brian Pratt. Oxfam, Oxford.

Nicoletti P, 1969. Further evaluation of serologic tests procedures used to diagnose brucellosis. Am J Vet Res 30: 1811.

Ning P, Guo M, Guo K, Xu L, Ren M, Cheng Y, Zhang Y, 2013. Identification and effect decomposition of risk factors for Brucella contamination of raw whole milk in China. PLoS One 8e68230.

Nofal AS, El-Leboudy AA, El-Makarem HSA, El-Rahman AHA, 2017. Prevalence of Brucella Organism in Milk and Serum Samples of Some Lactating Dairy Animals. Alexandria J Vet Sci 55.

OIE, 2002. OIE Animal Health Code. Also available online at: www.oie.int.

OIE, 2004. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 5th Ed. Paris, France.

Otlu S, Sahin M, Atabay HI, Unver A, 2008. Serological Investigations of Brucellosis in Cattle, Farmers and Veterinarians in the Kars District of Turkey. Acta Vet Brno 77:117–21.

Pfeiffer DU, 2002. Veterinary Epidemiology – An Introduction. Epidemiology division department of veterinary clinical sciences, The Royal Veterinary College, University of London UK.

Poester FP, Samartino LE, Santos RL, 2013. Pathogenesis and pathobiology of brucellosis in livestock. Rev Sci Tech 32:105–15.

Radwan AI, Bekairi SI, Mukayel AA, Al-Bokmy AM, Prasad PV, Azar FN, Coloyan ER, 1995. Control of Brucella melitensis infection in a large camel herd in Saudi Arabia using antibiotherapy and vaccination with Rev.1 vaccine. Rev Sci Tech 14:719–32.

Refai M, 2002. incidence and control of brucellosis in the near east region. Vet Microbiol, 90:81-110.

Sting R, Ortmann G, 2000. Experience with a simple ELISA for immunodiagnosis of Brucella in cattle, sheep and goats. Berliner Munchener Tierrztliche Wochenschrift 113:22-8.

Sutherland SS, Evans RJ, Bathgate J, 1986. Application of an enzyme linked immunosorbent assay in the final stages of a bovine brucellosis eradication program. Aust Vet J 63:412-5.

Sutra L, Caffin JP, Dubray G, 1986. Role of immuno-globulins in the Brucella Milk-Ring Test. Vet Microbiol 12:359–66.

Turkmani A, Ioannidis A, Christidou A, Psaroulaki A, Loukaides F, Tselentis Y, 2006. In vitro susceptibilities of Brucella melitensis isolates to eleven antibiotics. Ann Clin Microbiol Antimicrob 2;5:24.

USDA and APHIS. 1998. Brucellosis Eradication: Uniform Methods and Rules. USDA-APHIS, Washington D.C.

Wainaina M, Aboge GO, Omwenga I, Ngaywa C, Ngwili N, Kiara H, ... Bett B, 2020. Detection of Brucella spp. in raw milk from various livestock species raised under pastoral production systems in Isiolo and Marsabit counties, northern Kenya. Trop Anim Health Prod 52:3537-44.

Wasseif SM, 1992. Brucellosis in Sharkia governorate – an epidemiological study. Egypt J Occup Med 10: 247-58.

WHO, 2005. Brucellosis in humans and animals. WHO guidance, Geneva.

Yamazhan T, Aydemir S, Tünger A, Serter D, Gökengin D, 2005. In vitro Activities of Various Antimicrobials against Brucella melitensis Strains in the Aegean Region in Turkey. Med Princ Pract 14:413–6.