

Original Article

Human and animal brucellosis in the Sultanate of Oman: an epidemiological study

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Abstract

Introduction: Brucellosis is an infectious disease caused by direct contact with infected animals or animal products contaminated with *Brucella*. *Brucella* is a Gram-negative aerobic coccobacillus that infects different types of animals and is considered to be an important zoonotic disease. **Methodology:** *Brucella* were isolated from blood samples and identified following biochemical tests and agglutination with A and M monospecific antisera. Furthermore, *Brucella* antibody titers of the tested sera were obtained by the microtiter agglutination method (MAM). **Results:** The main *Brucella* species isolated in Oman was *B. melitensis*. However, in countries bordering Oman and their neighboring countries, both *B. melitensis* and *B. abortus* have been isolated and identified. A total of 412 human patients with suspected cases of brucellosis were admitted to the Department of Communicable Disease Surveillance and Control in the Dhofar Governorate for diagnosis and treatment. During the year 2015, a total of 343 human cases were positively diagnosed with brucellosis in the Dhofar Governorate. During the years 2015 to 2019, a total of 10,492 animals were examined in different Governorates of Oman for brucellosis. The results indicated that 1161 (11%) animals were serologically positive for brucellosis.

Conclusions: The results of this study confirmed that *Brucella melitensis* is the main species responsible for human brucellosis in Oman. It was not surprising that the Dhofar Governorate exhibited a high percentage of infected patients since it is culturally acceptable to drink raw camel milk (unpasteurized), unlike cow milk which is pasteurized.

Key words: brucellosis; zoonosis; infectious diseases; Oman.

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Introduction

The farming of sheep, goats, camels, and cattle has increased significantly during the last 30 years due to the significant increase in demand for animal milk and meat. Brucellosis is the main disease that affects the production of farm animals [1]. *Brucella*, the causative agent of brucellosis, is a Gram-negative aerobic coccobacillus that infects different types of animals [2-5]. Ten species of *Brucella* have so far been isolated and identified. These include *B. melitensis*, *B. abortus*, *B. suis*, *B. canis*, *B. neotomae*, *B. ovis*, *B. ceti*, *B. pinnipedialis*, *B. microti*, and *B. inopinata* [5]. However, only five species, *B. melitensis*, *B. abortus*, *B. suis*, *B. canis*, and *B. neotomae* have been reported to infect humans [5, 6].

The main reported cause of brucellosis in humans and animals in the Sultanate of Oman is *B. melitensis* [7,8]. However, both *B. melitensis* and *B. abortus* were reported as the main causes of brucellosis in other neighboring countries [2,4,8,9].

Available literature indicates that brucellosis affects various domestic, wild and marine animals. The most susceptible domestic animals to *B. melitensis* and *B. abortus* are sheep, goats, camels, and cattle [10-15]. The disease is transmitted from infected animals to humans through human ingestion of contaminated milk and milk products, by close contact with infected animals, and by handling material containing infected animal products [16].

Epidemiological studies on the distribution of brucellosis among humans and animals showed a steady, gradual increase in incidence rates in many parts of the world, especially the Mediterranean regions, parts of Asian and African continental areas, Eastern Europe, and Latin America [17].

The Bedouins are known to consume fresh non-pasteurized camel milk, that may harbor *B. melitensis*, thus leading to the spread of this disease to humans [9].

This study aims to find the seroprevalence of human and animal brucellosis in Oman between 2015-2019, to

identify the causative species of *Brucella*, and to develop a strategic plan for controlling brucellosis in the Sultanate of Oman.

Methodology

Descriptive epidemiological data

The data on human brucellosis in the Sultanate of Oman was based on patients with clinical signs of brucellosis referred to the local hospitals for diagnosis and treatment. The data were collected prospectively for 5 years. Diagnosis of brucellosis was based on clinical examination, bacteriological isolation, and serological results. During 2015, a total of 412 human patients were hospitalized due to a high risk of brucellosis. These patients had a history of being in contact with animals and/or had consumed non-pasteurized animal milk and milk products. They were referred to the Department of Communicable Disease Surveillance and Control in eight provinces in the Dhofar Governorate for diagnosis and treatment. All the patients who had clinical signs of suspected brucellosis were referred to hospitals for treatment. In addition, we obtained data from patients who had been admitted for treatment of brucellosis at local hospitals in the Muscat province between the years 2015-2019.

Microbiological analysis

Brucella was isolated from blood samples and/or fluid from local abscesses cultured on blood agar (Difco™, MD, USA), potato-dextrose agar (Difco™, MD, USA), and trypticase soy agar (Difco™, MD, USA). The plates were incubated at 37 °C in a CO₂ incubator for 24-72 hours. Growth was examined macroscopically and microscopically. The isolated *Brucella* was identified by colony morphology, Gram stain, biochemical tests, and agglutination with A and M monospecific antisera, according to the method described by Corbel *et al.* [2].

Brucella melitensis and *Brucella abortus* antibody titers of the tested sera were obtained by the microtiter agglutination method (MAM), which is considered one of the most widely accepted screening tests in human brucellosis. The stained *B. melitensis* (M antigen) and *B. abortus* (A antigen) suspensions were obtained from Welcome Research Laboratories (Beckenham, England), and the final dilutions of the sera were tested between 1:20 and 1:320. The titer used was the highest dilution that showed clear agglutination.

Veterinary data

To evaluate the seroprevalence of animal brucellosis in different farms in Oman, blood samples

were collected from 5123 goats, 4560 sheep, 551 cattle and 1646 camels, which represent 35 goat herds, 35 sheep flocks, 12 cattle herds and 35 camel herds. These blood samples were examined for brucellosis between 2015 to 2019. The animals were located in over seven governorates in the Sultanate of Oman. A history of the management system, abortion, and brucellosis vaccination program was recorded. Herd and flock sizes were between 20 to 400 animals. Five herds of goats, and five flocks of sheep had a history of abortion. Ten herds of goats and sheep were classified as large (over 200 animals), 10 herds as medium (50-200), and 15 herds as small (less than 50). Random blood samples were collected by using disposable syringes. Each animal was sampled once only during the study. The sera were stored at -80 °C until use. Data on the total animal population in the Sultanate of Oman and the percentage of vaccination against brucellosis were obtained from the Ministry of Agriculture, Fisheries, and Water Resources [18].

Serological testing

The Rose Bengal test (RB) and enzyme-linked immunosorbent assay (ELISA) were conducted on all sera collected from animals as described by Alton *et al.* [19]. The RB test was performed with the standard *Brucella* antigen (Diagnostic Lab, Becton, Dickinson and Company, NJ, USA). The indirect ELISA method utilized anti-*Brucella* IgG antibodies using kits (Jordan Bio Industry Centre (JOVAC), Amman, Jordan). S-19 antigen, positive and negative control sera were used to standardize the test. A reading of 0.32 or less was regarded as negative. In the case of human sera, a total of 40 IU per mL by the Tube Agglutination test (TA) was considered positive based on the recommendation by the World Health Organization [20].

Statistical analysis

The epidemiological background and clinical presentations were analyzed with regard to provinces, age, and gender. The data are presented as percentages.

Results

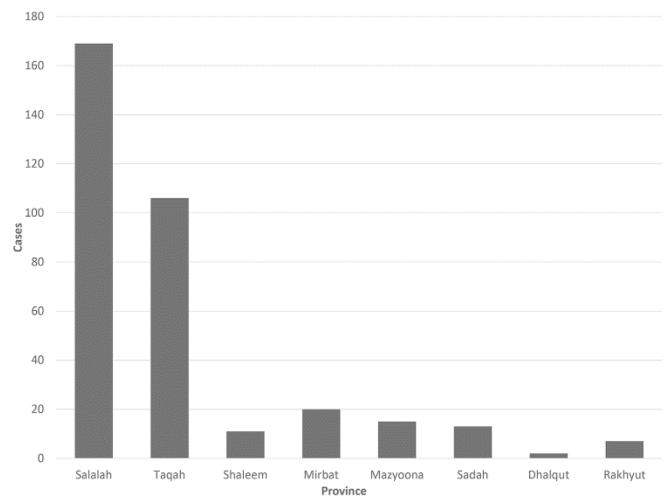
Human Brucellosis

During the year 2015, a total of 350 (343 from Dofar and 7 from Sultan Qaboos University Hospital [SQU]) human patients with clinical signs of brucellosis were referred to the Communicable Disease Surveillance and Control Department, Ministry of Health, Dhofar Governorate (Figure 1). Patient history indicated that consumption of unpasteurized raw animal milk and milk products was responsible for brucellosis

in 63% of the cases. 83% of patients had direct contact with animals, mainly camels, cattle, sheep, and goats. However, 5.6% of the brucellosis-positive cases had not ingested raw milk or come in direct contact with infected animals. All the patients with clinical signs of brucellosis were referred to local healthcare centers for diagnosis and treatment. *Brucella* was isolated from 7 patients and they had all the clinical signs of brucellosis including fever, malaise, body ache, sweats, and arthralgia. 91% of the patients had fever making it the most common presenting feature. The patients were divided into two groups based on their clinical symptoms. Group one had severe clinical signs characterized by fever, headache, and arthritis and included 5 (71.4%) of the patients in which *Brucella* species were isolated from the blood. Group two included two patients (28.6%) with no severe symptoms except enlargement and abscessation of lymph nodes on the axillary, neck and/or shoulder areas. One of these patients was a 38-year-old man presenting with an enlarged red swelling in the neck area. Physical examination indicated unilateral right-side lymphadenopathy that was positive for the serological *Brucella* test.

With regards to the incidence of brucellosis in eight provinces of the Dhofar Governorate, patients from Salalah province had the highest incidence (169 cases), followed by Taqah province (106 cases), and Dhalqut province (2 cases) (Figure 1). Among the 343 patients who were positive for brucellosis, 273 were male and 70 were female. In addition, 21.5% of the 343 patients were in the age group of 2 -10 years old, 50.1% were 11-30 years old, 25% were 31-50 years old, and 3.5% were ≥ 51 years old (Figure 2). Analysis of human seroprevalence of brucellosis by month indicated that

Figure 1. Human brucellosis by province reported by the Directorate General of Health Services, Dhofar Governorate, Department of Communicable Disease Surveillance and Control, 2015.



brucellosis was reported during the 12 months of the year, with the highest incidence rate between March and October (Figure 3). This could be due to the fact that camel milk is available in large quantities during these months and people drink fresh camel milk without pasteurization due to belief in traditional medicinal uses.

Animal brucellosis

The total number of goats, sheep, cattle, and camels examined for brucellosis in the Sultanate of Oman during the years 2015 to 2019 was 10,492 animals, of which 1161 (11%) animals were serologically positive for brucellosis (Table 1). In the Dhofar Governorate, serological testing of animals for brucellosis during the

Figure 2. Human brucellosis by age.

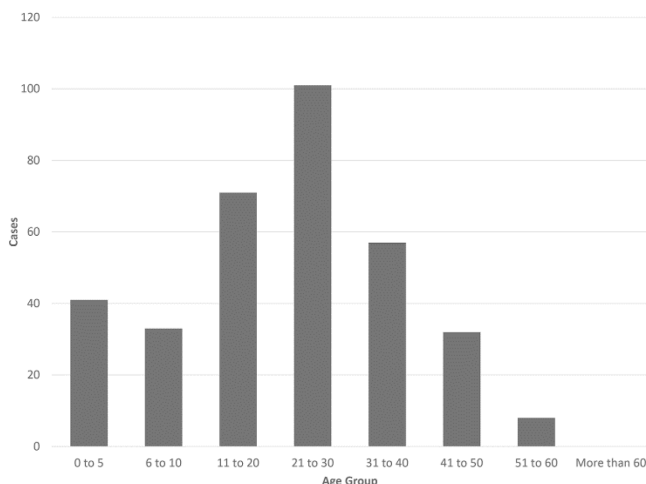


Figure 3. Incidence of human brucellosis by month.

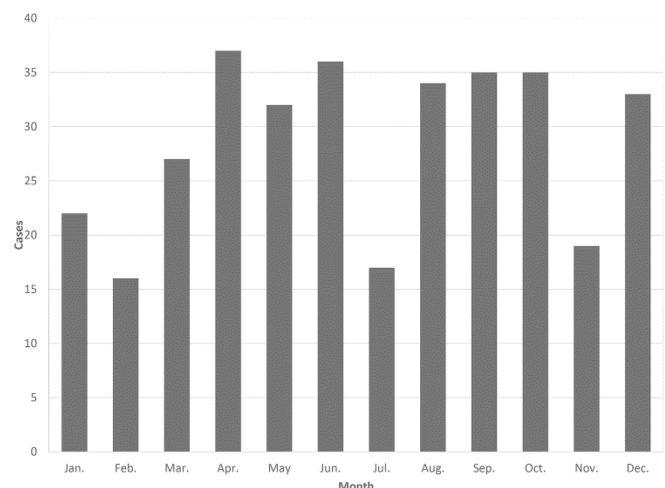


Table 1. The number of animals examined serologically for brucellosis and the number of positive results in percentage.

Governorate	Camel			Sheep			Goats			Cattle			All Animals examined + Positive + %
	Number examined	Number Positive	%	Number examined	Number Positive	%	Number examined	Number Positive	%	Number examined	Number Positive	%	
All Governorates	1646	336	20.4	4560	610	13.4	5123	421	8.2	551	37	6.7	11880 1404 11.8%
Muscat	141	44	13.2	50	4	8	50	4	8	121	6	4.9	
Dhofar	188	31	16.4	1000	153	15.3	750	180	24	80	4	5	
Batinah North and South	85	5	5.8	2200	341	15.1	2501	115	4.6	188	16	8.5	
Dakhiliyah	297	70	2.4	81	6	7.4	358	30	8.4	90	4	4.4	
Dhahira	41	7	17	488	16	3.3	806	42	5.2	45	5	11.1	
Sharqiyah North and South	664	147	11.2	730	88	12	380	29	7.6	20	2	10	
Wusta	230	32	13.9	11	2	7.5	278	21	7.5	7	0	0.0	

year 2015 indicated a seroprevalence rate of 20.9% in goats, 14.9% in sheep, and 9.2% in camels (Table 2).

This study used ELISA as the serological test to detect antibodies against *Brucella* in 11,880 blood samples collected from camels, sheep, goats, and cattle during the years 2015 to 2019. The results of the serological testing are shown in Tables 1 and 2. Cattle, camels, sheep, and goats showed high incidence rates (between 6.6% to 23.5%) during the years 2015 to 2019 in different Governorates of Oman.

Discussion

In 2004, Oman had approximately 1,543,707 goats, 357,621 sheep, 121,054 camels and 303,831 cattle [18]. Brucellosis is a widespread disease and of major economic importance in most countries around the world [21-25]. Reported cases of brucellosis among people and animals in Oman are high [26-29]. This is,

in part, because of the traditional feeding management system of animals practiced by animal owners that allows goats, sheep, cattle, and camels to graze on the same land during the daytime. Moreover, Oman shares borders with Yemen, Saudi Arabia, and the United Arab Emirates where a high incidence of brucellosis in animals was reported [30-32]. Ismaily *et al.* postulated that herding goats infected with brucellosis along with camels increases the chances of camels becoming infected and since *Brucella* is excreted in camel milk, and can cause subsequent human infections [33]. Moreover, animals belonging to many animal owners may graze in the same pasture, potentially spreading the organism between different farms. In addition, it has been postulated that infection occurs in animals of all ages but persists most commonly in sexually mature animals [34]. Furthermore, most of the animals abort in pastures. If the cause of abortion is the *Brucella*,

Table 2. Serological results in the percentage of positive cases of human and animal brucellosis in the Sultanate of Oman from 2015-2019. Data are presented as the total number of sera examined and the percentage of positive cases.

Year	Human	Camels	Sheep	Goats	Cattle
2015	412 (83.3%)	388	650	650	-
		36	97	136	-
		9.2%	14.9%	20.9%	-
2016	-	375	1388	2546	332
		45	101	114	12
		12%	7.3%	4.8%	3.6%
2017	-	331	828	985	122
		59	94	76	13
		17.8%	11.3%	7.7%	10.7%
2018	-	255	780	418	36
		36	182	48	4
		14.1%	23.3%	11.5%	11%
2019	9 77.7%	297	913	704	81
		70	136	47	6
		23.5%	14.9%	6.6%	7.4%
Total	421	1646	4559	5123	551
Positive Percentage	350 (83.1%)	246 14.9%	610 13.4%	412 8%	35 6.4%

contaminated placental and vaginal discharges of infected animals can spread the disease. As a result, other susceptible flocks and herds that graze in the same pastures can get infected. Infection of fetuses may occur in utero and may remain latent in newborns throughout their early life. These animals may remain serologically negative until their first parturition, at which time they may begin to shed *Brucella* and continuously contaminate the pastures [35]. Finally, *Brucella* is excreted into the milk of infected animals, without obvious signs of mastitis, and this increases the risk of human infection.

Brucellosis caused by *B. melitensis* is a highly infectious disease of domestic animals causing serious zoonosis worldwide [26]. It is regarded as an important re-emerging communicable disease in the Middle East and Mediterranean countries [8]. The prevalence varies considerably between animal herds and flocks, between areas, and between countries [2,36-37]. In Oman, positive cases of brucellosis in goats were first reported in 1979 in the Dhofar Governorate, located in the southern part of Oman on the border with Yemen (37). A few years later, Ismaily *et al.* reported a high incidence of brucellosis in goats, sheep, camels, and cattle [33]. Our data found 343 positive cases of brucellosis in different wilayats of the Dhofar Governorate during the year 2015. Traditionally, people consume camel milk unpasteurized. Moreover, camel milk is available all over the year directly from the camel owners, and as part of their hospitality, they encourage their guests to drink fresh milk, which is also believed to have medicinal properties against diabetes and cancer. Previous data reported by the Ministry of Health on human brucellosis in different wilayats in the Dhofar Governorate identified positive cases per 10,000 population: 305 cases in 1998, 309 in 1999, 302 in 2000, and 159 in 2001 [6]. Such a high incidence rate is associated with the high incidence rate of brucellosis in animals. This is partly explained because people consume non-pasteurized raw animal milk and milk products, have direct contact with infected animals and their secretions, and use a poor management system due to which animals are prone to infection. In Jordan, a retrospective study recorded 68 children with clinical signs of brucellosis in which 58.2% of the patients had consumed unpasteurized milk and dairy products [38]. The impact of brucellosis on camels, sheep, and goats is greater in terms of the adverse effects it may have on human health in the rural population of Oman due to the traditional mode of consumption of non-pasturized raw milk, and milk products of camels, sheep, and goat, especially because these animals harbor the *B.*

melitensis with no clinical signs [1,26,27,39-41]. The situation is serious because people in rural areas believe that drinking fresh raw milk has anti-diabetic and anti-cancer effects. This will make control of this zoonotic disease more critical and potentially cause high incidence rates on a yearly basis.

Diagnosis of brucellosis is mainly dependent on the isolation of *Brucella* and serological testing. In Oman, serological testing is the main method of diagnosing brucellosis. The Rose Bengal plate agglutination, serum tube agglutination, and ELISA tests are usually recommended for screening herds and individual animals. In recent years, ELISA has been used as a diagnostic test for brucellosis in living animals and has replaced the other serological diagnostic tests. In addition, Polymerase Chain Reaction (PCR) has been applied successfully on blood and lymph samples from living animals as a diagnostic test for brucellosis [42]. Gene probes and PCR have also been used successfully as effective typing methods for *Brucella* [43].

Many countries have successfully eradicated brucellosis from their animals. For example, Australia planned a successful eradication program to control brucellosis due to *B. abortus* [44]. In recent years, most of the published data indicated that *Brucella melitensis* has been the most common cause of brucellosis in camels, goats, and sheep [14,45-48]. In Oman, despite vaccination campaigns for sheep and goats with a reduced dose of Rev-1 strain, containing 1×10^3 colony-forming units of *B. melitensis*, brucellosis remains the principal cause of human and animal brucellosis with a high incidence rate [1,16,27,28]. An experimental study on the efficacy of the reduced Rev-1 vaccine in protecting sheep from brucellosis was done in Jordan [17]. The results indicated that sheep vaccinated with the Rev-1 vaccine are still susceptible to *B. melitensis* infection. The results of the experiment clearly indicated the low efficiency of the reduced-dose Rev-1 vaccine in protecting sheep and goats against brucellosis. Furthermore, the vaccine strain of *B. melitensis* was isolated from 3.4% of the aborted sheep fetuses [46]. Due to these results, it was recommended to stop vaccinating sexually mature animals with the reduced Rev-1 vaccine [17]. Thus, it is highly recommended to use the full dose Rev-1 vaccine that contains 1×10^9 CFU for all female sheep and goats at 3-6 months old and female camels at 6 months of age. For cattle, it is recommended to use a full dose of the S-19 *B. abortus* vaccine for female calves at 3-6 months age. Vaccines for humans against brucellosis are not yet available [49]. However, applying hygienic measures to limit human exposure to infected animals, consuming

only pasteurized milk and milk products, vaccinating animals, and the detection of brucellosis by serological tests are highly recommended as principal approaches to control brucellosis in Oman [50-53].

Conclusions

Brucellosis is an infectious disease of endemic nature in animals and is a high-risk for humans. It has been reported that the consumption of unpasteurized milk and milk products prepared from raw milk is the main source of human brucellosis. It is culturally acceptable to drink raw camel milk (unpasteurized), unlike cow milk which is pasteurized in Dhofar Governorate. Published data indicated that *Brucella melitensis* is the main species responsible for human brucellosis in Oman. Vaccination of goats, sheep, and camels with a full dose of the Rev-1 *B. melitensis* vaccine will help in controlling the disease in animals, and this will lead to control of human brucellosis in Oman.

Recommendations

Prevention of human brucellosis caused by *Brucella* depends upon the eradication of the disease in animals, especially goats, sheep, cattle, and camels. To avoid human infection with brucellosis, it is generally recommended to practice hygienic measures as a general precaution when visiting animal farms. Public awareness and education are also needed. Hygienic measures include regular hand washing after touching animal materials, avoiding consumption of fresh unpasteurized milk and milk products from animals that carry a high risk of *Brucella* infection, and proper disposal of aborted fetuses, placenta, and discharges from infected animals. Treatment regimens with different types of antibiotics are recommended for people with clinical signs of brucellosis. Identifying infected animals by serological testing is highly recommended in endemic areas. This should be carried out every year on individual animals or on a representative sample of the animals in every herd or flock in different locations in Oman. Since no commercial vaccines against human brucellosis are available, vaccination of all female animals in endemic areas with full-dose *Brucella* vaccines is highly recommended.

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