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Brucella Biovars Isolated from Domestic Livestock in Dhofar Province in the Sultanate of Oman

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Abstract: Isolation of Brucella microorganisms was attempted from a total of 200 different samples collected from cattle, camels, goats and sheep from Dhofar Province in Oman. The collected samples comprised milk, placentas, fetal membranes, vaginal secretions and aborted fetuses. Twenty eight Brucella isolates were obtained from all samples, 13 of them were isolated from cattle, 11 from goats and 4 from camels whereas no isolates were obtained from sheep. The most important source of isolation was milk samples from which 25 isolates were recovered (13.4% of the total milk samples examined). Isolation from other samples was performed with less frequency. Two isolates were recovered from placentas and one from uterine exudate. Identification and biotyping of the isolates resulted in only one biotype that is *Brucella melitensis* biovar 1. The public health hazards of animal brucellosis in Oman were discussed in the light of the results of this study, taking into account the dietary habits of the people. More bacteriological investigation of brucellosis was suggested to study ovine brucellosis as well as the role of other animal species in Dhofar, in the epidemiology of the disease in Oman.

Key words: *Brucella melitensis*, isolation, livestock, Dhofar, Oman

INTRODUCTION

Brucellosis is a bacterial zoonotic disease of worldwide distribution caused by the members of genus *Brucella*. Nine *Brucella* species are currently recognized these include the six classical species: *B. melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. canis* and *B. neotomae* (Verger *et al*., 1987) two species of marine mammal origin: *B. pinnipedialis* and *B. ceti* (Foster *et al*., 2007) and the recently characterized species *B. microti* (Scholz *et al*., 2008) which was isolated from the common vole. Each of the former three species (*B. melitensis*, *B. abortus* and *B. suis*) was classified farther into biovars, according to a set of tests including mainly agglutination with monospecific sera, dye sensitivity, CO_2 requirement and H_2S production (OIE, 2009).

Brucella species are Gram-negative aerobic bacteria. They appear microscopically as nonmotile coccobacilli, cocci or short rods. They react positively to catalase, urease and oxidase tests except *B. ovis* which is negative to both urease and oxidase tests and *B. neotomae* and some African isolates of *B. abortus* biovar 3 which are negative to oxidase test (OIE, 2009). Many strains require supplementary CO_2 for growth, especially on primary isolation. Brucella neither liquefy gelatin nor produce indole. On serum-dextrose agar and other clear media, smooth variants produce transparent, raised and convex colonies with an entire edge and smooth shiny surface. They appear pale honey in color by transmitted light (Corbel and Morgan, 1984).

Although, it is one of the ancient diseases that can possibly be traced back (Seleem *et al*., 2010) brucellosis is considered recently an important reemerging disease in the Middle East, North and East Africa, the Mediterranean countries, South and Central Asia and central and South America (Corbel and WHO, 2006; Maurin, 2005). Human and animal brucellosis were reported in all countries of the Middle East including those of the Gulf countries (Gul and Khan, 2007). In Bahrain the disease was reported for the first time in a human patient with a history of drinking unpasteurized goat's milk (Ali *et al*., 2007) whereas the disease was not yet reported in animals.

Brucellosis of livestock in Oman was investigated serologically as early as 1979 when Mackinon reported the first two positive sera from goat's serum (Nicoletti, 1986). This was followed in 1985-86 by a sero-surveillance which revealed the presence of Brucella antibodies in the sera of cattle, goats and camels and the sero-positive

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animals were mainly localized in Dhofar region (Ismaily *et al*., 1988). Bacteriologically, animal brucellosis was not comprehensively studied in Oman. Nicoletti was able to isolate and identify *Brucella melitensis* from goat's milk from Dhofar region (Nicoletti, 1986); no more reports were available on this aspect. This research is an attempt to investigate *Brucella* species involved in livestock brucellosis in Dhofar region at the Sultanate of Oman, aiming to provide important basic epidemiological information about the disease in the country which will be essential for designing a suitable and effective control program.

MATERIALS AND METHODS

Samples: The examined samples include aborted fetuses (stomach contents, spleens and lungs), retained and aborted placentas and fetal membranes, vaginal secretions collected in swabs from recently aborting females and milk samples from serologically positive lactating females. Samples were collected from cattle, camels, goats and sheep from Dhofar Province at The Sultanate of Oman (Table 1).

Isolation: Primary isolation was performed on Farrell (1974)'s Brucella selective medium. Briefly prepared by enrichment of each liter of sterilized basic medium (Brucella medium base, Oxoid) with 10% v/v sterile inactivated horse serum (GIBCO), sterile dextrose solution (5% w/v final concentration) and supplemented with Brucella selective supplement (oxoid). Each sample was inoculated onto two plates and incubated at 37°C one aerobically and the other microaerophilicly at 10% CO_2 tension. The incubated plates were examined daily for up to 10 days for the appearance of Brucella-like colonies before been discarded as negative. The Brucella-like colonies were sub-cultured for purification onto Serum Dextrose Agar (SDA) plates. Briefly prepared as described by Corbel *et al*. (1983) by supplementing each liter of sterile basic medium (blood agar base No. 2, Oxoid) with sterile dextrose solution (5% w/v final concentration) and 10% v/v sterile inactivated horse serum (GIBCO).

Identification: The purified isolates were classified up to the species level, according to the methods described by (Corbel *et al*., 1983) using a set of tests which include Gram's staining, modified Zeihl-Neelsen staining, hydrolysis of urea, hydrolysis of gelatin, methyl red reaction, Voges-Proskauer reaction, indole production, nitrate reduction and lysis by Tbilisi (Tb), Weybridge (Wb), Berkeley (Bk_2), Izatnagar (Iz) and R/C Brucellaphages strains at Routine Test Dilution (RTD). The isolates were also tested for catalase and oxidase production.

Typing: The isolated *Brucella* species were typed to the biovar level as described by Corbel *et al*. (1983). The tests performed comprise: H_2S production, CO_2 requirement, growth on media containing basic fuchsin (20 μg mL^{-1}), growth on media containing thionin (20 μg mL^{-1}) and slide agglutination with monospecific anti-A, anti-M and anti-R sera.

The monospecific antisera, Brucellaphages and reference Brucella strains were obtained from the FAO/WHO Collaborating Centre for Brucellosis reference and research, VLA, UK.

RESULTS AND DISCUSSION

After 72 h of incubation at 37°C, 28 isolates were recovered from a total of 200 samples. Twenty five out of the total number of the isolates were obtained from milk samples of eleven cows, ten goats and four camels. Whereas two isolates were recovered from aborted cow's placentas and one from a goat's uterine exudate following abortion (Table 1). The primary isolates on Farrell (1974)'s selective medium from both aerobic and microaerophilic incubation were all recovered as pure cultures. All colonies showed the characteristics typical of the genus *Brucella*. They were raised, convex, circular about 1mm in diameter with entire edges. In transmitted light, they had glistening shiny surface and appeared pale honey colored.

Microscopically, the organisms were Gram-negative coccobacilli. They resisted decolorization with diluted

Table 1: Samples collected from livestock in Dhofar Province for isolation of *Brucella* sp.

Animal species	Milk samples			Placentas			Aborted fetuses			Uterine swabs			Total		
	T	+	-	T	+	-	T	+	-	T	+	-	T	+	-
Cattle	92	11	81	2	2	0	3	0	3	3	0	3	100	13	87
Camels	27	4	23	0	0	0	0	0	0	0	0	0	27	4	23
Goats	42	10	32	0	0	0	0	0	0	6	1	5	48	11	37
Sheep	25	0	25	0	0	0	0	0	0	0	0	0	25	0	25
Total	186	25	161	2	2	0	3	0	3	9	1	8	200	28	172

T: Total samples examined; +: Positive samples (i.e., Brucella isolates); -: No isolation

Table 2: Biotyping results of *Brucella* species isolated from livestock in Dhofar Province

Isolate No.	Animal infected	Sample for culture	Shape and staining	Mod ZN	Oxidase	Cata-lase	Urease	H_2S production	CO_2 requirement	Aggluti-nation with monospecific antisera			Lysis by phages at RTD					Growth on		*Brucella* species	Biovar
										A	M	R	Tb	Wb	Bk_2	Iz	R/C	TH	BF		
Dhofar-01	Cow	Placenta	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-02	Cow	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-03	Cow	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-04	Cow	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-05	Cow	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-06	Cow	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-07	Goat	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-08	Cow	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-09	Cow	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-10	Camel	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-11	Camel	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-12	Goat	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-13	Goat	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-14	Goat	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	PL	PL	NL	-	+	B. *melitensis*	1
Dhofar-15	Goat	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	PL	NL	-	+	*B. melitensis*	1
Dhofar-16	Goat	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	PL	NL	-	+	*B. melitensis*	1
Dhofar-17	Goat	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-18	Cow	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-19	Cow	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-20	Cow	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-21	Goat	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-22	Goat	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-23	Goat	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-24	Goat	Uterine exudates	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-25	Camel	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-26	Camel	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-27	Cow	Milk	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1
Dhofar-28	Cow	Placenta	CB-	+	+	+	+	-	-	-	+	-	NL	NL	L	L	NL	-	+	*B. melitensis*	1

Mod. Z-N = Modified Ziehl-Neelson Stain. (Resist decolorization with 0.5% acetic acid); CB- = Gram negative coccobacilli; NL = No Lysis; L = Lysis; PL = Partial lysis; TH = Media containing thionin, concentration 1/50 000 w/v, BF = media containing basic fuchsin, concentration 1/50 000 w/v

acetic acid solution when stained by the modified Ziehl-Neelsen technique hence appeared red under the microscope.

As shown in Table 2 all isolates were oxidase, catalase and urease positive. They did not produce H_2S, did not require supplementary CO_2 for growth, resist the inhibitory effect of basic fuchsin dye (1/50000 w/v) in growth media but inhibited by an equal concentration of thionin dye in the growth media. In slide agglutination test all isolates were agglutinated with anti-M monospecific serum but were neither agglutinated with anti-A nor with anti-R monospecific sera. The isolates were all lysed with Bk_2 and Iz Brucellaphages at RTD but not with Tb, Wb and R/C phages.

From the obtained results, it was concluded that only one species, *Brucella melitensis* was encountered and that all strains were belonged to biovar 1.

The first 17 isolates (strains: Dhofar-01 to Dhofar-17) were retested for confirmation at AFSSA/UZB/OIE/FAO Brucellosis Reference Laboratory France. They were all confirmed as *Brucella melitensis* biovar 1 with the exception of strains (Dhofar-15) and (Dhofar-16) which were found to be dissociated to the rough form).

The epidemiological aspects form the basic knowledge which needs to be available before the formulation of any disease control program. Of these aspects are understanding of the etiology of the disease and the host animal species affected. Hence, isolation and biotyping of the organisms causing brucellosis of livestock in Oman are crucial for planning an effective program to control the disease. This research provided this information and therefore it may serve that need.

Official reports and diagnostic results from the Ministries of Agriculture and Health, in Oman, showed that brucellosis is endemic in Dhofar Province and that both animal and human brucellosis diagnosed in other parts of the country were almost all originated from Dhofar Province. Therefore, studying of the etiology of brucellosis in Dhofar Province will more or less represent the situation in whole of Oman.

Results from this study concur with those obtained by Nicoletti who isolated and identified *B. melitensis* in the milk of local goats from Dhofar Province before more than two decades (Ismaily *et al*., 1988; Nicoletti, 1986), it seems that no change had occurred in the etiological picture of the disease since then. Moreover, researchers found that *B. melitensis* is not only the cause of caprine brucellosis but it is also responsible for bovine and camels brucellosis in Dhofar. On the other hand human brucellosis in Oman was found to be due to infection with *B. melitensis* which was invariably isolated in bacteriologic cultures of specimens submitted for diagnosis in the major hospitals of the country (Scrimgeour *et al*. 1999).

The way by which the disease was first introduced into Dhofar is unknown but most probably it came across

the border with imported animals carrying the disease. The animal husbandry practices and the open pasture where all livestock species as well as other free-living animals such as donkeys and dogs intermix freely in this pasture, facilitates the transmission of brucellosis within the individuals of the same species and to those of other species. Moreover, introducing purchased or exchanged animals that were not certified free of brucellosis is regarded another means for spreading of the disease.

According, to the agricultural census 2005, sheep population in Dhofar is relatively small (7605 head). They comprise around 1.9% of the whole livestock population in Dhofar Province (data obtained from the ministry of agriculture and fisheries, Sultanate of Oman). Although, almost all sheep are flocked together with goats under the same management conditions and are grazing the same pasture, still no positive cases were yet detected among the individuals of this livestock species during the period of this study. No logical explanation could be concluded for this result. Hence, more extensive research is suggested to study ovine brucellosis in the country.

The role of other animal species in the epidemiology of the disease such as donkeys and dogs which live as free nondomesticated animals in the pastures of Dhofar should also be investigated. On the other hand, the role of other domestic animals such as horses and pigs on the epidemiology of brucellosis in Oman could be negligible as horses are mainly reared in closed farms or as riding horses with police and other military forces and pigs are not reared as one of the farm animals in the country.

Isolation of Brucella organisms with a relatively high rate (25/186 = 13.4%) from milk samples (Table 1) is of great zoonotic importance. Where drinking of raw camels' milk is a deep-rooted local custom all over the country. Some people prefer to drink goats' milk partially boiled by dropping only red-hot stones in the raw milk, this partial boiling do not generate enough heat to kill the bacteria. Moreover, it is a custom in Dhofar to produce milk byproducts such as butter, quail and sour milk, from raw cows and goats' milk which if collected from infected animals will constitutes an additional public health hazard.

CONCLUSION

Serological studies showed that brucellosis is prevalent in Oman, particularly in Dhofar (Ismaily *et al*., 1988; Idris *et al*., 1993). The fact that no species other than *B. melitensis* was yet isolated from the livestock in Oman during the earlier decades and that biovar 1 is the only biovar encountered according to this study, hypothesize that *B. melitensis* biovar 1 could be the sole causative organism responsible for brucellosis in Oman. Nevertheless, isolation is suggested to be continued in Dhofar Province and to be extended to the other regions of the country, before confirming or rejecting this hypothesis.

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