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A cross-sectional seroepidemiological study of camel (*Camelus dromedarius*) brucellosis and associated risk factors in the Sultanate of Oman

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Abstract

Brucellosis is a globally distributed and economically devastating zoonotic disease of multiple species, including camels. Human and livestock brucellosis is prevalent in Oman, especially in southern Dhofar governorates of Oman, where camels share habitat and have close contact with other susceptible species. We conducted a randomized cross-sectional sero-epidemiological study to investigate the seroprevalence of brucellosis in camels of Oman. The sera from 2,250 camels from 552 geographically marked farms were screened through Rose Bengal plate agglutination test and later confirmed by the competitive ELISA (COMPLIZA, VLA, UK). In total, nine [0.4%, confidence interval (CI) 0.4, 0.8] camels from eight (1.5%, CI 0.6, 2.8) herds were tested positive for brucellosis. The highest prevalence was recorded in Dhofar (3.7%, CI 1.4, 7.9) and the lowest in Sharqiyah (1.3%, CI 0.0, 7.2) governorate ($p = 0.052$). All seropositive camels were of local breed and females. Seroprevalence was higher (0.5%, CI 0.2, 1.0) in adults (>4 yr of age) as compared with young (≤ 4 yr of age) camels (0.2%, CI 0.0, 0.8). The results of binary logistic regression indicated that camel herds located in south (Dhofar) [odds ratio (OR) 6.39, CI 1.01, 40.67], practice of open replacement system (OR 14.49, CI 1.83, 114.51) and with history of abortions (OR 8.01, CI 1.34, 47.77) were more likely to test positive for brucellosis. We conclude that brucellosis is endemic at a low level in camels of Oman and a control strategy based upon test-and-slaughter/inclusion of camels in the current vaccination program after carefully planned vaccine evaluation studies could be considered to control it.

Keywords: Brucellosis, Camel, Oman, Risk factors, Seroepidemiology.

Introduction

Brucellosis causes direct and indirect losses to livestock through abortions, premature births, metritis, reduced fertility, and reduced milk yield (Seleem *et al.*, 2010; Gwida *et al.*, 2012). It is regarded as an important re-emerging communicable disease in the Middle Eastern and Mediterranean countries (Brown *et al.*, 2014). Nearly, every case of human brucellosis has an animal origin and, therefore, control is primarily a veterinary concern (Nicoletti, 2002). Although several studies have concluded that the consumption of raw camel milk and camel meat are potential sources for transmission of the bacterium to humans (Omer *et al.*, 2007; Hadush *et al.*, 2013), no specific programs for the control and eradication of the disease in camels have been implemented yet (Sprague *et al.*, 2012).

The disease has been reported frequently in camels, particularly if there has been contact with infected small ruminants (Erdenebaatar *et al.*, 2004; Dawood, 2008). The incidence of the clinical disease is reported to be rare in camels; however, the possible shedding of organisms in milk may lead to disease transmission in humans (Corbel *et al.*, 2006).

Human and livestock brucellosis caused by *Brucella melitensis* is endemic in Oman and records from the

Ministry of Health and Animal Health Research Center point toward the endemic human and livestock brucellosis in the Southern Dhofar governorate (Ismaily *et al.*, 1988; Idris *et al.*, 1993; Scrimgeour *et al.*, 1999). A retrospective study examining 3 yr of data from the Dhofar Human Hospital revealed that 63% of cases were due to consumption of raw milk, especially from cattle or camels, and 83% of patients had a history of contact with live animals (El-Amin *et al.*, 2001). In 1986, a study conducted in Oman found that 3.6% of sampled camels were seropositive for brucellosis (Ismaily *et al.*, 1988). Subsequent surveys reported variable seroprevalence of 18.2% (1999), 9.1% (2000), 7.1% (2001), and 1.07% (2002) in studies conducted by Ministry of Agriculture (MAF, 2003). A national brucellosis control program was implemented in 2003 in the endemic region (Dhofar governorate) of Oman. This control strategy included carpet vaccination (Rev. 1) except in camels due to lack of optimal data of safety, potency, and protective efficacy (vaccination-challenge trial) of Rev. 1 in dromedary (Radwan *et al.*, 1995). Most recently, Rev. 1 has been found safe and immunogenic in lactating and young camels (Benkirane *et al.*, 2014).

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Nonetheless, the vaccine efficacy is yet to be elucidated both in control (vaccination challenge trial) and field trials before it could be considered for a brucellosis control program in camels. Despite all the fright about brucellosis in camels of Oman, before the study reported here, the prevalence of brucellosis in camels in Oman has not been investigated on epidemiological grounds. This study was planned to explore the prevalence of brucellosis in camels of Oman and identify the risk factors.

Materials and Methods

Study design and sampling

A cross-sectional serological study was conducted in all the governorates of Oman. The sample size was calculated at an expected prevalence of 50% (unknown disease prevalence), 95% confidence levels, and 5% error margin (Thrusfield, 2007). The required sample size was to include at least 385 herds of camels in the

study. This number was further stratified according to the population proportion of camels in each governorate and wilayats (district) of Oman. Inside wilayats, camel herds were randomly selected from different locations. Random selection of the herds and camels was carried by using the Survey Toolbox software (Cameron, 1999). The blood samples were drawn from 2,255 (254 males and 2,001 females) camels originating from 552 randomly selected geographically marked farms/herds (Fig. 1). The sampled camels were further divided into two age groups viz., young (less than equal to 4 yr, $n = 684$), and adults (above 4 yr, $n = 1,566$) of age. A large proportion of camels belonged to local breeds ($n = 2,226$) followed by imported ($n = 18$) and cross breeds ($n = 6$). Epidemiological data were collected from the sampled farms and animals to build a geodatabase.

Serological diagnosis

Sera were initially screened through Rose Bengal plate agglutination test (RBT) and later confirmed

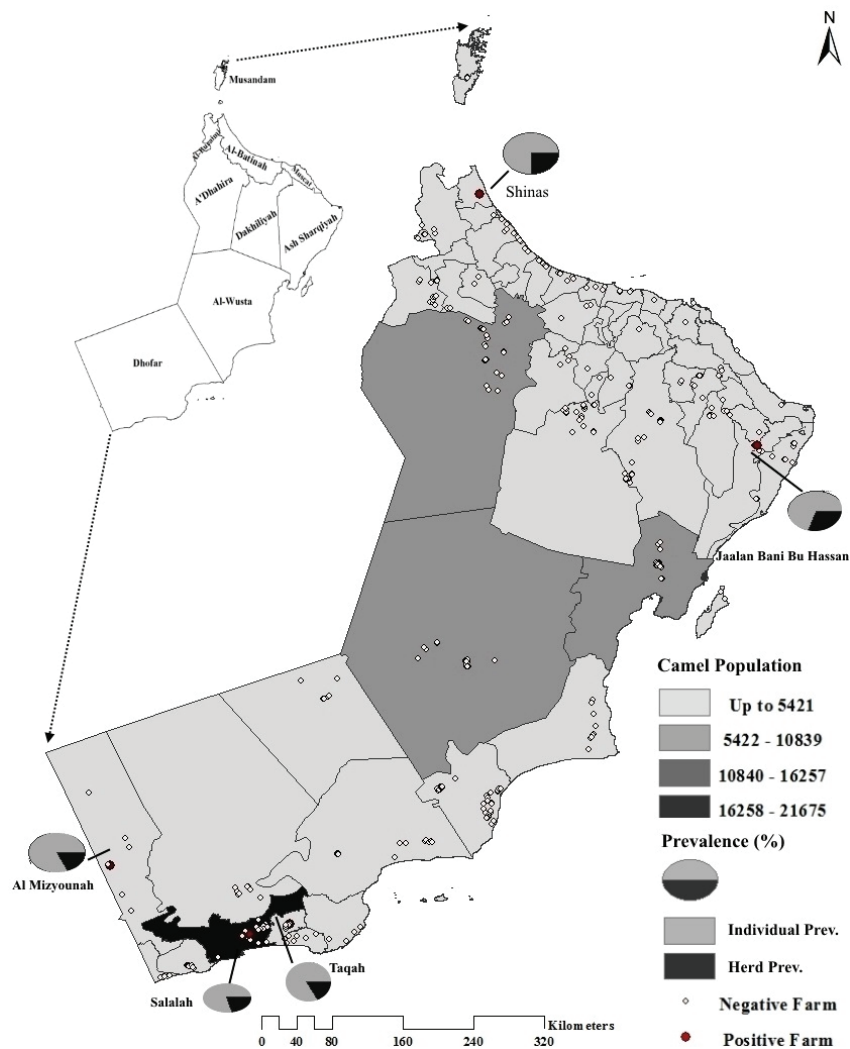


Fig. 1. Distribution of camel population in different wilayats, location of sampled and brucellosis positive camel herds along with herd and individual prevalence in Oman.

with a commercially available competitive ELISA (COMPELISA-400, VLA, UK) for the presence of anti-brucella antibodies (Perrett *et al.*, 2010). The tests were performed by strictly adhering to the instructions of the manufacturer.

Data analysis

Prevalence along with 95% confidence intervals (CIs) was computed. Initially, a univariable analysis was conducted, and odds ratio (OR) along with 95% CI was calculated for different variables. All variables with $p \leq 0.20$ based on the Wald statistics were used to construct a backward stepwise binary logistic regression model. The fit of the final model was assessed by observing values of the Hosmer–Lemeshow test, the Nagelkerke *R* square test and the observed *versus* predicted values (residual statistics), to identify outliers at 0.5 cut off point (Urdaz-Rodríguez *et al.*, 2009). All relevant analyses were performed by using IBM SPSS Statistics 17.0 for Windows® (IBM Corporation, New York, USA).

Results

In total, 2,255 camels were sampled during this study. However, one of the owners indicated that five of his camels were vaccinated against brucellosis by attending veterinarian recently, along with other ruminants. Therefore, these samples were omitted, and analysis was performed on 2,250 (252 males and 1,998 females) samples. In the initial screening through RBT, 10 samples were found positive. However, when subjected to cELISA, 9 out of the 10 RBT positive samples gave a positive reaction. The overall herd-level seroprevalence was 1.5% (CI 0.6, 2.8), and the highest prevalence was recorded in Dhofar governorate (3.7%, CI 1.4, 7.9) followed by Al Batinah (2.5%, CI 0.1, 13.2) and Ash Sharqiyah (1.3%, CI 0.0, 7.2) governorates ($p = 0.052$) (Table 1). In the north of Oman, two camel

herds were found positive in the wilayats of Shinas (Batinah) and Jaalan Bani Bu Hassan (Al Sharqiyah). In Dhofar (South), six herds were positive for brucellosis in three wilayats (Al Mizyounah, Salalah and Taqah) (Fig. 1).

At animal-level, seroprevalence recorded was 0.4% (CI 0.2, 0.8), and the highest was recorded in Dhofar (0.8%, CI 0.3, 1.6) and Al Batinah (0.8%, CI 0.0, 4.6) governorates followed by Ash Sharqiyah (0.4, CI 0.0, 2.2), $p = 0.052$. All seropositive camels were female (0.45%) and of local breed (0.4%, CI 0.2, 0.8). The prevalence was slightly higher in camels above 4 yr of age (0.51%, CI 0.2, 1.0) than those below 4 yr of age (0.14%, CI 0.0–0.8), $p = 0.207$. In infected herds, the within-herd prevalence varied from 1.7% to 20% ($p = 0.386$). The majority (62.5%, $n = 5$) of the positive camels were kept in mixed herds with other ruminants (cattle, goat, and sheep).

The influence of individual-level variables (age, sex, and breed) and herd level variables (herd size, cohorts, housing, management, replacement, history of abortion in a herd, and location) on the seroprevalence of brucellosis in camels are summarized in Table 2. The analysis of individual-level variables indicated that adult camels (>4 yr) were more likely to test positive than young camels (≤ 4 yr) (OR: 3.51, CI 0.44, 28.12), $p = 0.237$. No analysis was possible for sex, breed, and individual animal abortion history because all positive camels were females of local breeds with no prior history of abortion.

At herd-level, univariable analysis indicated that herds; with more than 15 heads (OR 4.19, CI 0.84, 20.95), where camels were kept only (OR 3.26, CI 0.39, 26.74), managed separately (OR 1.27, CI 0.30, 5.36), with open replacement system (OR 3.70, CI 0.73, 18.85) and loose housing (OR 5.95, CI 7.27, 48.71) were more likely to

Table 1. Individual and herd-level seroprevalence of brucellosis in camels ($n = 2,250$) randomly sampled from different governorates of Oman.

Governorate	Individual		Herds	
	Number positive/ number tested	Prevalence (95% CI)	Number positive/ number tested	Prevalence (95% CI)
Al Batinah	1/118	0.9 (0–4.6)	01/40	2.5 (0.1–13.2)
Al Buraimi	0/140	0 (0–2.6)	0/43	0 (0–8.2)
Al Dakhiliyah	0/138	0 (0–2.6)	0/41	0 (0–5.6)
A'Dhahira	0/135	0 (0.3–2.7)	0/40	0 (0–8.8)
Dhofar	7/904	0.8 (0–1.6)	6/162	3.7 (1.4–7.9)
Musandam	0/6	0 (0–45.9)	0/2	0 (0–84.2)
Muscat	0/15	0 (0–21.8)	0/4	0 (0–60.2)
Sharqiyah	1/247	0.4 (0–2.2)	1/75	1.3 (0–7.2)
Al Wusta	0/547	0 (0–0.7)	0/145	0 (0–2.5)
Total	09/2250	0.4 (0.4–0.8)	8/552	1.5 (0.6–2.8)

Individual-level prevalence was not significantly different among various governorates, $F = 2.56$, $P = 0.109$. Herd-level prevalence was not significantly different among various governorates, $F = 3.79$, $P = 0.052$.

test positive for brucellosis. However, the association was not significant ($p > 0.05$). Camel herds dwelling in southern Dhofar governorate (OR 7.46, CI 1.49, 37.37) and with the history of abortions (OR 8.71, CI 2.12, 35.81) were significantly ($p < 0.05$) more likely to test positive for brucellosis (Table 2).

Of the seven variables analyzed through univariable analyses (Table 2), abortion history in herd ($p = 0.003$), herd size ($p = 0.081$), replacement system ($p = 0.116$), housing ($p = 0.096$), and location of herd ($p = 0.014$) were found to be associated with brucellosis (Wald $p \leq 0.2$). These variables were used to construct the initial multivariate model (Table 3) and the final binary logistic regression model indicated that camel herds located in south (Dhofar) (OR 6.39, CI 1.01, 40.67), practicing open replacement system (OR 14.49, CI 1.83, 114.51) and with history of abortions (OR 8.01, CI 1.34, 47.77) were the variables associated with the high prevalence of brucellosis in camel herds of Oman (Table 3). The Hosmer and Lemeshow test ($\chi^2 = 1.313$,

$p = 0.519$) and Nagelkerke R Square (0.209) indicated that it is a suitable model to predict seroprevalence of brucellosis in camel herds of Oman.

Discussion

Camels play an important social and economic role for pastoralists in many regions of the world and are a valuable source of milk, meat, transport, leather, and wool as well as beings used for recreational activities (Rollefson, 2000). However, diseases, such as brucellosis, can affect production and productivity (Tibary *et al.*, 2006). RBT is economical, simple, and rapid screening assay performed with a stained *Brucella abortus* (S99 or S1119-3) suspension at pH 3.6–3.7 and plain serum. Because of high sensitivity (>99%) and the rise of false positive reactions in large ruminants, the present OIE guidelines recommend that all RBT positive results be confirmed, by quantitative assays, including CFT and ELISA (Corbel *et al.*, 2006). Therefore, we used cELISA for the confirmation of RBT results.

Table 2. Univariable analysis of the risk factors for seropositivity to brucellosis in camels ($n = 2,250$) of Oman.

Variable	Category	Pos./Tested	Prevalence (95%CI)	p value	OR (95% CI)	p value
Individual level						
Age groups	>4 yr	08/1569	0.5 (0.2–1)	0.208	3.51 (0.44–28.09)	$\chi^2 = 1.397$ $p = 0.237$
	≤4 yr	01/684	0.2 (0–0.8)			
Breed	Local	9/2226	0.4 (0.2–0.8)	-	-	-
	Imported	0/18	0 (0–18.5)			
	Cross	0/6	0 (0–45.9)			
Sex	Male	0/252	0 (0–1.5)	-	-	-
	Female	9/1998	0.5 (0.2–0.9)			
Herd level						
Location	South (Dhofar)	6/162	3.7 (1.4–7.9)	0.004	7.46 (1.49, 37.37)	$\chi^2 = 5.95$ $p = 0.014$
	North (Rest of Oman)	2/390	0.5 (0.1–1.8)			
Herd Size	>15 camels	6/233	2.6 (1–5.5)	0.038	4.19 (0.84–20.95)	$\chi^2 = 3.044$ $p = 0.081$
	≤15 camels	2/319	0.6 (0.1–2.2)			
Camel kept with	Camels only	7/378	1.9 (0.7–3.8)	0.244	3.26 (0.39–26.74)	$\chi^2 = 1.216$ $p = 0.270$
	with ruminants	1/174	0.6 (0–3.2)			
Managed	Separately	5/314	1.6 (0.5–3.7)	0.747	1.27 (0.30–5.36)	$\chi^2 = 0.104$ $p = 0.747$
	Mixed	3/238	1.3 (0.3–3.6)			
Replacement	Open herds	2/47	4.3 (0.5–14.5)	0.093	3.70 (0.73–18.85)	$\chi^2 = 2.47$ $p = 0.116$
	Close herds	6/505	1.2 (0.4–2.6)			
Housing	Loose	7/301	2.3 (0.9–4.7)	0.059	5.95 (7.27–48.71)	$\chi^2 = 2.766$ $p = 0.096$
	Confined	1/251	0.4 (0–2.2)			
Abortion history	Yes	4/60	6.7 (1.8–16.2)	< 0.001	8.71 (2.12, 35.81)	$\chi^2 = 9.015$ $p = 0.003$
	No	4/492	0.8 (0.2–2.1)			

Table 3. Final binary logistic regression model for predicting brucellosis at herd level in camels in Oman.

Category	Exposure variable	Comparison	OR	95%CI	p value
Location of herd	South Oman	North Oman	6.39	1.01–40.67	0.049
Replacement	Open herds	Close herds	14.49	1.83–114.51	0.011
Abortion history	Yes	No	8.01	1.34–47.77	0.022

In the present study, 1.5% of herds had seropositive animals. Few studies have reported herd-level prevalence in camels (Radwan *et al.*, 1992; Omer *et al.*, 2000; Megersa *et al.*, 2005; Al-Majali *et al.*, 2008; Mohammed *et al.*, 2011); however, the majority of these were not truly representative of the population and targeted specific high-risk components of the population, and hence reported higher values (10.2%–54.2%). A low herd-level seroprevalence (<2%) was observed in this study as compared to previous estimates (3.5%) in Oman (Ismaily *et al.*, 1988).

Since the sampling plan was constituted to detect at least a single animal positive with the expected prevalence to be 50%, a low-level individual seroprevalence (0.4%) was recorded during the study. Individual camel seroprevalence ranging from 1.6% to 12.1% have been reported in other studies (Radwan *et al.*, 1992; Kudi *et al.*, 1997; Abou-Eisha, 2000; Omer *et al.*, 2000; Megersa *et al.*, 2005; Al-Majali *et al.*, 2008; Dawood, 2008; Balcha and Fentie, 2011; Mohammed *et al.*, 2011). Low-level individual prevalence ranging from 1.4% to 2.0% was reported by the studies from Saudi Arabia, Iran, Abu Dhabi, Egypt, Ethiopia, and Somalia (Baumann and Zessin, 1992; Afzal and Sakkir, 1994; Khadjeh *et al.*, 1999; Abou-Eisha, 2000; Megersa *et al.*, 2005; Mohammed *et al.*, 2011). However, some studies have recorded higher level of overall prevalence, including 7.3% in Egypt (El-Boshy *et al.*, 2009), 19.4% in Jordan (Dawood, 2008), 30.5% in Sudan (Omer *et al.*, 2007), 4.2% in Ethiopia (Teshome *et al.*, 2003), 3.8% in Iraq (Jawad, 1984), 7.5% in Nigeria (Kudi *et al.*, 1997), and 8% in Pakistan (Straten *et al.*, 1997). The differences in the prevalence reported between the countries are likely to be associated with different environmental and management conditions, the type of study conducted, and the diagnostic test used.

Camels in Dhofar (South of Oman) governorate were more likely to be seropositive (3.7%, OR: 7.46) than camels from other governorates. This finding highlighted the endemic nature of brucellosis in Dhofar as reported by others (Ismaily *et al.*, 1988; Mehta and El-Mauly, 1990). Approximately, 45% of camel population in Oman is in Dhofar, and effective control of brucellosis is essential to maintain the productivity of this population. Multispecies contact, a higher population density of livestock, and communal grazing along with faulty management practices adopted by farmers may have contributed to the establishment and maintenance of brucellosis in this governorate (Kudi *et al.*, 1997; Mohammed *et al.*, 2011).

In the current study, evidence of infection was only detected in females. Other studies have also reported higher seroprevalence in females (Tolosa *et al.*, 2008; Bayemi *et al.*, 2009; Hadush *et al.*, 2013). Because of their usefulness in the production herds, females generally have a longer lifespan than males, and this may have increased exposure to the bacterium (Mekonnen *et al.*, 2010). All seropositive camels were of local breeds. No previous studies have reported significant differences between breeds for brucellosis and it is likely that management and husbandry factors are more important than breed in determining the probability of a camel being infected with *Brucella* (Radwan *et al.*, 1992; 1995).

In our study, the seroprevalence increased with the age of camels. Studies conducted elsewhere have also reported a higher prevalence in adults as compared to younger camels (Megersa *et al.*, 2005; Dawood, 2008; Balcha and Fentie, 2011). The growth and multiplication of *Brucella* are enhanced by age and sexual maturity and is dependent on the increasing level of sex hormones and erythritol (Poester *et al.*, 2013). Additionally, with age, animals are more likely to be exposed to the organism (Dhand *et al.*, 2005).

In the present study, herds containing more than 15 heads of camels were found more likely (OR 4.19) to contain seropositive animals than smaller (≤ 15 heads) herds. Similar observations were reported by others (Abbas and Agab, 2002; Al-Majali *et al.*, 2008; Balcha and Fentie, 2011; Mohammed *et al.*, 2011; Hadush *et al.*, 2013). Larger herd size increases the opportunity for contact within infected animals, mainly if abortions have occurred (Abbas and Agab, 2002).

We found that camels kept (OR 1.9) and managed (OR 1.6) separately were more likely to test seropositive. Inter-species transmission of disease is a well-documented phenomenon (Erdenebaatar *et al.*, 2004; Dawood, 2008) and our findings differ with that of Musa and Shigid (2001) and Mohammed *et al.* (2011), who reported a higher level of seropositivity in camels raised along with cattle. Moreover, results of several studies have documented that camels kept with small ruminants are also more susceptible to get the infection (Ismaily *et al.*, 1988; Radwan *et al.*, 1992; Abou-Eisha, 2000; Al-Majali *et al.*, 2008). The difference recorded here could be related to the variations in the herd structure in Oman as compared to the studies conducted elsewhere.

In the present investigation, we found a statistically significant association (<0.001) between seropositivity and herds with a history of abortion. Association

between *Brucella* infection and abortion in camels has been described in other studies (Musa and Shigid, 2001; Mohammed *et al.*, 2011) and is a well-recognised sign in most of *Brucella* infections.

We found that herds with an open herd replacement system (OR 3.70) and loose camels (OR 5.95) were more likely to test seropositive. The selection of breeding stock, introduction of new animals without prior testing, and lack of quarantine might have resulted in the higher prevalence of brucellosis in these herds. Moreover, transhumance is much more pronounced in the pastoral areas of Oman, especially in Dhofar, where the herds of camel graze with other ruminants at common pasture, consequently facilitating the spread of the organism due to increased exposure. Similar was reported in Nigeria (Kudi *et al.*, 1997).

The present study documented that camel brucellosis is endemic, especially in Dhofar governorate of the Sultanate of Oman, *albeit*, at a very low level. Moreover, due to the limited number of positive samples and a wide confidence interval observed during the logistic regression analysis, the results of risk factor analysis should be interpreted with caution. This low-level seropositivity offers the promise of its control through a test-and-slaughter strategy. However, in case of prized racing camels (sometimes worth million dollars per head), adoption of this policy may not be feasible until a test based upon direct detection of the pathogen is used to confirm the disease. The inclusion of camel in the ongoing vaccination program with Rev. I vaccine in Dhofar warrants execution of carefully planned trials, as Rev. I organism (*Brucella melitensis*) is known to be excreted in the milk and camel herders often prefer to drink raw (unpasteurized) camel milk. Owing to the uncontrolled movement of animals, the risk of spread of brucellosis in disease-free governorates/regions (North of Oman) cannot be overlooked.

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Conflict of interest

The authors declare that they have no conflict of interest.

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