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## RESEARCH ARTICLE

## Bacteriological and serological study on brucellosis infection in camel (*Camelus dromedaries*), Al-Hodeida governorate, Yemen

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### Abstract

Camels are highly susceptible to brucellosis caused by *Brucella melitensis* and *Brucella abortus* however, diagnosis of camel brucellosis is difficult because most camels appear normally and there are no clear clinical signs on affected camels. Study of camel brucellosis especially on Al-Hodeida governorate and the association of *Brucella spp.* with abortion cases in camel's population are very limited. Total 295 samples were collected from 100 camels and 195 samples were subjected for isolation other 100 serum samples were examined by Rose Bengal Plate Test (RBPT), febrile agglutination tests to determine prevalence of brucellosis. Result recoded high prevalence of brucellosis in camels by using RBPT, culture of vaginal swabs, Milk ring test (MRT) and culture of whole blood sample with prevalence 0.11, 0.10, 0.05 and 0.02 respectively. Eight camel's abortion cases have been recorded in current study and it were positively associated with *Brucella spp.* Infection. In conclusion, high prevalence of brucellosis needs serious controlling measurement to avoiding economical losses and zoonosis hazard aspect. Both *Brucella melitensis* and *Brucella abortus* is a major cause of brucellosis in camels rather than highly association with abortion cases.

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## INTRODUCTION

Brucellosis is a global zoonosis disease associated with significant morbidity in humans and livestock (Boschioli *et al.*, 2001; Gwida *et al.*, 2012). Brucellosis, also known as "undulant fever", "Mediterranean fever" or "Malta fever". It's a worldwide distributed and can spread among camels and other farm animals through direct contact with blood, placenta, fetuses or uterine secretions, or through consumption of contaminated raw animal products. Consumption of unpasteurized milk and milk products from camels and other farm animals are considered to be the main route of infection as well as an occupational hazard in human (Almuneef *et al.*, 2004; WHO, 2005; Corbel, 2006). Although there has been great progress in controlling the disease in many countries, there still remain regions where the infection persists in domestic animals and, consequently, transmission to the human population frequently occurs (Corbel, 2006).

Expansion of animal industries and urbanization, and the lack of hygienic measures in animal husbandry and in food handling partly account for brucellosis remaining a public health hazard. Expansions of international travel which stimulates the taste for exotic dairy goods such as fresh cheeses which may be contaminated, and the importation of such foods into *Brucella*-free regions, also contribute to the ever-increasing concern over human brucellosis (Corbel, 2006; Ahmed *et al.*, 2010). There are many species of *Brucella* genus can cause brucellosis in humans and animals i.e. *B. melitensis*, *B. abortus*, *B. ovis* ...etc (Radwan *et al.*, 1995; Corbel, 2006; Gwida *et al.*, 2012). Several studies reported that, *Brucella melitensis*, is the predominant species in goats and sheep, cattle, camel

and human (Refai, 2002; Almuneef *et al.*, 2004). Control of brucellosis mainly based on vaccination and/or elimination of infected animals whereas in human, prevention of infection is primarily based on raising awareness, food-safety measures, occupational hygiene and laboratory safety. Carroll, (1950) has made some recommendations for the management of herds that are free of brucellosis, which include serologic sampling for every six months, sanitary management of new animals introduced into the herd and, in the case of new animals being bought; they should be certified as free herds from brucellosis and quarantined. Yemen is well-known for its rural culture and traditional lifestyle, where different livestock species are kept together and people live in close proximity to their livestock. Brucellosis is the likely cause of health impact and economic losses to owners and their animals; therefore, this study is aimed to study prevalence of brucellosis in camels at Al-Hodeida governorate and to isolate and identify of *Brucella species* that cause brucellosis in camels. Moreover, the study was designed to determine the association between brucellosis in camels and abortion cases recorded in the area of study during study time at Al-Hodeida governorates, Yemen.

## Materials and Methods:

### Study area

Al-hodeida is bordered the red sea and its part of Tihama region. It is very hot at summer and moderate in winter. Majority of people working in agriculture and fishing.

### Samples collection

Camels in Al-hodeida governorate follow the grassing and water. The samples were collected from that places where most camel herds gathering for grassing and water. The study was including north and south of Al-Hodeida governorate. The north reign gathering place includes herds of camels coming from Al-Jatameiah, al-mhub, Al-Madalah, Al-Jabanah, Dear Eisa, Al-qabsah, Al-homrah and Arj villages. The south region was representative by Beat Al-faqeh which include Beat Al-faqeh eastern region (Al-hwilah, demlah and Almazebah villages) and Beat Al-faqeh western region which called Al-mawaheeb. Two hundred and ninety five samples were collected and brought to faculty of Agriculture & Veterinary Medicine for processing and laboratory microbiological examination/tests. Samples were collected as follow:

#### Serum samples:

Total of 100 blood samples were collected from 100 camels in sterile tube containers and properly labeled with necessary information and subjected for RBPT test and febrile agglutinin test of *Brucella melitensis* (M) and *Brucella abortus* (A) according to corbel *et al.* (1996). Briefly, 40µl from serum was mixed with 40µl from RBPT reagent (Quimica Clinica Aplicada S.A. ®. Spain) and the mixture were rotating for 3 minutes. The positive result was recorded when clear agglutination was appeared. Febrile agglutinins test for both *Brucella melitensis* and *Brucella abortus* was carried out according to manufacturing structure (Quimica Clinica Aplicada S.A. ®, Spain). Briefly, all samples, reagents and controls were brought to room temperature and the bacterial suspension was softly homogenized then 20µl of serum and each control on different zones of slid and one drop of bacterial suspension was added to each zone and mixed with aid a disposal stirrer. After one minute slide was checked for absent or present of agglutination. Present of agglutination is equivalent to a titer of 1:80 approximately by the tube technique.

#### Whole blood samples:

Total of 100 blood samples were collected from 100 camels in sterile tube containers and properly labeled with necessary information for analysis. Whole blood samples were subjected for blood culture on tryptose soya broth and subculture on day 2,7,14 and 21 to Brucella supplement agar (Himedia®, India) and the procedures were done according to Nielsen and Duncan (2000).

#### Vaginal swabs:

Total of 56 vaginal swabs were collected and subjected for culturing of *Brucella species* under aerobic and non aerobic condition. Isolation and identification of *Brucella species* was done according to Al-Garadi *et al.* (2011).

#### Milk samples:

Twenty nine milk samples were collected from camel's aseptically in proper containers and properly labeled with necessary information. Milk ring test (MRT) and isolation were done to all samples according to (Corbel, 2006; OIE, 2008; Al-afifi, 2009).

Abortion cases:

Eight cases have been recorded in current study one of them was recorded during collection of samples.

### Statistical Analysis

Statistical analysis of *Brucella spp.* Seroprevalence and chi square were used to determine association measurement was performed in the SPSS® software (Release 18.0 standard version, SPSS Inc., Chicago, Illinois).

### Result:

Bacteriological examinations have been done from direct vaginal culture swabs, whole blood, and milk samples. The result revealed that the direct culture of vaginal swabs is the best culturing method for isolation of *Brucella species* subsequently whole blood culture methods. However, there is no isolate from milk samples as shown in table 1.

**Table 1:** Culture results for isolation and identification of *Brucella spp.* at Al-hodeda governorate

Sample type	Positive no.	Negative	Total sample	Prevalence%
Vaginal swabs	6	50	56	10.7
Milk sample	0	39	39	-
Whole blood	2	98	100	2
Total	8	187	195	4

All isolate were grown on supplemented Brucella agar after 3 days, all of them were Modified Ziehl Nielsen stain (MZNS) positive and rapid positive urease test. Only two isolate show late positive urease test and produce H<sub>2</sub>S. Other biochemical test as in table 2.

**Table 2:** Biochemical tests for identification of *Brucella species*

Test	Result
MZNS	+
Appearance under the microscope	Coccobacilli
Urease	+
TSI	Grow
Oxidase	+
Citrate reduction	+
Motility	-
Primary culture growth after(day)	2-3

Eleven camels out of hundred camels examined were seropositive for *Brucella species* antibody; the overall seroprevalence of brucellosis in Al-Hodeida governorate was 11%.table 2.

**Table 3:** Seroprevalence of *Brucella spp.* infection in camels in Al-Hodeida governorate, western Yemen:

Test type	Total sample	Positive no.	Seroprevalence %
RBPT	100	11	11
A-fibril antigen	100	5	5
M-febrile antigen	100	6	6
MRT	39	2	5.1

Four aborted camels were seropositive for *Brucella species* which had history or have been recorded in current study. Therefore, exposure to *Brucella species* is positively associated with abortion cases in camels which are not commonly event in camels with highly odd ratio as in table 4.

**Table4:** Rose Bengal plate test for sero-positive brucellosis camels and its association with recording abortion cases:

Cases	Brucella +	Brucella -	Total	OR	X <sup>2</sup>	P value
Abortion	4	4	8	12.14	13.5	0.002
Non abortion	7	85	92			
Total	11	89	100			

## Discussion:

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 one of the major problems considered to be a predisposing factor leading to infertility and sterility along with the possible transmission of infection to man (FAO/WHO, 1986). Brucellosis in camels has been reported in Saudi Arabia, Kuwait, Oman, Iraq, Iran, Sudan, Egypt, Libya and Somalia. It has been reported even in racing camels in the United Arab Emirates (Refai, 2002). Brucellosis is classical zoonosis; the major source of infections remains contact with infected animals or handling of carcasses. Less frequently it is acquired through food (Kiel and Khan *et al.*, 1989; Cooper, 1992 and Stohr *et al.*, 1997).

Theoretically, all three known *Brucella species* can cause infection in camels (Higgins, 1986). *Brucella abortus* and/or *Brucella melitensis* have been isolated from milk, vaginal swabs, aborted fetuses, lymph nodes and hygromas of infected camelids from different countries.

In current study *Brucella species* was isolated from vaginal swabs and whole blood samples from 8 camels from which have isolation rate (10.7%) and (2%) respectively however, no isolate was obtained from milk samples this may be due to less sample size. Most samples was attempted to be *Brucella melitensis* except two isolate which show different biochemical profile would be *Brucella abortus* more confirmatory result is highly recommended. Radwan *et al.* (1995), who examined a large camel herd with 2536 dromedaries in Saudi Arabia from which a 12% abortion rate and a Brucella sero-prevalence of 8% were reported, isolated B. melitensis, biovars 1,2 and 3 from aborted camel fetuses. Similar differences in the sero-prevalence have been reported from Saudi Arabia by Radwan *et al.* (1992) Moustafa *et al.* (1998) reported on a serological survey in dromedaries and a brucellosis eradication

campaign in the eastern region of the UAE during a 5-year period. The highest prevalence was in 1991 with 5.8% reactors, whereas the lowest was in 1996 with 0.01%. Since no camels have been culled due to brucellosis, it is believed that the reduction in camel brucellosis was caused by the reduction in brucellosis in sheep and goats.

In this study 3 screening tests (RBPT, A & M febrile agglutinin test and MRT) were performed to evaluate the states of brucellosis in camels at Al-Hodeida governorate, they revealed a prevalence of 11%, 5-6% and 0% subsequently. This reflects the real situation of brucellosis among the contact farm animals which pay the attention to study the role of camels in transmitting brucellosis to other farm animals and vice versa. The prevalence rate of serologically positive sera measured by RBPT was 11% (table 3). The prevalence rate among camels was bit higher than those in Saudi Arabia reported by Radwan *et al.*, (1995) but is similar to that sero-prevalence founded in Somalia reported by Ahmed and Ibrahim (1980) and Bornstein (1984). Also the sero-prevalence of brucellosis in camels in observed here was found to be higher than that reported by Afzal and Sakkir (1994), Moustafa *et al.* (1998) and Refai (2002). In Yemen previous study was reported brucellosis prevalence of camel which was not recorded any cases of brucellosis by using ELISA as a diagnostic test AL-Shamahy (1999). These differences in sero-prevalence may be due to the difference in number of samples collected and the difference of diagnostic techniques used in each study. *Brucella species* is highly associated with abortion cases recorded in this study (table 4). According to various researchers, brucellosis in breeding camelids occurs in all of the known forms, whereby abortion is its most obvious manifestation (Acosta *et al.*, 1972; WHO/FAO, 1986; Radwan *et al.*, 1995).

## Conclusion

This survey confirmed the presence of *Brucella spp.* infection in Al-Hodeida region in Yemen, showing a significant prevalence rate in camels (11%). Intervention strategies should include safe breeding procedures, regular serology testing, slaughtering of infected animals and vaccination of uninfected herds of camels. The present study indicates that brucellosis seroprevalence in camels in Al-Hodeida governorate is quite high which it is likely to be a risk factor for human infection with *Brucella spp.* Therefore, it is imperative to take prevention and control measures to reduce brucellosis prevalence in camels in this governorate. This study throws a strong highlight on that camel should be included in the national program for control and eradication of brucellosis in Yemen, especially, this disease could be transmitted from animals or their products to human. Further seroprevalence study including more population and covering Yemen governorates is highly recommended.

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