MEDDOCS Open Access Publisher

ISSN: 2640-1223

Journal of Veterinary Medicine and Animal Sciences

Open Access | Research Article

Prevalence and Control of Brucellosis in Saudi Camel Herds

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Received: Dec 01, 2021

Accepted: Jan 17, 2022

Published Online: Jan 21, 2022

Journal: Journal of Veterinary Medicine and Animal Sciences

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: Brucellosis; Camel; Control; Herd; Prevalence.

Abstract

Background: The occurrence of brucellosis in camel might leads to significant economic lose in affected herd and it is associated with human brucellosis in Saudi Arabia due to habit of raw milk consumption. Thus, it is important to monitor the prevalence of *Brucella* infection to control it and to avoid economic loss.

Aim: The objectives of this study were to determine the prevalence and to control *Brucella* infection in Saudi camel herd.

Methods: Herd were grouped into group1 (H1), group2 (H2) and combined seronegative animals of H1 and H2 (Hc). Serum and milk samples were collected and analyzed twice-using Rose Bengal test, Competitive Eenzyme Linked Immunosorbent Assay (cELISA) and Real Time Polymerase Chain Reaction (rtPCR) to determine the sero-prevalence and to identify species of *Brucella* respectively.

Results: *Brucella* sero-prevalence in first and second test were 18% and 60% in H1, 3.6% and 4.6% in H2 and 62% and 29.7% in Hc. *Brucella melitensis* was the only species detected by rtPCR in milk but not in vaginal discharge samples. The prevalence of *Brucella melitensis* in H1 were 67% and 3% for first and second test respectively.

Conclusion: The reduction of sero-prevalence in the Hc and prevalence of *Brucella melitensis* in H1 milk support that test and slaughter strategy might help successful disease control and eradication if it is applied as early as disease prevalence is low.

Cite this article: Mohammed Babiker MH, Alamin A, Qasim I, Alsahaf A, Abdulal A, et al. Prevalence and Control of Brucellosis in Saudi Camel Herds. J Vet Med Animal Sci. 2022; 5(1): 1098.

Introduction

Brucellosis was first reported in camel in 1931 in Russia [1]. Since then, it has been reported from all camel-keeping countries [2]. Including Saudi Arabia [3]. Brucellosis in camels is caused mainly by *Brucella abortus* (B. *abortus*), and *Brucella melitensis* (*B. melitensis*), and it has the same health impact as those in bovine and small ruminants, such as abortion, stillbirth calves, fetal death, reduction in milk yield and infertility leading to significant economic loss [4]. In Saudi Arabia camel brucellosis may leads to human brucellosis due to habit of camel milk consumption. Thus, it is important to control Brucellosis in infected herd to avoid economic loss and the risk of zoonotic source to human.

It is well established that control of animal brucellosis depends on sensitivity of infected animal detection to determine the real prevalence; and *Brucella* species identification. Using a combination of serological tests such as Rose-Bengal test or Serum Agglutination Test (SAT) is an essential tool to screen large number of animals. These tests must be accompanied by another serological confirmatory test such as cELISA [5]. Moreover, the important of *Brucella* prevalence is positively associated with *Brucella* species; although none of the serological tests can differentiate between *Brucella* species due to cross reaction; PCR overcome the cross reactivity within *Brucella* species and with gram negative bacteria and enhance sensitivity of animal detection [6,7]. Using rose benghal and sero-agglutination test; seroprevalence of camel brucellosis was 4.3% in small Saudi camel herd and 8.6% in intensively raised animals [3]. Furthermore, the author reported that *Brucella melitensis* was the species associated with camel brucellosis in these herds which can represent a serious public health risk [3]. Thus, it is important to determine the precise brucellosis prevalence with *Brucella* species identification in the herd to better implement control strategy measure.

The selection of control strategies to brucellosis depends on the herd size, disease prevalence, and *Brucella* species. The prevalence of camel brucellosis follow two distinct patterns and it is positively associated with herd size. Low seroprevalence 5% appear to be associated with nomadic kept camels whereas high prevalence 8-15% was found in camel kept intensively. Successful strategies to control brucellosis in cattle or small ruminant herd include slaughter of identified infected animals, vaccination or combination of the two methods. Vaccination against brucellosis is practiced in countries with high prevalence, either in immature animals only whereas in other countries vaccination is practiced regardless sex or age. The 'test-and-slaughter' method is expensive and only recommended and feasible in countries where prevalence is not exceeding 2% [8,9]. In camel, unlike cattle or small ruminant, there is no specific strategy, to control or eradicate camel brucellosis. Despite those dromedaries were successfully vaccinated with B. *abortus* strain19 and with *B. melitensis* Rev1 [10,11]. Vaccination protocols are acceptable method, additionally test and slaughter can accelerate control of brucellosis, reduce economic and public impact. This study was conducted to investigate the prevalence and to control brucellosis in camel herd using test and slaughter of adult and vaccinate of young camels.

Materials and methods

Study area

The study area is located in Al- Muzahimiyah governorate (24°28' 17" North; 46°16' 51" East), about 59 kilometers southwest to Riyadh, Saudi Arabia. It is one of the areas with a high density of dromedaries camel.

Study herd

The herd is composed of total 1700 camels and it is divided to four sub-herds groups kept in 10 pens as follows:

- The original herd (H0): The herd is composed of 300 camels including Maggater, Maggahiem and cross of these two breeds and kept in two pens with separate workers. The herd used for milk production. This herd was not included in the control strategy, but prevalence and species of *Brucella* were investigated.
- **Herd1 (H1):** A total of 900 imported camels added to the farm and kept in 3 separated pens.
- **Herd2 (H2):** Atotal of 500 imported camels added to the farm and kept in 3 separated pens.
- **Combined Herd (Hc):** Seronegative animals of H1 and H2 were mixed together in one group to form combined group (Hc).

Herd management

The overall herd under closed system with zero grazing. The herd is fed with roughage including alfalfa, barley and concentrated feed. The herd is supervised by a veterinarian and vaccinated against enterotoxaemia and pasteurellosis but not against brucellosis. However, the newly born calves are vaccinated against *Brucella* using Rev1vaccine produced locally. The herd was unclassified based on age or physiological status.

Description of herd investigation

During the period of March 2017 to January 2019 the veterinarian of studied herd conducted MEWA to assist in checking for important diseases in the original herd and in imported camels. The MEWA team suggested four diseases to be investigated which were as follows Brucellosis, Q-fever, MERS, and Blood parasites. Animals positive for trypanosome were treated by one single dose of Quinapyramine sulphate while animals tested positive for Q-fever, MERS were kept untreated. For brucellosis the original Herd (H0) and Herd1 (H1) were screened twice before all animals were screened again to exclude positive animals from the herd to slaughterhouse.

Samples types and method of *Brucella* testing

For seroprevalence, serum was collected twice from all animals and tested using rose-bengal test and the results were confirmed by Competitive Enzyme Linked Immunosorbent Assay (cELISA). Rose-Bengal test was used according to European commission manual for animal health. In brief, serum and Rose-benghal antigen reagent brought to room temperature before each serum sample was mixed with equal volume of *Brucella* antigen reagent (30 µl) on one of the divided wells (2 cm) of white flat tile plate. The mixture agglutination results were read within 4 minutes for each sample and controls of positive and negative were included to optimized results reading. A confirmatory commercial cELISA (INgezim *Brucella* Compac 2.0,10 BRU.K3. INGENASA Company) was used for all rose-benghal positive samples as manufacture instructions. In brief, 100 µL of previously diluted sera in wash solution was placed into duplicate wells, A 100 µL of horseradish peroxidase conjugate was added and incubate the mixture for one hour. The mixture in

each well was washed 4 times and 100 µL substrate was added to each well before it the plate was incubated for 10 minutes at room temperature. A 100 µL of stop solution was added and the plate was read at 450 nm within 5 minutes. The percentage of inhibition in each sample was calculated as follow: PI = 100×(1-(OD sample/OD negative control). A sample give more than 40% inhibition was considered positive.

For *Brucella* species identification a representative samples including milk and vaginal cotton swabs from seropositive animals were collected and tested using Real Time Polymerase Chain Reaction (rtPCR) commercial kit for detection *B. melitensis* DNA (LightMix kit *Brucella melitensis* cat No. 40-0648-32), as recommended by manufacturer instruction In brief, DNA was extracted using MagNA pure Compact Nucleic Acid isolation Kit I, the DNA was digested using proteinase K, boiled, a designed primer target A85bp long fragment of the 25k Da outer membrane protein gene from *B. melitensis*. The DNA was denatured, amplified, melted then cooled. Thereafter, the hyperdization probes with LightCycler® Red 690 detected in chanel 705.

Results

Prevalence of camel brucellosis in original herd (H0) and herds under control (H1, H2 and Hc):

Before incorporation of control measure; initial testing of the herds indicates blooming of outbreak in the studied herd. The seroprevalence of camel brucellosis in the primary Herd (H0) was 12% where the representative milk samples revealed that 33% of the samples were *Brucella melitensis* using rtPCR (Table1). Likewise, seroprevalence of H1 on two consequence testing were 2.9% and 20% respectively. However, the Real Time Polymerase Chain Reaction (rtPCR), showed that *Brucella melitensis* was the only species detected in milk of H0 and H1. The prevalence of *Brucella melitensis* in (H1) camel milk was 67% and 3% on the first and second tests respectively (Table 1).

Figure 1: Initial Prevalence of camel Brucellosis in the original camel herd (H0) and H1.

Herd	Type of sample	Type of test	Number of tested animals	Number of +ve animals	Prevalence (%)
H0	Serum	ELISA	66	8	12.1
	Milk	RT PCR	6	2	33.3
H1	Milk	RT PCR	6	4	67
			32	1	3
	serum	ELISA	101	3	2.9
			272	54	20

Discussion

This study describes a trial to control brucellosis in open camel herd in high camel density area. We used Rose-Bengal Test (RBT) as screening test and Competitive Enzyme-Linked Immunosorbent Assay (cELISA) as confirmatory test to increase sensitivity of *Brucella* seroprevalence antibody detection in animal's samples. Moreover, we identified the circulated *Brucella* strain by rtPCR. In addition, we reduced the seroprevalence of camel brucellosis in herd using slaughtered of all tested positive and vaccinate the newly camel calves.

The recommended serological tests for *Brucella* detection in ruminants are Complement Fixation Test (CFT), Rose-Bengal Test (RBT), Sero-Agglutination Test (SAT) and Competitive Enzyme-Linked Immunosorbent Assay (c-ELISA) [12]. Despite none of the serological brucellosis tests are validated for use in camels yet. However, it was found that a combination of different serological tests can increase diagnostic efficacy in camels [13]. Initial screening of the original camel Herd (H0) revealed 12% of camel samples were seropositive for brucellosis where the representative milk samples revealed that 33% of the samples were *Brucella melitensis* by rtPCR (Table1). Similarly, two initial consequence screening of newly introduced herd H1 revealed an increase in *Brucella* seroprevalence from 2.9% to 20%; an indicative of *Brucella melitensis* outbreak blooming in both original (H0) and imported Herd (H1). Thereafter, the owner decided to screen all animals and to get rid of all infected animals due to the desire to combine imported camels with original herd. Since the original herd was not served by the same labors that serve H1; It is not possible that introduction of newly animals bring the infection to the original Herd (H0) alternatively the later herd was infected before or both herds were infected. The spread of camel brucellosis during sexual activity plays a subordinate role, but the primary shedding routes of *Brucella* organisms remains the uterine fluids (lochia) and placenta expelled from infected animals [14]. The seroprevalence of brucellosis in H1 and H2 were 18% and 3.6% on the first test respectively. After removal the positive animals; the seroprevalence of *Brucella* in H1 was 62% while in H2 was 4.8%. However; when seronegative animals were combined in one herd Hc; the seroprevalence reduced from 62% to 29.7% on two respective tests (Table 2). The severely increase in *Brucella* prevalence of H1 from 18% to 62% but not in H2 might attributed to intensive breeding of camels which increase the chance of transmission of infection between animals. These results are consistent with that the intensive system is associated with more incidences of diseases in Saudi Arabia previously reported Radwan et al, 1992). Similarly, [15] reported a higher incidence of camel brucellosis in intensively farmed camels than in free-grazing desert camels. Camel brucellosis caused by *B. melitensis* and B. *abortus* has been reported in all camel-rearing countries, except Australia and the incidence appears to be closely related to the breeding and husbandry practices [4]. The results of the real time polymerase chain reaction (rtPCR), showed that *Brucella melitensis* was the only species detected in milk of H0 and H1. The prevalence of *Brucella melitensis* in (H1) camel milk was 67% and 3% on the

Thereafter, the owner decided to screen and to get rid of all infected animals. The seroprevalence of brucellosis in H1 and H2 were 18% and 3.6% on the first test respectively. After removal the positive animals; the seroprevalence of *Brucella* in H1 was 62% while in H2 was 4.8%. However; when seronegative animals were combined in one herd Hc the seroprevalence reduced from 62% to 29.7% on two respective tests (Table 2).

Figure 2: Competitive Eenzyme Linked Immunosorbent Assay (cELISA) Seroprevalence of camel Brucellosis in the studied camel herds

Camel Herd group	Tests Group	Number of tested animals	Number of +ve animals	Prevalence (%)
H1	Frist test	950	172	18
	Second test	818	508	62
H2	Frist test	470	17	3.6
	Second test	453	22	4.8
Hc	First test	712	444	62
	Second test	424	126	29.7

first and second tests respectively (Table 1). These results are in agreement with Radwan et al [3] who reported that 26% of 100 milk samples were *B. melitensis* biovars (1 and 2(in seropositive Saudi Arabian dromedaries.

The choice of control strategy depends on a number of considerations, such as prevalence in different animal species, human clinical incidence and the capacity of veterinary services [13]. Marta et al. [16] reported three essential components to control brucellosis, these are strict biosecurity at the farm level, immunization of the susceptible population and test and slaughter programs. Dromedaries were successfully vaccinated with B. *abortus* strain 19 and with *B. melitensis* Rev1, but still very little is known about the optimal vaccination age in camels and their serological response. The control method of brucellosis used in Saudi Arabia is vaccination of the young animals especially in sheep and goat. However, we assumed that test and slaughter method and vaccination of young animals can be more efficient to eradicate the brucellosis in studied herd, because it's under good management there is no contact with other herds. The failure of eradication of disease from the study herd can be attributed to improper management, such as delay in separation and removal of positive camels from farm by sending them to the abattoir, improper disinfection of the farm after disposal of infected animals. The Interval periods between tests were not regular and not as recommended by OIE which should not exceed one month between each two successive tests. The last test done to the mixed herd (Hc) revealed that the prevalence was decreased to half (from 62 to 29.7%) which was due to strict removal and slaughter of all positive animals from the farm. This suggests successful resolution by test and slaughter strategy. In conclusion, to free camel herd from Brucellosis, we recommend regular testing of all animals, beside vaccination of newly camel calves.

Conclusion

To achieve free camel herd from Brucellosis, we recommend regular testing of all animals, removal and slaughter of all positive animals, vaccination of newly camel calves beside good management of the farm.

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