**Prevalence and Control of Brucellosis in Saudi Camel Herds**

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**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.

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

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, Magghahiem 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): A total 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 Immunosorbert 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×(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 hybridization probes with LightCycler® Red 690 detected in channel 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).

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].

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Number of tested animals</th>
<th>Number of +ve animals</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H0</td>
<td>ELISA</td>
<td>66</td>
<td>8</td>
</tr>
<tr>
<td>Milk RT PCR</td>
<td>6</td>
<td>2</td>
<td>33.3</td>
</tr>
<tr>
<td>Milk RT PCR</td>
<td>6</td>
<td>4</td>
<td>67</td>
</tr>
<tr>
<td>serum ELISA</td>
<td>101</td>
<td>3</td>
<td>2.9</td>
</tr>
</tbody>
</table>

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

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).

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

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

**References**


