Serodiagnosis for brucellosis in camels by rose Bengal and C-ELISA test in Iraq

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Abstract
Brucellosis is an occupational disease for veterinarians, farmers, and meatpacking workers. It causes heavy economic losses in livestock in developing countries and has an important public health issue for dairy product consumers. Little information is known about the camel brucellosis and its effect on human health. A study of the seroprevalence of camel brucellosis had been conducted in Al-Najaf province during the period from February 2018 to March 2019. One hundred seventy-two camels were involved in this study with ages between 3 to 9 years. The serum samples were screened by using the Rose Bengal plate test (RBPT) with positive samples tested again with the competitive ELISA test (cELISA). Our results show that out of 172 camel sera collected, 6 (6.97%) were positive for Brucella antibodies by Rose Bengal plate test of which 4 (4.6%) was confirmed by cELISA. Furthermore, on age distribution, for RBPT, the positive results were 4 camels (4.6%) and 2 camels (3.1%) for older and younger animals respectively, while in cELISA, 3 (3.4%) older camels were positive with only 1 (1.1%) younger camels. The result was statically significant (P<0.05). The study concluded that Brucella antibodies are present in camel and they were probably infected due to contact with infected animals, and the cELISA test is a good test for confirmation of the RBPT.

Keyword: Brucella, camel, seroprevalence, Rose Bengal, cELISA

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Introduction
Camels have been considered as one of the important financial gains for the nomadic population, and also as a support for the national economy in several developing countries for their role in transport, and food supply in form of meat and milk (1, 2). Brucellosis is a debilitating zoonotic disease due to bacteria of the genus Brucella and Camels are highly susceptible to brucellosis caused by Brucella melitensis and Brucella abortus (3). There are approximately 58,000 camels in Iraq, according to the FAO statistics of 2011 (4). Many factors can cause variation in the prevalence of brucelloses such as climatic conditions, geography, sex, species, age, and diagnostic tests applied (5).

The disease causes abortion and birth of non-viable offspring is female, orchitis, and epididymitis in male, and cause infertility in both (6). Camels are not the primary hosts of Brucella, but they are susceptible to both B. abortus and B. melitensis infections (7). Difficulties can arise for the diagnosis of camel brucellosis, especially as this disease exhibits only a few clinical signs in contrast to its clinical course in cattle (8). This study was carried out for:

1- Monitoring of brucellosis in camels by various methods.
2- Detect the relationship between infection and age of the animals.
3- Constituting baseline data for further study of Brucella infections in Al-Najaf province.

Materials and Methods
Samples collection
For the experimental animals in the current study; 172 camels of both sexes were selected randomly from different places in Al-Najaf province, Iraq, from February 2018 to March 2019. About 10 ml of blood without anticoagulant was collected from the jugular vein at the time of slaughter using sterile plain evacuated tubes. The tubes were labeled with the animal’s sex and age (3-6 years and 6-9 years).
The samples were kept at 4°C overnight then centrifuged at 3000 rpm for 10 minutes for separation of serum. The collected sera coded according to age and stored at −20°C till analysis for serodiagnosis. 

**Serological Tests**

Initially, the antibodies to *Brucella species* were detected by Rose Bengal plate test (RBPT) using a commercially available test kit, which was carried out according to Alton et al. (1988). The positive sera tested by (RBPT) were retested by the cELISA Kit and performed by following the procedures described by the manufacturers (*serelisa brucella* kit-synbiotic-Europe Code: ASBRU3). 

**Statistical analysis:**

Descriptive statistics were calculated using Open Epi version 3.03 to determine the level of *Brucella* seroprevalence in relation to age and sex of camels. A P-value lower than 0.05 was considered statistically significant. 

**Results and Discussion**

Brucellosis is a highly contagious infectious disease that has economic and zoonotic importance. It affects the productivity and reproductive efficiency of animals through the reduction of milk production after abortion, loss of offspring, and infertility (temporary or permanently) (5, 33). The RBPT is widely used as a screening test for brucellosis because of its simplicity, rapidity, and field suitability. However, the result of RBPT requires confirmation by complement fixation test (CFT) or enzyme-linked immunosorbent assay (ELISA) as recommended by (9, 32). The evaluated cELISA kit seemed to be rapid, simple, sensitive, and specific for detecting antibodies to *Brucella*. 

The overall seroprevalence rate of brucellosis in examined camels was (6.97%) and (4.65%) through RBPT and cELISA, respectively, in which 8 animals out of 172 were positive for RBPT and 4 animals were positive for cELISA (Table 1, 2). 

**Table 1: Results of serological diagnosis of brucellosis by RBTP and confirmed by cELISA in camels in different locations in the Al-Najaf province**

<table>
<thead>
<tr>
<th>Test name</th>
<th>Number of examined samples</th>
<th>Number of positive samples</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBTP</td>
<td>172</td>
<td>6</td>
<td>6.97%</td>
</tr>
<tr>
<td>cELISA</td>
<td>172</td>
<td>4</td>
<td>4.6%</td>
</tr>
</tbody>
</table>

**Table 2: Prevalence of brucellosis in camels according to age**

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of positive RBPT</th>
<th>Percentage %</th>
<th>Number of positive cELISA</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-6 years</td>
<td>2</td>
<td>3.1%</td>
<td>1</td>
<td>1.1%</td>
</tr>
<tr>
<td>6-9 years</td>
<td>4</td>
<td>4.6%</td>
<td>3</td>
<td>3.4%</td>
</tr>
</tbody>
</table>

The general prevalence of brucellosis of the current study is in agreement with the study conducted in Libya (10), Sudan (11), Egypt (12) and Afar region of Ethiopia (13) with prevalence rates of 4.1%, 5.5%, 7.3%, and 7.6%, respectively. This may be due to similarities in ecological and geological environments and the management of livestock in these areas. Nevertheless, the result of this study is higher than the observations recorded by (14, 22), with prevalence rates of 0%, 3.03%, 1.4%, 1.86%, 2.2%, 2%, 3.6%, and 0.3 – 1.9% in Ethiopia, Kirkuk province in Iraq, Saudi Arabia, Saudi Arabia, Nigeria, Ethiopia, Southern Ethiopia, and Somalia, respectively. While it was lower than ratios of 17.2% recorded by (5) in Pakistan, 7.5% recorded by (13) in Kano, 11.42% mentioned by (24) in Nigeria, (25) in Kenya, 19.4% (26) Jordan, 30.5% (27) in Sudan, and 38.0% (28) in Sudan also. All these variations in seroprevalence among different regions and times could be due to variations in animal management and husbandry practices, the number of susceptible camels, presence of high number of camels in the herds and mixing of aborting camels with normally parturient camels and difference in number of herds involved in sample unit, virulence of the organisms, absence of veterinary services, type of applied diagnostic tests, close contact with infected domestic and wild animals, population intensity, lack of...
awareness about the disease in camels (14, 29). In relation to husbandry practices, these animals are usually kept overcrowded and reared in open systems with different ages and without differentiation of aborted and pregnant ones and even males and females are housed together with high stocking density, all these factors play important roles in the spread of the infection. Another reason affecting the prevalence rate is the traditional farming; farmer does not have knowledge about brucellosis and usually keep Brucella infected animals for breeding purposes which serves as a source of infection. On age distribution, 3 (3.4%) older camels (aged 6-9 years) were positive by only one younger camel (aged 3-6 years) (1.1%) was positive as shown in (Table 2). The result was statically significant (P<0.05). Our results are in agreement with (19), who reported that adult camels had more infection rate than young camels with ratio of (12.5%) for 3 adult camels and (0.6%) for one camel respectively, (26) who recorded a higher prevalence rate in adult (64.8%) than in young camels (35.2%) in southern province of Jordan, (13) also reported a higher infection rate in adult (13.8%) than in young camels (0%) in Afar region in Ethiopia, and (30) also mentioned higher prevalence range of (6.8% - 9.2%) in older camels than younger age (4.2%). This difference in infection rate may be due to the Brucella bacteria having a higher tendency to the reproductive organs, and the young animals are not sexually mature so it may be resistant to Brucella infection for this reason (31). Seroprevalence of camels brucellosis appeared to follow two distinctive patterns, low prevalence below 5% in nomadic or extensively kept camels and high prevalence 8–15% in camel kept intensively or semi intensively (18). The infection is caused by different biotypes of B. abortus and B. melitensis (2). In conclusion, our study showed that Brucella antibodies are present in camels in Al-Najaf city with the infection possibly as a result of contact with infected camels. Although there were variations in the prevalence rates when compared to similar studies in the same and other areas, the reason could due to the fact that the present study is an abattoir survey. The cELISA test is a good test to confirm the rose Bengal plate test.

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References