SEROPREVALENCE OF BRUCELLOSIS AMONG SUSPECTED CASES OF CAMELS.

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ABSTRACT

In the current study, a serological survey on camel brucellosis was carried out for a total number of 1148 camels (Camelus dromedaries) employing the rose bengal test (RBT) for infected-herd screening. The suspected positive RBT serum samples were confirmed by applying slow agglutination test (SAT), rivanol test (RT) and indirect enzyme linked immunosorbent assay (iELISA) as confirmatory tests. From suspected positive RBT she-camels, milk samples were collected under sterile conditions and subjected to isolation and identification of Brucella organisms by culturing of milk samples onto Brucella agar selective media, then the isolated pure colonies were identified biochemically. The results revealed that, out of 1148 camel serum samples, 164 (14.29%) were positive for RBT. Out of the 164 suspected positive RBT serum samples, 133 (11.58%), 128 (11.15%) and 142 (12.37%) were positive for SAT, RT and iELISA respectively. Out of the 90 she-camel milk samples (She-camels giving suspected positive RBT samples) only two isolates of Brucella were isolated and identified as Brucella melitensis biovar-3 with a percentage of 2.2%. The results of the present investigation revealed that, the Brucella spp. exists within the camel population in Egypt. This finding highlights the need for further research, including the isolation and characterization of the causative agents, reliable epidemiological studies and the need to implement a transparency policy and effective control measures in Egypt. Moreover, the health authorities should include camels in national programs and apply intervention strategies in order to prevent and control brucellosis in the future.
INTRODUCTION

Camels are the most robust animal species in production and survival under harsh environmental conditions. Although many pastoral groups and communities throughout the world depend on camels for their livelihood, the health status of camels has not yet received proper attention from researchers and scientists. Camels are highly susceptible to brucellosis caused by *Brucella melitensis* and *Brucella abortus*. Difficulties can arise in diagnosis of camel brucellosis, especially as this disease provokes only few clinical signs in contrast to its clinical course in cattle and so, many infected camels are silent carriers of brucellosis. (Gwida et al., 2012). Because none of the commonly used serological test can be perceived as a perfect test for *Brucella* diagnosis in camel and most serological tests used for camels have been directly transposed from cattle without adequate validation. Of imminent concern is the fact that, brucellosis can be easily transmitted from animals or their products to humans mainly via milk leading to a high number of human brucellosis cases and serious public health problems. Farmers from nomadic areas believe that, raw camel milk has a curative effect on the digestive system (Kiel and Khan, 1999 and Gwida et al., 2011). The infection of camel herds depends on the *Brucella* spp. prevalent in other animal species sharing the same habitats, and on husbandry methods (Musa et al., 2008). The infection seems to be widespread among camel herds in Africa and on the Arabian Peninsula (Gwida et al., 2012). Classical tests for the diagnosis of brucellosis i.e. culture and phenotypic characterization, are laborious, time-consuming, pose the risk of infection, and can generate discordant results. Isolation of the causative agent often fails in routine diagnosis. Serological tests such as rose bengal test (RBT), slow agglutination test (SAT) and rivanol test (RT) are widely used for the detection of antibodies to *Brucella* spp. especially at herd level. However, their specificity is limited by antigenic cross-reactivity and the false positive results encountered in infections with other organisms (Mert et al., 2003; Thirlwall et al., 2008 and Varshochi et al., 2011). The specificity of these serological tests is particularly important during a program of *Brucella* eradication. Indirect enzyme linked immunosorbert assays (iELISAs) have been developed and used in various countries for sero-diagnosis of brucellosis in cattle, and other animals (Omer et al., 2001 and Mainar-Jaime et al., 2005). Furthermore, the World Organization for Animal Health (OIE) has approved an indirect ELISA (iELISA) for testing serum and milk (Gall and Nielsen, 1994; Vanzini et al., 2001,
and OIE, 2008). However, such kind of work in camels is limited. Therefore, the objective of this study was to use a sensitive and specific diagnostic tests, to estimate the animal- and herd-level seroprevalence of camel brucellosis in Egypt to highlight the epidemiologic, economic and public health impact of camel brucellosis as a basis for designing effective control strategies.

MATERIALS AND METHODS

Serological tests:
A total of 1148 serum samples were collected from camels (Camelus dromedaries), then they were subjected to RBT, the positive results of RBT were confirmed by SAT and RT according to Alton et al., (1988). In RBT, any degree of agglutination was considered as positive results. For SAT, visible agglutination at dilution of 1/40++ or more was considered positive. For RT, complete agglutination at dilution of 1/25++ or more was considered positive. Then, iELISA was applied for suspected RBT serum samples, an iELISA kit (Brucelisa) provided by the VLA, (an executive agency of the Department for Environment, Food and Rural Affairs), which contained all the necessary reagents. The test was performed according to the manual which is accompanied with the kit (Ekgatat et al., 2010).

Bacteriology:
Camel milk samples were collected under sterile conditions from she-camels that gave suspected positive RBT and submitted to bacteriological examination. They were cultured onto Brucella selective medium and incubated at 37°C in condition of 10% CO₂ for 5 days. The plates were examined after 3 days and then it was observed in each day to observe the development of the bacterial colonies. The suspected colonies were identified biochemically using phenotypical methods (morphology, CO₂ requirements, H₂S production, urease, catalase and oxidase activity, nitrate reduction, lactose fermentation, citrate utilization, grow in presence of thionine and fuchsin dyes (at different concentrations: 1: 50,000 and 1: 100,000), lysis by Tbilisi phage and agglutination with A and M anti-sera) MacMillan (1990).
RESULTS

Serological results:
Results of different used serological tests revealed that out of 1148 camel serum samples, 164 (14.29%) were positive for RBT. Out of the 164 suspected positive RBT serum samples, 133 (11.58%), 128 (11.15%) and 142 (12.37%) were positive for SAT, RT and iELISA respectively. Tables (1, 2, 3, 4).

Table (1): Correlation between RBT and SAT results.

<table>
<thead>
<tr>
<th>Suspected positive RBT (n=164) (14.29%)</th>
<th>SAT positive Titres (n=133) (11.58%)</th>
<th>Negative Titres</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*1/10 *1/20 1/40 More than1/40</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>14 (1.22%) 17 (1.48%) 119 (10.36%)</td>
<td>14 (1.22%)</td>
</tr>
</tbody>
</table>

n: Total Number
RBT: Rose Bengal Test.
SAT: Slow Agglutination Test

Table (2): Correlation between RBT and RT results.

<table>
<thead>
<tr>
<th>Suspected positive RBT (n=164) (14.29%)</th>
<th>RT positive Titres (n=128) (11.15%)</th>
<th>RT Negative Titres</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/25 1/50 1/100 1/200</td>
<td>36 (3.13%)</td>
</tr>
<tr>
<td></td>
<td>108 (9.41%) 11 (0.96%) 9 (0.78%)</td>
<td>0.0</td>
</tr>
</tbody>
</table>

n: Total Number
RBT: Rose Bengal Test
RT: Rivanol Test

Table (3): Correlation between RBT and iELISA results.

<table>
<thead>
<tr>
<th>Suspected positive RBT (n=164) (14.29%)</th>
<th>iELISA positive results</th>
<th>iELISA Negative results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>142 (12.37%)</td>
<td>22 (1.92%)</td>
</tr>
</tbody>
</table>

n: Total Number
RBT: Rose Bengal Test
iELISA: Indirect ELISA
Table (4): Comparison between the different used serological tests (SAT, RT and iELISA) results of the suspected RBT (n=164).

<table>
<thead>
<tr>
<th>Test</th>
<th>Results</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>SAT</td>
<td>*133</td>
<td>0.0</td>
<td>(11.58%)</td>
</tr>
<tr>
<td>RT</td>
<td>128</td>
<td>36</td>
<td>(11.15%)</td>
</tr>
<tr>
<td>iELISA</td>
<td>142</td>
<td>22</td>
<td>(12.37%)</td>
</tr>
</tbody>
</table>

n: Total Number  RBT: Rose Bengal Test  RT: Rivanol Test  iELISA: Indirect ELISA  *: Titre considered negative results "14 (1/10) and 17 (1/20) titres"

**Bacteriology:**
Out of the 90 she-camel milk samples (She-camels giving suspected positive RBT samples) only two isolates of *Brucella* were isolated which were identified as *Brucella* melitensis biovar-3 with a percentage of 2.2%.

**DISCUSSION**

Brucellosis in livestock causes enormous losses for economies of developing countries and poses a severe health risk to consumers of dairy products. Little information is known especially on camel brucellosis and its impact on human health. Control of brucellosis in livestock and human depends on the reliability of the methods used for detection and identification of the causative agent. However, diagnosis of brucellosis in camels is frequently difficult. The disease can mimic many infectious and non infectious diseases. Characteristic clinical signs of brucellosis in camels are often lacking and diagnostic methods are not evaluated yet. In the present study, the seroprevalence of camel brucellosis was 14.29%, 11.58%, 11.15%, and 12.37% using RBT, SAT, RT and iELISA respectively. Tables (1, 2.3, 4)
These results indicated that, many infected camels might be silent carriers for brucellosis and their products may pose a serious health problem for consumers. These observations were supported by Abu-Damir et al. (1989) and Gwida et al. (2011). This seroprevalence showed high incidence than that previously reported in Egypt. This might be attributed to the fact that, the camels were imported from Sudan and Somalia where a high prevalence of camel brucellosis is known (Azwai et al., 2001; Queipo-Ortuno et al., 2005; and Al-Ruwaili et al., 2012). Nevertheless, seroprevalence of brucellosis in camels has to be examined in confirmatory studies to evaluate the importance of the disease in camel population. Other factors that might influence the prevalence of camel brucellosis included management system, the herding of different species together, use of common pastures and water sources, age, breed, sex, lactation, status and season (Mohammed et al., 2011 and Gumi et al., 2013). Moreover, Lack of proper surveillance and control measures in most parts of Africa may be contributing to this increase, as may the importation of animals and their products from more developed countries despite the preventive and control measures in such countries (McDermott and Amiri, 2002; schelling et al., 2003; Mai et al., 2012 and Bekele et al., 2013). It was advisable to combine at least two serological test methods to screen brucellosis on herd level. This finding was in accordance with the procedure of monitoring in other animal species. Thus, the sensitivity will be increased. Having in mind these facts, a camel posing a risk for consumers was considered either to have Brucella DNA in its blood samples or being positive for the presence of antibodies confirmed by two independent serological test systems (Gwida et al., 2011 and 2012). The different serological tests which were applied in the current study, giving no relevant difference in results, inline with the report of Gómez et al. (2008) and Gwida et al. (2011). Nevertheless, the highest positivity was with RBT (14.29%), that many of the RBT results were falsely positive because of its relatively low specificity and very high sensitivity, Moreover, the cross reactivity with other Gram-negative bacteria which has lipopolysaccharides (LPS) Ochains similar to those of brucella. These organisms have agglutinins capable of reacting with Brucella antigens, thus giving false positive reactions. Despite these limitations, the RBT may be used as a screening test to ascertain exposure of animals to infection due to Brucella species (Kaltungo et al., 2013). The seroprevalence rate was lower with the SAT (11.58%) than RBT (14.29%) (Table, 2) and this finding may be
attributed to the increase in specificity of this test. The results agreed with the finding of (Bertu et al., 2010; Dashti et al., 2012 and Kaltungo et al., 2013). Moreover, SAT specificity was likewise dependent on the cutoff titre used and the prevalence of the disease in the population tested (Pabuccuoglu et al., 2011; Shemesh and Yagupsky, 2011 and Asaad and Alqahtani, 2012). The lowest percentage of the serological tests used in the current study was that showed by RT (11.15%) (Table 3). This finding was coincided with results recorded by Mikolon et al. (1998) and Acosta-González et al. (2006) who stated that, the Rivanol test detects principally IgG1, and to a lesser extent IgG2, because initial treatment of sera with Rivanol removes IgM by precipitation, reduces the reactivity of IgG2, and promotes the reactivity of IgG1. This gives the rivanol test low sensitivity but high specificity. The obtained results of RBT and iELISA were (14.29%) and (12.37%) respectively (Table 4). Various studies declared that, a combination of RBT for screening infected herds and the iELISA for identifying infected individuals was considered to be a quite appropriate and effective diagnostic tool for large-scale serological survey of brucellosis (Kashiwazaki et al., 2012). In contrast, others found more camel reactors to ELISA than RBT (Gumi et al., 2013) where iELISA was reported as a highly specific and highly sensitive test. Nevertheless, lower seroprevalence with SAT than that with iELISA due not only to the intrinsic factors of each test but also to the immunoglobulin classes that, the tests target (Nielsen,2002). Culture-positivity was accepted as the gold standard in the diagnosis of brucellosis (Ciftçi et al., 2005). In the present study, two isolates were isolated out of 90 milk she-camel samples (2.2%) and were identified as B.melitensis biovar-3. This finding was reported by Bassiony et al. (1996) and Ahmed et al. (2010). While, Ben Shimol et al. (2012) isolated B.melitensis biovar-1. On the other hand, Gwida et al. (2011) declared that, although their results revealed only B. abortus in camel serum, this fact does not ensure that these camels are infected with B. abortus only since B.melitensis was isolated from camels of the same origin earlier. Previous reports described this Brucella species (B. melitensis biovar-3) as the most prevalent in Egypt (Refai, 2002; Samaha et al., 2008 and Menshawy et al., 2014). Thus, the infection of camel herds depends on the Brucella spp. prevalent in other animal species sharing the same habitats, and on husbandry methods (Musa et al., 2008). The results of the present investigation revealed that, the Brucella spp. exists within the camel population in Egypt. This finding highlights the need for further research, including the isolation and
characterization of the causative agents, reliable epidemiological studies and the need to implement a transparency policy and effective control measures in Egypt. Moreover, the health authorities should include camels in national programs and apply intervention strategies in order to prevent and control brucellosis in the future.

**Conclusion:**
From the above mentioned results, it can be concluded that, for diagnosis of camel brucellosis, sera must be examined by more than specific test as (ELISA) beside screen test (RBT).

**REFERENCES**


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