Seroprevalence of Camel Brucellosis in Egypt

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1. Abstract

The current study was performed to evaluate the presence of *Brucella* antibodies in serum obtained from camels in Egypt. Sero- prevalence study was carried out in three governorates of Egypt during the period of October 2016 to December 2017. A total of 312 serum samples were collected from provinces of Matrouh, Aswan, and Giza. Samples of serum were screened using the Rose Bengal plate test (RBPT) then positive samples confirmed by ELISA and CFT.   Results revealed that out of 312 camel sera collected, 89 (28.5%) were positive for *Brucella* antibodies by Rose Bengal plate test then when confirmed by CFT and ELISA the results revealed 81 (91.01%) were positive by CFT and 87 (97.75%) were positive by ELISA, respectively. The results of this study confirm that RBPT, CFT, and ELISA can be used for diagnosis of camel brucellosis and understanding the epidemiology of camel brucellosis for performing future effective control programs in Egypt.

Keywords: Camel, Brucellosis, serological tests.

2. Introduction

Camels play a vital socio-economic role within the agriculture system in many parts of the world. Many people depend on the camel for meat, milk, and hair production as well as it stills an important mean of drought and transportation **(Elsawalhy et al., 1996).**

Brucellosis was reported in camels as early as 1931 **(Solonitsuin, 1949).** In all camel-rearing countries except Australia, camel brucellosis had been reported. It is an insidious disease rarely developed clinical signs and mainly causes problems in the diagnostic laboratory because there is no sufficiently validated test **(Wernery 2014; Sprague et al., 2012).** Camel brucellosis caused mainly by one of three Brucella species (*B. abortus, B. melitensis, B. ovis*) **(Higgins, 1986; Seifert, 1996).** *B. melitensis* had been mainly reported in Africa and the Middle East while B. abortus is widespread in the former USSR **(Wernery, 2014).** Camels mainly infected via spill-over from sheep, goat, and cattle in contact with it. As that all *Brucella* spp. and biovars infecting other ruminants have also been isolated from camels **(Sprague et al., 2012)**. Brucellosis causes significant loss of productivity in camels through late first calving age, long calving interval time, low herd fertility, and

comparatively low milk production **(Wernery and Kaaden, 2002).** Individual cases of abortion, fetal death, mummification, delayed sexual maturity, infertility, stillbirth, mastitis, orchitis, and joint disease might be encountered in naturally infected camels with *B. abortus* **(Higgins, 1986; Obeid et al., 1996; Musa and Shigidi, 2001).** The prevalence of camel brucellosis was up to 40% in certain regions and poses problems to camel keeping countries **(Wernery, 2014).** Regarding the origin of infection, there are few reports, camel to camel transmission or the persistence of disease in the herd **(Schulze zur Wiesch et al., 2010; Gwida et al., 2012; Sprague et al., 2012; Wernery, 2014).** Camel brucellosis was an insidious disease since it hardly provokes any clinical signs **(Musa and Shigidi, 2001).** Prevalence of brucellosis in apparently healthy camels indicates that many infected camels might be silent carriers for brucellosis and their products may pose a serious health problem for consumers **(Bekele, 2004).** Also, the disease has an impact on the export and import of animals constraining livestock trade **(Radostits et al., 2006).** Using serological tests for diagnosis of cattle brucellosis may be adequate for the diagnosis of brucellosis in camels.

However, there is no validation for brucellosis serological test for camel sera was done **(Gwida et al., 2012).** In Egypt, several studies on the seroprevalence of camel brucellosis had been done by **(El-Sawally et al., 1996; Abdel Moghney, 2004; El-Sayed et al., 2017).** The majority of studies on camels brucellosis use a combination of serological methods to increase the efficacy of diagnosis CFT recommended by many authors as it the most sensitive and specific test for brucellosis **(Wernery, 2014).** Although CFT was recognized as a good test when correctly performed, it has many practical drawbacks: it was cumbersome, time-consuming and difficult to standardize

(Uzal, 1995). To overcome the problems of other serological tests different enzyme-linked immunosorbent assays (ELISA) have been developed. Also, ELISA could detect *Brucella* carriers which were seronegative by RBT and CFT **(Van Aert et al., 1984).** The aim of this investigation was to assess the seroprevalence of camel brucellosis in three different locations (Matrouh, Giza, and Aswan) provinces using RBPT, CFT, and ELISA in order to gain data aid us for making future effective control of camel brucellosis in Egypt.

3. Materials and methods

The study was assessed and agreed by the Animal Care and Welfare Committee Ethics, University of Sadat City, Egypt.

Animals

A total of 312 camel serum samples of mixed ages and both sexes were collected from three provinces (Matrouh, Aswan, and Giza) in Egypt during the period of (October 2016 to December 2017). All of these animals were not vaccinated against brucellosis. All camels were clinically normal at the time of sampling and according to the owners; none had previously shown clinical signs of brucellosis.

Blood sera

The blood samples were collected from camel jugular vein. The collected samples were kept in the refrigerator overnight giving the chance for the serum separation then centrifuged at 3000 r.p.m. for five minutes. Clear sera were siphoned off and stored in cryotubes at -20_o C until its use for serological studies **(Alton et al., 1988).**

All collected Serum samples were initially screened by RBPT using RBPT antigen according to **(Alton et al. 1988).** Samples positive by RBPT were examined by

complement fixation test (CFT) and Enzyme-linked immunosorbent assay (ELISA).

Rose Bengal plate test

Briefly, 30 µl of RBPT antigen produced by (CZV veterinaria, S.A. Aptdo. Pontevedra, Spain) were added to an equal volume of serum on a ceramic tile. The sera and the antigen were mixed with an applicator stick and rocked gently. It was observed for four minutes for agglutination. The result was graded as +1, +2 or + 3 based on the degree of agglutination **(OIE, 2013)**

Complement fixation test

Antigens used for CFT was supplied by (Institute Pourquier, France). According to OIE, Positive and negative control sera are the national reference sera standardized. Positive control sera contain 595 International CFT Units (ICFTU) per milliliter for CFT **(OIE, 2000).**

ELISA

Indirect enzyme-linked immunosorbent assays (ELISA) (for the detection of antibodies in serum and milk) and ELISA with the ability to distinguish vaccinated animals from animals infected with *Brucella* spp. was performed according to

The manufacture instruction of kit; SERELISA® Brucella OCB Ab Mono Indirect (ASBRU30CB); Synbiotics Europe 2, rue A.fleming 69007 Lyon- France..

4. Results and Discussion

The prevalence of camel brucellosis in different three governorates by serological tests as presented in (Table 1). Using RBT as a screening test was positive (28.52%) (89 positive from 312 serum samples) and (71.47%) was negative (223 negatives from 312 serum samples). And out from (89) positive samples by RBT revealed that (81) samples were positive by CFT (91.01%) and (8) samples showed negative results by CFT (8.99%) (Fig.1). Also, out from (89) positive samples by RBT revealed that (87) samples

were positive by ELISA (97.75%) and (2) samples only showed negative results by ELISA (2.25%). The square test showed a highly significant correlation of all tests with the latent class ($p < 10_{12}$ or lower) as presented in (Table 2).

Brucella infection in farm animals was 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 **(Wasseif, 1992)**. Brucellosis in camels has been reported in Saudi Arabia **(Alshaikh et al., 2007)**, Kuwait **(AL-Khalaf and EL-Khaladi, 1989)**, Jordan **(Dawood, 2008)**, Yemen **(AL-Shamahy, 1999)**, Iran **(Ahmad and Nemat, 2007)**, Sudan **(El-Ansary et al., 2001)**, Egypt **(Abdel Moghney, 2004)**, Libya **(EL-Boshy et al., 2009)** and Somalia **(Ghanem et al., 2009)**. It has been reported that even in racing camels in the United Arab Emirates **(Moustafa et al., 1998).** In the diagnosis of brucellosis, serological investigation still has played a dominant role **(Konstantinidis et al., 2007).** RBPT was used as screening for the diagnosis of brucellosis **(Farina, 1985)**. In the present study RBPT, CFT and ELISA were used as screening and confirmatory tests for diagnosis of camel brucellosis and detection of naturally infected cases in a total of 312 dromedary camels during the period between October 2016 to December 2017 from three provinces (Matrouh, Aswan, and Giza) in Egypt. In the present study screening test as (RBT) was performed in the serum samples and revealed an overall prevalence (28.52%). In Egypt, the seroprevalence of camel brucellosis has been reported by different authors at different localities using different tests. The present results were higher than that recorded by **(Abdel Moghney, 2004)** (9.26%), **(Al-**

Gaabary and Mourad, 2004) (6.75%) and **(El-Sawally et al., 1996)** (11.3%). The differences in seroprevalence observed from the previous researchers might be due to differences in herd size, camel origin, tests used, management conditions, season variation and the presence or absence of infectious foci, such as Brucella infected herds, which could spread the disease among contact herds. High prevalence appears to be due to the fact that these camels were imported from Sudan which is known to have a high prevalence of 23.80% **(Musa et al., 2008)** and 37.5% **(Omer et al., 2010).** These studies attributed insufficient preventive measures, the lack of adequate control programs and uncontrolled animal transportation across "open" borders. Moreover, we used CFT as a confirmatory test for the positive serum samples **(OIE, 2012).** In the present study, 89 positive serum samples by RBT were examined by two confirmatory tests (CFT, ELISA) and seroreaction was (91.01%) and (97.75%) positive results respectively. The higher reactions were recorded for ELISA followed by CFT. The present results were higher than those recorded by **Abdel Moghney (2004)** (10.3%). According to the available literature, Sharkia Governorate recorded an incidence of 8%, Kaliobia 4%, and Dakahlia 6% **(Barsoum et al, 1995),** Behira 8.74% **(Abdel Moghney, 2004).** While in the present study (Matrouh, Aswan and Giza) provinces recorded an incidence of (28.52%), (91.01%) and (97.75%) for RBPT, CFT, and ELISA test, respectively. This difference in the results may be due to escaping of some imported positive reactors during quarantine measures, lacking of a national program for camel brucellosis eradication including periodical testing and slaughtering of positive cases, absence of a vaccination program for camels according to Egyptian field strains and which proved with imported camels. Matrouh and Aswan provinces considered on the

borders of Egypt and the majority of camels slaughtered in Egypt are coming from neighboring countries, which will be a mode of transfer of infection if they have the micro-organisms **(Abdel Moghney, 2004).**

In the present investigation, all camels were clinically normal at the time of sampling and according to the owners, none had previously shown clinical signs of brucellosis. Results of this investigation indicate that many infected camels might be silent carriers for brucellosis and their products may lead to a serious health problem for human. Our observations are supported by a study **(Abu Damir et al., 1990)**.

Conclusion

The results of this investigation revealed that camel brucellosis is prevalent in the studied areas in Egypt. The prevalence of brucellosis using RBT was (28.52%) among the examined camels in three provinces (Matrouh, Aswan, and Giza) in Egypt. The Rose Bengal plate antigen test is a rapid test and can be used as a screening test for Brucella. A confirmatory test of high specificity and sensitivity such as CFT and ELISA can be used for the diagnosis of brucellosis. The present data highlights the need for further research, including the isolation and characterization of the causative agents, reliable epidemiological studies, implement a transparency policy, and effective control measures in Egypt

5. References

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Items	Serological tests					
	RBPT		CFT		ELISA	
No. of tested samples	312		89		89	
Results	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
	89	223	81	8	87	2

Table 1: Seroprevalence of brucellosis among camels based on different serological tests

Test	Correlation %		
	Total	Positive	Negative
RBPT	99 %	99 %	97 %
ELISA	98 %	98 %	99 %
CFT	97 %	97 %	98 %

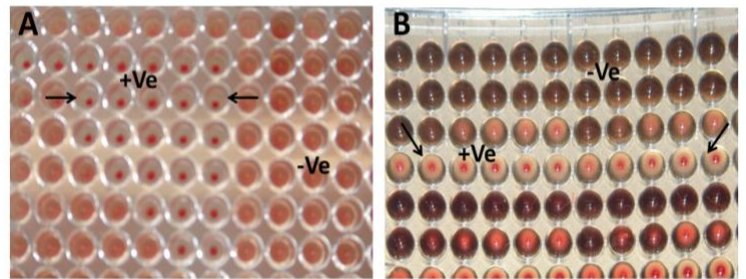**Table 2:** Correlation of tests calculated according to the latent class model

Fig.1: Plate complement fixation. (A, B) showed the positive sample (aggregation of sheep RBCs at the bottom of well) (black arrow) and the negative samples (hemolysis of RBCs).