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Full Length Research Paper

Sero-prevalence of camel brucellosis (*Camelus dromedarius*) and phenotypic characteristics of *Brucella melitensis* biovar 3 in Shalateen City, Red Sea Governorate, Egypt

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The objective of this study was to estimate the sero-prevalence of brucellosis in camels in Shalateen city, Red sea Governorate. A total of 801 Sera were collected from apparently healthy dromedary camels from 2014 to 2015 spring. Sera were consequently serologically tested and confirmed using Rose Bengal plate test (RBPT), buffer acidified plate antigen test (BAPAT) and complement fixation test (CFT). 103 (12.90%), 93 (11.60%), and 92 (11.50%) were positive for RBPT, BABAT and CFT, respectively. Young camels were more sero-positive than old one (13.30 vs.10.80%). In addition, females were more sero-positive than males (19.10 vs. 7.10%). Moreover, *Brucella melitensis* biovar 3 was isolated from stomach content of aborted camel fetus. Statistically, the apparent prevalence (AP) was estimated to be 11.50%, while true prevalence (TP) was 13.60% (95% CI: 11.20 to 16%; $P < 0.05$). There was non statistical significant association between different age groups, while a highly significant difference were detected between seasons and genders. This study documented a high prevalence of camel brucellosis in the area of study and there is a need for planning and implementation of joint programs by stakeholders in prevention and control of the disease as well as raising public awareness in decreasing the distribution of the disease.

Key words: Camel brucellosis, Egypt, serological tests, sero-prevalence, Shalateen.

INTRODUCTION

Camel brucellosis is an insidious disease, since it hardly provokes any clinical signs (Musa and Shigidi, 2001). The disease is caused by *Brucella abortus* (*B. abortus*), *Brucella melitensis* (*B. melitensis*) and *Brucella ovis* (*B. ovis*) affecting mainly the dromedary camels (Seifert,

1996). In camels, the manifestation of the disease is mild or even asymptomatic with abortion if compared to cattle. So it may silently affect the reproductive performance of camels through low herd fertility and relatively low milk production (Gwida et al., 2012).

The disease can also have an impact on export and import of animals constraining livestock trade (Radostits et al., 2006). However, information about economic losses due to camel brucellosis is scarce. Although camels are not the primary host of *Brucella*, *B. abortus* and *B. melitensis* isolated from milk, aborted fetus, lymph nodes and vaginal swabs (Radwan et al., 1992; Gameel et al., 1993; Agab et al., 1994; Abou-Eisha, 2000; Hamdy and Amin, 2002; El-Gohary et al., 2016; El-Diasty et al., 2016; El-Hady et al., 2016). Disease transmission depends on *Brucella* spp. being prevalent in contact animals (Musa et al., 2008).

Brucellosis may spread from camels to humans, either through direct contact or via raw milk consumption especially in Arabian and African Countries (Cooper, 1991; Al-Juboori and Baker, 2012). The uncontrolled movement of camels from *Brucella* infected areas to *Brucella* free areas is considered the major obstacles in brucellosis eradication program (Radostits et al., 2006). Most of the reports addressed the seroprevalence of brucellosis in camels; this is not surprising due to the relative ease by which samples can be obtained and handled. The complement fixation test (CFT), is a recommended test for international trade as required by the World Organisation for Animal Health (OIE).

Serological diagnosis of brucellosis depends mainly on detection of IgG₁ immunoglobulin because most of cross reactive bacteria share the IgM antibody with *Brucella* species, also IgG₂ and IgA were inconstant and small in amount so, trials were made to eliminate IgM and to detect IgG₁ (Radostits et al., 2006). Serological tests used for diagnosis of brucellosis in cattle may also be adequate for diagnosis of brucellosis in camels. However there is no validation for brucellosis serological test for camel sera done (Gwida et al., 2012). *B. melitensis* biovar 3 were isolated from camel stomach contents and swabs of lungs, livers, spleens of aborted fetuses and infected joint (Al-Majali et al., 2008; Musa et al., 2008).

Finally, it must be kept in mind that the serodiagnosis of brucellosis is additionally impaired by the allegedly strong cross-reactivity between *Brucella* spp. and *Yersinia enterocolitica* O:9 and other gram-negative bacteria (Emmerzaal et al., 2002). Therefore, the present study was aimed to determine the seroprevalence of brucellosis in dromedary camels imported from Sudan at Shalateen quarantine in Egypt.

MATERIALS AND METHODS

Study area

Shalateen is a town north of the Halayeb Triangle, Egypt. It is

located 520 km south of Hurghada and serves as the administrative center of all Egyptian territory up to the border between Egypt and Sudan including the villages of Abu Ramad, 125 km to the southeast; Halayeb, 165 km to the southeast; Ras Hadarba 200 km to the southeast. Ras Hadarba or Cape Hadarba lies on the shores of the Red Sea to the southeast of the city of Halayeb and to the east of mount Hadarba from which it takes its name.

The village of Ras Hadarba lies on north of the borders between Egypt and Sudan which run along the 22°N parallel of latitude; Marsa Hameera, 40 km to the north; and Abrak, 90 km to the west. The first three towns (Abu Ramad, Halayeb and Ras Hadarba) are located within the disputed Halayeb Triangle. In Egypt, the number of camels was estimated to be 120,000 heads (SADS, 2009). About half of the camels in Egypt are present in the Shalateen area (Mahran, 2004).

Study design and samples size estimation

A cross-sectional study was designed and adopted in this survey participating with camel owners. It was carried out from spring 2014 to 2015 in Shalateen quarantine. The sample sizes for animals for serological studies, serum samples from camels for molecular studies, were calculated by the formula of multistage random sampling (Thrusfield, 2005).

Each animal was examined clinically and information on different aspects of age, gender, date of sampling, and history of abortions was also recorded. Samples from camels were screened serologically for the presence of *Brucella*.

Sample collection

Blood samples

Blood samples were taken from examined animals; about 10 mL of jugular vein blood were collected in sterile silicon coated vacuum tubes 'vacutainers' (catalogue no. 02-683-60, Becton Dickinson, 38241 Meylan, Cedex, France), identified, kept in a slant position in the shade for about 2 h for complete clotting and transferred on ice packs to the laboratory avoiding shaking.

Samples were kept overnight at 4°C to allow separation of serum, centrifuged at 1000 g for 10 min to obtain amber clear serum. Sera were kept at -20°C each in 2 aliquots in sterile Bijou bottles until examined. Sera were screened for *B. abortus* antibodies by RBPT, BAPAT and CFT and positive sera were kept for further serological diagnosis.

Tissue samples

Mesenteric, retropharyngeal and supramammary lymph nodes of suspected camels were sampled at postmortem examination. Fetal stomach contents were collected carefully by heating the outer surface of the abomasum by heated spatula, sterile syringe was then introduced from the sterile point to obtain some of the fetal stomach contents.

Serological tests

All collected sera were initially screened by RBPT using RBPT antigen according to Alton et al. (1988) and OIE (2012). Antigens

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Table 1. Seroprevalence of camel brucellosis in Shalateen city as determined by BAPAT, RBPT and CFT in relation to season.

Season	Camels examined	BAPAT		RBPT		CFT	
		Pos.	%	Pos.	%	Pos.	%
Spring 2014	145	18	12.40	17	11.70	17	11.70
Summer 2014	49	2	4.10	2	4.10	2	4.10
Autumn 2014	233	40	17.20	36	15.50	36	15.50
Winter 2015	96	3	3.130	3	3.130	3	3.13
Spring 2015	278	40	14.40	35	12.60	34	12.20
Total	801	103	12.90	93	11.60	92	11.50

Pos. = Number of animals positive for brucellosis; P<0.05: significant differences between different seasons.

for BAPAT and RBPT were obtained from Veterinary Sera and Vaccine Research Institute (VSVRI), Abbassiya, Cairo 11517, Egypt. Antigen for CFT was kindly supplied by the National Veterinary Services Laboratories (NVSL), Ames, IA 50010, USA. In CFT, titers of 1/4 were regarded as suspicious, while titers of 1/8+ or above were considered as positive.

Sera that tested positive to RBPT and BAPAT were further tested using CFT for confirmation and standard *B. abortus* antigen S99 (CVL, New Haw Weybridge, and Surry KT15 3NB, UK). Preparation of the reagent was evaluated by titration and performed according to protocols recommended by World Organization for Animal Health (OIE, 2004). Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above were classified as positive and lack of fixation/complete hemolysis was considered as negative.

Bacteriological examination

Swabs from stomach contents of two aborted feti, also, samples of fetal membranes and uterine discharges of two aborted cows were

taken under complete aseptic condition for culture of *Brucella* spp. This was performed according to the recommendations of the FAO/WHO Expert Committee on Brucellosis (Alton et al., 1988; OIE, 2012) using direct culture on *Brucella* Agar Media containing *Brucella* selective antibiotics (Oxoid, England).

The plates were examined for *Brucella* colonies. The suspected colonies were identified and typing on the base of colonial morphology, urease, CO₂ requirement, susceptibility to *Brucella* phages, growth in the presence of thionin and basic fuchsin dyes (1:25000, 1:500000, 1:100000), production of H₂S, and antigenic are characteristics using specific antisera (A, M, R).

Data analysis

Descriptive and analytic statistics were computed using software SPSS®Version20. The degree of association was computed using odds ratio (OR) signified by 95% confidence intervals (Thrusfield, 2005). True Prevalence was estimated according to Rogan and Gladen (1978) from the following equation:

$$\text{True prevalence (TP)} = \frac{\text{Apparent prevalence} + \text{combined specificity of RBPT and CFT-1}}{\text{Combined sensitivity of RBPT and CFT} + \text{combined specificity of RBPT and CFT-1}}$$

RESULTS

Seroprevalence

Eight hundred and one (801) camels were examined for brucellosis in Shalateen Quarantine from spring 2014 to 2015. 103 (12.90%), 93 (11.60%) and 92 (11.50%) were positive for RBPT, BABAT and CFT, respectively (Table 1). These results reveal that the apparent prevalence (AP) was estimated as 11.50% by CFT, while TP was estimated as 13.60% (95%; CI: 11.20 to 16%). Among the total 103 camels positive for the disease in Shalateen quarantine, 42 (16.90%) were at 1 to 2 years old, 33 (11.20%) at 2 to 4 years old and 28 (10.80%) at the breeding age (Table 2).

By CFT, brucellosis-infected camels were observed in 36 (17.10%) out of the 507 examined male camels while 56 (19%) out of the 294 examined female camels were

positive for brucellosis. There was no significant difference between different age groups while a highly significant difference was detected between different sexes (P<0.05) (Table 3).

Bacterial isolation

A smear from one fetal stomach contents showed partially acid fast organisms. *B. melitensis* biovar 3 was isolated from stomach content of this aborted fetus; the morphological, cultural, biochemical and serological identification of the isolated *Brucella* strain. One *Brucella* isolates could be recovered from the stomach content of one aborted foetus by culture on artificial media, followed by isolates identification by its morphology and growth characteristics of the colonies and biochemical tests.

This isolate was typed as *B. melitensis* biovar 3 based

Table 2. Seroprevalence of camel brucellosis in Shalateen city as determined by BAPAT, RB PT and CFT in relation to different age group.

Age	Camels examined	BAPAT		RBPT		CFT	
		Pos.	%	Pos.	%	Pos.	%
1- 2 years	248	42	16.90	33	13.30	33	13.30
2-4 years	294	33	11.20	32	10.80	31	10.60
≥ 4years	259	28	10.80	28	10.80	28	10.80
Total	801	103	12.90	93	11.60	92	11.50

Pos. = Number of animals positive for brucellosis, P> 0.05: no significant differences between different age groups.

Table 3. Sero-prevalence of camel brucellosis in Shalateen city as determined by BAPAT, RB PT and CFT in relation to sex.

Sex	Age	Number of examined animals	CFT		Total number	Total pos.	
			Pos.	%		Number	%
Male	1- 2 years	168	10	6	507	36	7.1
	2-4 years	177	9	5.10			
	≥ 4years	162	17	10.50			
Female	1- 2 years	80	23	28.80	294	56	19
	2-4 years	117	22	18.80			
	≥ 4years	97	11	11.30			

Pos. = Number of animals positive for brucellosis, P<0.05: significant differences between the two sexes.

on as if it does not required CO₂ for growth, negative for H₂S production, grow in the presence of thionin and basic fuchsin dye (1:250000 and 1:500000), urease positive after 20 h , phage (Izatnagar) lyses and agglutinated only with A and M monospecific antisera (Table 4).

DISCUSSION

During the last few years, camel brucellosis has been a subject for many researches in many countries of the world especially those rearing racing camels such as the Arabian Gulf countries as well as other countries where camels constitute an important part of their livestock in many African and Asian countries (Yasmin and Remya, 2011).

Serological investigation still has played a dominant role in diagnosis of the disease (Konstantinidis et al., 2007). BAPAT, RBPT and CFT were used as screening for diagnosis of brucellosis (Morgan et al., 1969; Hunter and Allen, 1972; Farina, 1985). Moreover we used CFT as confirmatory test for the positive serum samples (OIE, 2012). In the present study, BAPAT, RBPT and CFT were used as screening and confirmatory tests for diagnosis of camel brucellosis and detection of naturally infected cases in a total of 801 dromedary camels during the period between 2014 and 2015 from Shalateen quarantine.

The overall prevalence of camel brucellosis was 12.90, 11.60 and 11.50% as determined by BAPAT, RBPT and CFT, respectively. Statistical analysis revealed that AP was estimated as 11.50%, while true prevalence (TP) was estimated as 13.60% (95% CI: 11.20 to 16%). High prevalence appears to be due to the fact that these camels were imported from Sudan which is known to have high prevalence of 12.30, 15.50 and 30.50% in 2004, 2005 and 2006, respectively as recorded by (Omer et al., 2007); 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. Chi square analysis for comparison between seasonal occurrences of *Brucella* infection revealed high significant differences between different seasons (P ≤ 0.05). The prevalence was being high in spring and autumn (Abdel-Raouf and El-Naggar, 1964; Shalash, 1965; Musa and Abusineina, 1978; Mares, 1954).

In Egypt, the sero-prevalence 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-Boshy et al. (2009) (7.35%). However, this results is in agreement with those of Hamada et al. (1963) (10.29%), Ahmed and Nada (1993) (11.6%) and El-Sawally et al. (1996) (11.3%),

Table 4. Phenotypic characteristics of *Brucella* isolates (*Brucella melitensis* biovar 3) recovered from stomach content of aborted fetus of she-camel.

Strain source		CO ₂ requirement	H ₂ S production	Urease	Growth on dyes					Lysis by macrophage				MS			Conclusion
					Thionin			Basic fuchsin		Tb		Iz1	R/C	A	M	R	
					a	b	c	b	c	RTD	RTD 10 ⁴	RTD	RTD				
Field strain	One Stomach content	-	-	+ in 20 h	-	+	+	+	+	-	-	+	-	+	+	-	Brucella melitensis biovar 3
Reference strains	<i>Brucella melitensis</i> Ether	-	-	+in 18-24 h	-	+	+	+	+	-		-	+	-	+	+	Brucella melitensis biovar 3
	<i>B. abortus</i> 544	-	+	+in 2 h	-	-	-	+	+	+	+	+	-	+	-	-	Brucella abortus 1
	<i>B. suis</i> 1330	-	+++	++ in <15 min	+	+	+	-	-	-	+	+	-	+	-	-	Brucella suis 1

RTD: routine test dilution a: 1:25000 b: 1:500000 c: 1:100000 Tb: Tbilisi Iz1: Izatnagar R/C: R/C: Rough *Brucella*. B: *Brucella* MS=Monospecific sera.

respectively. The differences in sero-prevalence observed from the previous researchers, might be due to differences in herd size, camel origin, tests used, management conditions, and the presence or absence of infectious foci, such as *Brucella*-infected herds, which could spread the disease among contact herds.

The RBPT detected 93 (11.6%) reactors lower than BAPAT which detects 103 (12.9%) reactors, this variation on the incidence of positive reactors may be attributed to the difference in the acidity of their antigen as reported by Davis (1971) and Corbel (1973). The acidic pH of the RBPT antigen (3.65±0.05) inhibits more amount of IgM fraction (Alton et al., 1988). The test is an excellent screening test but may be oversensitive for diagnosis in individual animal particularly vaccinated animals (World Health Organisation, 2006).

IgG₁ was the main immunoglobulin measured by the CFT with a possible cause that IgM is denatured during the test (MacMillan, 1990). CFT was only measured IgG₁ while IgG₂ and IgA do not fix complement (Curtain, 1971; Cho and Ingram, 1972). The results from the CFT may be adversely affected by IgG₂ interference (prozone effect) and by anti-complementary activity (Plackett and Alton, 1975). The CFT should be

used only as a confirmatory test (Al-Dahouk et al., 2003).

All examined camels were clinically normal at the time of sampling. 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 (Abu Damir et al., 1989; Bekele, 2004). Non pregnant camels experimentally infected with *B. abortus* had no clinical manifestations and only negligible pathological changes were found (Abu Damir et al., 1989). On the contrary, 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 according to their age was determined. In young camels (less than 2 years old), 42 (16.9%) and 33 (13.3%) were positive for BAPAT and RBPT, respectively, and 33 (13.3%) samples were confirmed as positive reactor for CFT, while in the adult mature camels (2 to 4 years old), 33 (11.2%) and 32 (10.8%) were positive for BAPAT and RBPT, respectively, and 31 (10.6%) samples were confirmed as positive reactors for CFT. In

addition, the examined adult mature camels at the breeding age (more than 4 years old) were positive for BAPAT, RBPT and CFT (28, 10.8 and 10.8%, respectively). Chi square analysis for comparison between occurrences of *Brucella* infection at different age groups revealed that there is no significant difference between different age groups, which suggests that all ages of camels were susceptible to brucellosis.

Brucellosis can affect camels at an early life probably through sucking and persisted into adulthood. This is confirmed by highly significant infection rate in she-camels in this study. Also younger animals may be infected through transmission from adults during the long journey from Sudan to Shalateen quarantine through contact with other herds around source of water. The result is supported by those of Higgins (1986) who reported that young camels under 11 month were resistant to brucellosis because sex hormones and erythritol tend to increase by age and sexual maturity.

Sero-prevalence of camel brucellosis according to their sex was recorded. In male camels examined, 36 (7.1%) were positive for CFT while in she-camel examined, 56 (19%) were positive for CFT. Chi square analysis for comparison between occurrence of *Brucella* infection by

different tests and sexes revealed that there is a high significant difference between male and females. These results may be associated with the effect of erythritol (Smith et al., 1962). Reduction of immunity in females during lactation, pregnancy and other reproductive stress may also contribute to higher prevalence in female camels (Gyles and Prescott, 2004). These results agreed with Bekele (2004) and Hadush et al. (2013) from Ethiopia, Yagoub et al. (1990) and Agab et al. (1994) from Sudan, and Ajogi and Adamu (1998) and Junaidu et al. (2006) from Nigeria. On the other hand, others results shows equal distribution between both sexes (Abu-Damir et al., 1989; Abbas et al., 1987).

In the present study, our trials to isolate the organism from the stomach content of one aborted fetus has been successful and the morphological, cultural, biochemical and serological identification of the isolated *Brucella* strain revealed isolation of *B. melitensis* biovar 3. This biovar of *B. melitensis* was previously identified and considered as the prevalent type in Egypt in different animals as recorded by (Sayour, 2004; Hoda et al., 2006; Khoudair and Sarfenaze, 2007; El-Diasty, 2009; Rehab, 2011; Abdel Hamid, 2012; Menshawy, 2013; Affi et al., 2015). Originally *B. melitensis* affects mainly sheep and goat. Such inter-species transmission situation may be the outcome of close contact between sheep, goats and camels (Musa et al., 2008).

This may explain the occurrence of this biotype in camels in the current study which consider the most dominant biotype of *Brucella* isolated from both animals and human in Egypt as reported by (Mohamed and Eisa, 2004; Soliman, 2006; El-Diasty, 2009; El-Sayed et al., 2011; Abdel Hamid, 2012; Afifi et al., 2015, El-Diasty et al., 2016, El-Hady et al., 2016).

The isolation of *Brucella* from lymph nodes failed, and this may have occurred if the number of viable organisms in the examined samples is low or contaminated with other bacteria which may prevent *Brucella* growth (Seleem et al., 2010). The specificity of serological tests cannot usually be determined by bacteriological isolation because some animals that yield negative culture results are in fact infected (Alton et al., 1975; Poster et al., 2010).

Conclusion

It is concluded that brucellosis is present at a level of 11.6% (as determined by CFT) among the examined camels in Shalateen city. A combination of several serological tests such as BAPAT and RBPT, followed by a confirmatory test of high specificity such as CFT can be used for diagnosis of brucellosis. One isolate of *Brucella* are typed as *B. melitensis* biovar 3. This is represented as zoonotic threat to the public health.

Routine screening of animals for brucellosis is crucial that may help to detect positive cases and reduce the risk

of transmission of the disease. Effective implementation of control measures including test and culling of the infected animals, quarantine and movement controls may prevent the spread of infection. Applications of hygienic measures which help in the control of brucellosis in camels imported from Sudan are considered as the main source of infection and contamination of environment in Egypt. 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.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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