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Cross-sectional Study of Camel (*Camelus dromedaries*) Brucellosis In the Red Sea State, Sudan

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ABSTRACT

The study was conducted in Red Sea State to determine sero-prevalence of camel (Camelus dromedaries) brucellosis based on Modified Rose Bengal Plate Test (mRBPT) and Competitive Enzyme Linked Immuno-Sorbent Assay (cELISA).A total of 400 sera were collected from dromedary camel from different localities in the Red Sea State namely: Port Sudan, Algonob and Alowlib, Sawakin, Halayib and Animal quarantine in Sawakin. The overall sero-prevalence rate in the state using modified Rose Bengal Plate Test (mRBPT) was 10.8% (No .of positive cases = 43). The sero-prevalence rate in the different localities as follow: in Halayib was 3.3% (No. of positive cases = 13), 2.2% (No. of positive cases = 9) in Port Sudan, 2.8% (No. of positive cases =11) in Algonob and Alowlib, 0.5% (No. of positive cases = 2) in Sawakin and 2.0% (No. of positive cases = 8) in Animal quarantine. Statistically the difference between the sites was not significant (Chi- square = 3.641 df = 4 P-value = 0.457 > 0.05. Furthermore, sero-prevalence rate in females was 8.3% (No. of positive cases =33) and in males was 2.5% (No. of positive cases = 10). Strong relationship was obtained for sex and occurrence of the camel brucellosis (Chi- square = 8.414 df = 1 P-value = 0.004 < 0.01e). Application of logistic regression model revealed that sex could be a risk factor for the disease (Odds Ratio = 2.868 & 95% CI = 1.372 – 5.996). In contrast, no association was observed for age and presence of the disease (Chi-square = 3.506 df = 2 P-value 0.173 > 0.05). Hence, risk estimate statistics cannot be computed for the age. A total of the 400 serum samples collected from Red Sea State, only 43 samples that were positive with mRBPT were chosen for examination with cELISA as confirmatory test; the later test showed 30 sero-positive. Sero-prevalence rate of camel brucellosis using mRBPT was relatively high in the State. Hence, comprehensive control programme which include serological diagnosis followed by vaccination are recommended.

Keyword : Camel (Camelus dromedaries) brucellosis, mRBPT, cELISA, Sudan.

INTRODUCTION

Brucellosis is primarily a disease of animals which can be transmitted to man either directly or indirectly and it continues to be a zoonosis of worldwide public health and economic importance. The causative bacterium was named in honour of Sir David Bruce the discoverer of *Br. melitensis*.

The hallmarks of animals' brucellosis are abortion, infertility and reproductive failure (Philip, 2003). The genus *brucella* contains 10 recognized species including: *Br. melitensis* the main causative agent of brucellosis in sheep and goat, but the infection also occurs in cattle, camels and wild animals (Alton 1985, and Elberg, 1983), *Br. abortus* the cause of contagious abortion in cattle, can also infect bison, buffalo, camels, horses, chamois, dogs, fox and water buck, while infection of sheep and goats and pigs is rare (Elberg, 1983). Although *Br. suis* infects swine, infection of other animals such as dogs, carbion, reindeer and rabbits has also been reported (Elberg, 1983). *Br. canis* causes a highly infectious form of brucellosis in dogs, *Brucella neotoma* infects the desert rat (*Neotoma lepida*), and *Br. ovis* infect rams, *B. ceti* and *B. pinnipedialis* (marine mammals), *B. microti* (common vole) and *Brucella inopinata*, associated with a human infection (OIE, 2009).

There are two species of camels in the Genus *Camelus*, the dromedary or one humped camel (*Camelus dromedarius*) and the bacterian or two-humped camel (*Camelus bacterianus*). In Africa and Middle East there are around 17 million dromedaries, seven countries in Africa (Somalia 6 million, Sudan 3 million, Mauritania, Chad, Ethiopia, Eritrea and Kenya around 1 million each) have more than 80% of the camels in the world. Northern African countries (Morocco, Tunisia, Algeria, Libya and Egypt) have a total population reaching 830,000 camels. Saudi Arabia, Yemen and the United Arab Emirates in the Middle East account for more than 90% of the total camel population in the world (El Tayeb, 2003). In the Sudan camels are spread in a belt configuration, it extends between latitude 14º N to 16º N (Wardeh, 1989). Moreover, camels in Sudan are concentrated in two main regions; the Eastern regions, where camels are found in the Butana plain and the Red Sea hills, and Western regions are found in Darfour and Kordofan (Agab, 1993). Sudanese camels belong to the species *Camelus dromedarius*. These camels are owned and raised by nomadic tribes, who migrate north and south according to the season in search of water and pasture and escape from insects.

The Sudanese camel (*Camelus dromedarius*) plays an important role in the economy as a source of meat, milk, wool, hides and traction power. Camels constitute around 6% of the number of animals producing milk and meat in the Sudan. Its meat comprises 8.8 % of the national annual meat consumption (Haroon, 1991) at the official slaughterhouses and probably a similar percentage at the traditional markets. Camels are, as well, a good source for a substantial income of foreign currency. The main marketing areas are Egypt, Libya, Saudi Arabia and the Gulf Emirates. Camels are adapted to live in arid zones and facing hard conditions of life, their health and productivity are seriously affected by many debilitating diseases. Camels represent 6.06% (280154/1538845) of the ruminants in the Red Sea State (Anon, 2010). In addition, Red Sea State has a suitable environment for camels to live.

OBJECTIVE:

1. To determine the sero-prevalence of camel brucellosis using modified Rose Bengal Plate Test (mRBPT) and (cELISA) in Red Sea State, Sudan.
2. To determine some of the risk factors that associated with camel brucellosis in Red Sea State, Sudan.

MATERIALS AND METHODS

Study site

Red Sea State is located in the north eastern part of Sudan (latitude 17° to 22° north, longitude 33° to 38° in the east) with the land area of 210.410 km^2. Red Sea State constitutes approximately 10%.of the total area of Sudan and 63% of the Eastren region. It is delimited by Kassala State and

Eritrea in the south, River Nile State in the west, Egypt in the north and the Red Sea in the east. It is dividing into 10 localities: Port Sudan, Sawakin, Sinkat, Tokar, Halayib, Ageeg, Alganab and Alawlieb, Haya, Derodieb and Gebiet. The principal types of livestock found in the state are cattle, sheep, goats and camels. Camels represent 6.06% of the ruminants in the Red Sea State (Anon, 2010). Red Sea State has three sea ports, Port Sudan, Sawakin and Osaif. Port Sudan is the main port and capital of the Red Sea State, it is a coastal city located on the western coast of the Red Sea. Port Sudan has an arid climate with very hot summer from July to September and a moderate cool Winter from October to February. Ambient temperatures in winter are about 30°C and about 45°C in summer. The average ambient temperature in Port Sudan is 28.4°C. Over 90% of the annual rainfall occurs between October and January, mostly in November. Topographically the state can be divided into three plains. The coastal part which is considered as the lowest land of the state at the sea level and extends from Agig to Halaib. Mountain part from Dordeeb up to the south of Halaib. Following the mountains is the desert on the western borders of the state. On the south east direction of the state in Toker there is an irrigated scheme by rain water through flow of Baraka and Langreb streams. These temporary Khors drain water from the highlands during October up to January. Another Khor is Arbaat which joins the Red Sea, 18 kilometers north to Port Sudan. The vegetation cover in the state is poor, most of which is found around the streams and Khors at the mountains and in the southern part of the state. The dominants trees are *Acacia* spp.

Production System

Most of these camels were kept in defined farms throughout the year for milk production; they were kept under a semi-intensive system and allowed for limited outdoor grazing. They are fed dry grass and supplemented some time by dates and water is available.

Study Population

The study population was camels regard less to age, sex, breed, and season. Only sex and age of the camel and production system were recorded. Description of the target population with regard to site is presented in Table 1.

Table 1: Description of study population in study site

Unit	Site					Total
	Algonob and alowlib	Port Sudan	Animals quarantine	Sawakin	Halayib	
	Count (%)					
Sex						
male	21 5.0%	28 7.0%	94 23.5%	0 0.0%	33 8.2%	176 44.0%
female	92 23.0%	51 12.8%	10 2.5%	23 5.8%	48 12.0%	229 56.0%
Total	**113** **28.2%**	**79** **19.8%**	**104** **26.0%**	**23** **58.0%**	**81** **20.2%**	**400** **100.0%**
Age						
1– 3 years	17 4.2%	23 5.8%	0 0.0%	0 0.0%	6 1.5%	46 11.5%
4– 7 years	45 11.2%	40 10.0%	101 25.2%	8 2.0%	32 8.0%	226 56.5%
> 7 years	51 12.8%	16 4.0%	3 0.8%	15 3.8%	43 10.8%	128 32.0%
Total	**113** **28.2%**	**79** **19.8%**	**104** **26.0%**	**23** **5.8%**	**81** **20.2%**	**400** **100.0%**

Research Hypothesis

It is important to determine the current situation of Camel (*Camelus dromedaries*) brucellosis in the Red Sea State and some risk factors that associated with occurrence of the disease. An assumption of high sero-prevalence rate of the disease with some risk factors such as age, sex and production system were considered.

Sampling Method

Selection of the camels was done at different levels (sites, herds and camels itself) with support or willingness of the owners and this method called Non-Probability Multi- Stage Cluster Sampling as described by THrusfied (2007)

Collection of the Samples

A total of 400 blood samples were collected from camels in Red Sea State from September to December 2014. The blood samples were taken aseptically from the jugular vein (about 5 ml) using vaccutainer tubes.

Separation of Sera

Following the collection of blood samples, the vaccutainer tubes were placed vertically in ice boxes and transported to the laboratory with care to avoid haemolysis. In the laboratory the samples were kept overnight at 4° C to allow separation of serum. After centrifugation at 3000 rpm per 10 minutes, the sera were separated, poured into small tubes and kept in refrigerator at -4° C till tested.

Serological Tests

Two serological tests were used for detection of *Brucella* antibodies in serum; namely Modified Rose Bengal Plate Test (mRBPT) at Veterinary Research Laboratory, Port Sudan, Red Sea State, and Enzyme Linked Immune-Sorbent Assay (competitive ELISA) at the *Brucella* Department, Veterinary Research Laboratory Institute, Soba, Khartoum.

Modified Rose Bengal Plate Test (mRBPT)

This was similar to the classic Rose Bengal Plate Test (RBPT) but differed in the volume of antigen used which was half or third of the serum volume (antigen to serum was 1:3). This procedure was deemed suitable for detection of weakly positive samples.

Test procedure:

The serum samples and the antigen were removed from the freezer (-20˚C) and brought to room temperature (22±4˚C) to thaw; only sufficient antigen for the day's tests was taken from the refrigerator and left to warm up. An amount of 75µl of each serum sample was placed on a porcelain plate. The antigen bottle was shaken well, but gently, and 25 µl of the antigen was placed near each serum spot. Immediately after the last drop of antigen had been added to the plate, both the serum and antigen were mixed thoroughly using disposable clean wood or glass rod for each spot to make a circular or oval zone or using Rose Bengal shaker machine. The mixture was rocked gently for 4 minutes. Any visible clotting was considered positive. The positive test was compared with a control negative test to confirm it.

Competitive Enzyme Linked Immune-Sorbent Assay (cELISA)

Test procedure:

1. Prepare the conjugate solution
2. Add 20 µL of each test serum per well. Leave column 11 and 12 for controls.
3. Add 20 µL of the negative control to wells A11, A12, B11, B12, C11 and C12.
4. Add 20 µL of the positive control to wellsF11, F12, G11, G12, H11 and H12.

5. The remaining wells have no serum added and act as the conjugate controls.
6. Immediately dispense into all wells 100 µL of the prepared conjugate solution. This gives a final serum dilution of 1/6.
7. The plate is then vigorously shaken (on the microtitre plate shaker) for 2 minutes in order to mix the serum and conjugate solution. Cover the plate with the lid and incubate at room temperature (21˚C±6˚C) for 30 minutes on a rotary shaker at 160revs/min.
8. Shake out the contents of the plate and rinse 5 times with washing solution and then thoroughly dry by tapping on absorbent paper towel.
9. Switch on microplate reader and allow the unit to stabilize for 10 minutes.
10. Immediately before use prepare the substrate and chromogen solution by dissolving one tablet of urea H_2O_2 in 12 ml of distilled water. When dissolved add the OPD tablet and mix thoroughly. This can take a few minutes; the use of a magnetic stirrer will greatly increase the speed with which it dissolves. Add 100µL to all wells.
11. Leave the plate at room temperature for a minimum of 10 minutes and a maximum of 15 minutes.
12. Slow the reaction by adding 100µL of stopping solution to all wells.
13. Remove condensation from the bottom of the plate with absorbent paper towel. Read plate at 450nm wave length.
14. The lack of colour development indicated that sample tested was positive. A positive negative cut off was calculated as 60% of the mean of the optical density (OD) of the four conjugate control wells. Any test sample that gave an OD equal to or below that value was regarded as positive or otherwise negative.

Evaluation of the Test Results

The test results of each plate were evaluated by checking the following values:

1. Binding Ratio = $\frac{\text{Mean of 6 negative control wells}}{\text{Mean of 6 positive control wells}}$
2. The binding ratio must be greater than 10.
3. The mean OD of 6 negative control wells must be greater than 0.7.
4. The mean OD of 6 positive control wells must be less than 0.1.
5. The mean OD of the 4 conjugate controls must be greater than 0.7.
6. Any test plate results which did not comply with the above values were rejected and the samples were re examined.

Data Management and Analysis

Statistical analysis was done using IBM SPSS Statistics version 20.0. Descriptive statistics was used as count and percent for the variables. While analytical statistics such as chi-square was employed to demonstrate the association between some factors and occurrence of the camel brucellosis. For quantification of positive association, logistic regression model was used. Then the Odds Ratio

(OR) was obtained for some factors. When Odds Ratio greater than one, the variable could be a risk factor for the presence of the disease.

RESULT AND DISCUSSION

A total of 400 sera were collected from dromedary camel from different localities in the Red Sea State namely: Port Sudan, Algonob and Alowlib, Sawakin, Halayib and Animal quarantine in Sawakin. The overall sero-prevalence rate in the state using modified Rose Bengal Plate Test (mRBPT) was 10.8% (No .of positive cases = 43). The sero-prevalence rate in the different localities as follow: in Halayib was 3.3% (No. of positive cases = 13), 2.2% (No. of positive cases = 9) in Port Sudan, 2.8% (No. of positive cases =11) in Algonob and Alowlib, 0.5% (No. of positive cases = 2) in Sawakin and 2.0% (No. of positive cases = 8) in Animal quarantine. Statistically the difference between the sites was not significant (Chi- square = 3.641 df = 4 *P*-value = 0.457 > 0.05 (The results are summarized in Table 2).

Furthermore, sero- prevalence rate in females was 8.3% (No. of positive cases =33) and in males was 2.5% (No. of positive cases = 10). Strong relationship was obtained for sex and occurrence of the camel brucellosis (Chi- square = 8.414 df = 1 P-value = 0.004 < 0.01). Application of logistic regression model revealed that sex could be a risk factor for the disease (Odds Ratio = 2.868 & 95% CI =1.372 – 5.996) (The results are presented in Table 3).

Moreover, the sero-prevalence rate in age group (1-3 years) was 0.5% (No. of positive cases = 2), age group (4 - 7 years) was 5.8% (No. of positive cases = 23) and age group (> 7 years) was 4.5% (No. of positive cases = 18). Statistically, no association was observed for age and presence of the disease (Chi-square = 3.506 df = 2 *P*-value 0.173 > 0.05). Hence, the risk estimate statistics cannot be computed (The results are shown in Table 4).

A total of the 400 serum samples collected from Red Sea State, only 43 samples that were positive with mRBPT were chosen for examination with cELISA as confirmatory test; the later test showed 30 sero-positive.

Table 2: Sero- prevalence rate of Camel brucellosis using modified Rose Bengal Plate Test (mRBPT) in selected sites of Red Sea State, Sudan.

Site	mRBPT Negative	mRBPT Positive	Total	Chi-square	df	*P*-value
	Count (%)					
Algonob and alowlib	102 25.5%	11 2.8%	113 28.2%			
Port Sudan	70 17.5%	9 2.2%	79 19.8%			
	96	8	104			

Animals quarantine	24.0%	2.0%	26.0%	**3.641**	**4**	**0.457** **a**
Sawakin	21 5.2%	2 0.5%	23 5.8%			
Halayib	68 17.0%	13 3.3%	81 20.2%			
Total	**357** **89.2%**	**43** **10.8%**	**400** **100.0%**			

mRBPT: modified Rose Bengal Plate Test

df: Degree of Freedom

x : Sero- prevalence calculated from No. of positive cases as percentage

a : *P*-value > 0.05 (Not significant)

Table 3: Sero- prevalence rate of Camel brucellosis according to sex using modified Rose Bengal Plate Test (mRBPT) in selected sites of Red Sea State, Sudan.

Sex	**mRBPT** Count (%) **x**		**Total**	**Chi-square**	**df**	***P*-value**	**Odds Ratio(OR) 95% CI**
	Negative	**Positive**					
Male	166 41.5%	10 2.5%	176 44.0%				
female	191 47.8%	33 8.3%	224 56.0%	**8.414**	**1**	**0.004** **a**	**OR = 2.868** **b** **95% CI** **(1.372 – 5.996)**
Total	357	43	400				

	89.2%	10.8%	100.0%		

mRBPT: modified Rose Bengal Plate Test

df: Degree of Freedom

x : Sero- prevalence calculated from No. of positive cases as percentage

a : *P*-value < 0 .01 (Highly significant)

95% CI : 95% Confidence Interval

[b] : Odds Ratio (OR) > 1 and could be risk factor

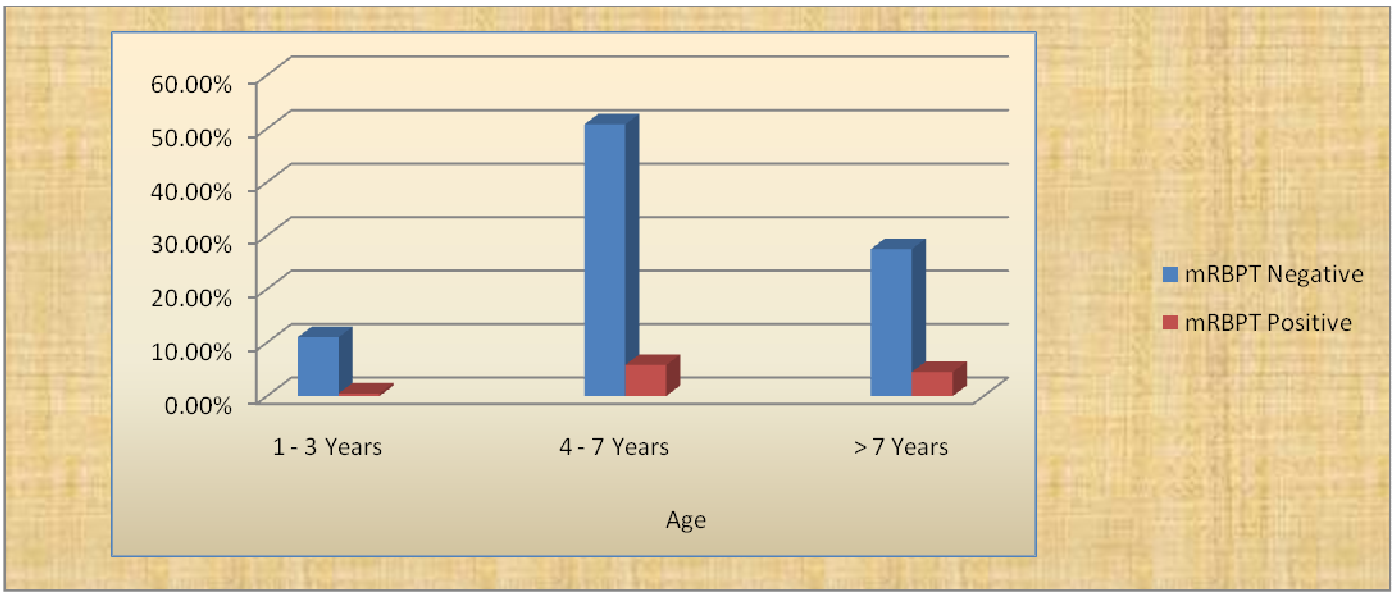

Figure 1: Sero- prevalence rate of Camel brucellosis according to age groups using modified Rose Bengal Plate Test (mRBPT) in selected sites of Red Sea State, Sudan.

Chi-square = 3.506 degree of freedom = 2 *P*-value 0.173

P-value > 0.05 (Not significant)

Odds Ratio (OR) : Risk estimate statistics cannot be computed

Discussion

Camels in the Sudan are not vaccinated against brucellosis. Similarly no work has been done on the species resistance of camels to brucellosis. In the present investigation the overall sero-prevalence of brucellosis was found to be (10.8%) which comparable to that obtained by Bitter (1986), in the Eastern Sudan, who reported 16.5% and 32.5%, and reported by Fayza *et al* (1990) 15.04%, in Khartoum State. However, the sero-prevalence rate considerably higher compared to that reported by Osman and Adlan, (1987) in Eastern Sudan, 8% and with the prevalence rate reported by Yagoub et al., (1990) in Eastern Sudan, 6.95% and Musa (1995),7.75% and that found by Raga (2000), 6.2 in Darfur State and Tag Elsir (2002), 6% Kassala State.Camels are infected by lateral infection from the primary host of *Br. abortus* (cattle), and *Br. melitensis* (sheep and goats). So, the

prevalence rate of brucellosis in camels increases when herded with these animals. Similarly, Musa (1995) reported 23% prevalence rate in area where camels were reared with cattle, 1.9% and 4.8% in herds newly introduced into such areas.

As seen from the results, the sero-prevalence rate in males was (2.5%), and in females was (8.3%). In contrast, Musa (1995) reported 7.05% in males and 7.69% in females, and that obtained by Raga (2000) in the same area in Darfur States. She found prevalence rate 11.4%in males and 4.2% in females. On the other hand, age was found not associated with presence of the disease. Our finding disagree with Agab *et al.,* (1995) who noticed that the disease increased with the age of 10 years after which the incidence declined. The disagreement could be to outcome of data analysis, because low numbers of camels were obtained for young animal.

In the present study, two types of serological tests were used for the diagnosis of brucellosis were used, and the results showed that the mRBPT was less sensitive than cELISA. The cELISA was used as confirmatory test because of its high sensitivity and specificity in detection of *Brucella* antibodies. According to the OIE (2009) only samples positive with mRBPT were confirmed by the cELISA as more false positive samples by the mRBPT. Similar findings were reported by Nielsen (2002). Serological diagnosis of brucellosis began more than 100 years ago with simple agglutination tests. Since, then it was realized that the serological tests were susceptible to false negative and false positive reactions. For instance, exposure to cross reacting microorganisms (Nielsen, 2002). Thus, cELISA has been shown to be a highly sensitive technique and suitable for large-scale screening of camel brucellosis, but availability of the diagnostic tests was a constraint in this study.

Human and animal populations in the Red Sea State are exposed to brucellosis by direct contact or by consumption of animal products or both without much awareness about the disease. Records from the Veterinary Research Laboratory in Port Sudan showed that between May 1998 - March 2007 there was 54.6% *Brucella* positive cattle, 9.1% camels, 25.0% sheep, 31.3%, goat and 66.7% equines. In the two last years 29.0% of cattle were *Brucella* sero-positive, 33.0 % camels, 12.0% sheep, 2.5% goats and 0% equines were positive for brucellosis. The high prevalence rate of brucellosis in Red Sea State in our study may be due to the poor management, crowding of animals in small and closed farms as well as poor hygienic measures. To date there is no annual vaccination against brucellosis in Red Sea State.

In conclusion, the sero-prevalence of camel brucellosis in Red Sea State as detected by the Modified Rose Bengal Plate Test (mRBPT) was relatively high. However, cELISA was found to be more sensitive than mRBPT. On the other hand, government should be considered the following recommendations:

1. Using vaccination to increases the resistance to infection, the program should be adopted by the Ministry of Animal Resources.
2. Improvement of veterinary extension services particularly education of animal owners about bases of hygiene measures.
3. Other preventive measures by government such as control of animal's movement across the borders should be followed.

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