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Sero-epidemiological Study on Camel Brucellosis in Somalia

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Abstract:

Brucellosis is one of the most important zoonotic diseases worldwide. The disease was not fully assessed in Somalia particularly after the civil war of 1990s. The present study was conducted from December, 2015 to March, 2016 in order to determine the seroprevalence and possible risk factors associated with camel brucellosis in Mogadishu city of Somalia. Questionnaire survey was also used to evaluate the knowledge-attitude-practice (KAP) among camel owners. A total of 180 camel sera were randomly sampled and tested using Rose Bengal Plate Test (RBPT), Modified RBPT (mRBPT), Serum Agglutination Test (SAT) and Competitive Enzyme Linked Immunosorbent Assay (cELISA). The investigated camels were apparently healthy above two years of age with no history of vaccination against brucellosis. The overall seroprevalence of camel brucellosis was 4.4% at individual level and 31.3% at herd level. The

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Kappa statistics indicated that there was perfect agreement between mRBPT, SAT and cELISA (k=0.841) while the RBPT has a Kappa value of (0.589) which was found to be a moderate level of agreement when compared to the cELISA. Questionnaire survey among the camel owners determined that camels with proved reproductive problems were culled by 77% of the respondents which is a good practice that might have contributed to the low prevalence of brucellosis in the present study. Conversely, 100% of the respondents confirmed of consuming raw milk of camel as well as bare hand contact of abortion materials with abortion cases reported by 63% of them. Furthermore, 77% of the respondents did not know brucellosis and its zoonotic risk. Thus, these factors can play a vital role of transmission of this disease among Somali people. It was concluded that Brucella infection exists in camels in Mogadishu, Somalia, and mRBPT is as sensitive as SAT and cELISA techniques. Moreover RBPT is very sensitive test validated and its antigen standardized for bovine brucellosis. Therefore, the study recommends a wider epidemiological surveillance and further validation of diagnostic serological tests in camels and other ruminants as well as human with isolation and identification of the infective Brucella organism strains and further biovars which enables best options for selection of brucellosis control strategy suitable to Somalia context. Hence, improvement of the public awareness on zoonotic potential of the disease is also recommended.

Key words: *Brucella,* Seroprevalence, Risk factors, zoonotic, disease control, Mogadishu, Somalia.

INTRODUCTION

Somalia takes the first place in the world in possessing over six million one humped camels [1]**.** The dromedary is an important livestock species in Somalia and plays a vital role as food and in the national economy of the country [2]. They have also social and cultural importance to the pastoralists of the country for payment of bride-wealth, known as "**yarad**" in Somali and compensation of injured parties in tribal feuds, known as

"**mag**" in Somali; blood-money [3]**.** The camel in Somali pastoralist is the most valuable animal for all and a large herd is a sign of strength, power and prestige. Camels are not primarily disposable income as they have a great potential for survival in long periods of drought as a recurrent phenomenon in the country [4]. The available data on Somali livestock population are poor and quite old. According to FSAU-FAO data (1999), the camel population of Southern zone (including Banadir region) is about 1.2 million heads [1].

Brucellosis is a worldwide zoonotic disease affecting both human and animals including camels. It causes heavy economic losses to the livestock industry and also poses serious human health hazards [5]. The *Brucella* infection in camels is caused by different biotypes of *B. abortus* and *B. melitensis* [5, 6, 7, 8]. It is characterized by abortion, retained placenta, uterine infection, foetal death, mummification and delayed maturity [5, 9]. Infertility, arthritis and hygroma were also reported [10]. The infection rate was higher in intensive camel production system [10, 11]. In countries with more of extensive form of husbandry like Somalia the prevalence rate is low [9, 11, 12, 13].Previous serological surveys in different camel rearing areas of Somalia reported prevalence rates ranging from 0.3% to 10.4%using different serological techniques [14, 15, 16].

In Somalia, despite the presence of the largest population of camels in the world and its economic and social importance to the pastoral and agropastoral Somali communities [1, 2, 3], livestock management as well as programmes to control infectious diseases like brucellosis have declined after collapse of central veterinary services in the country due to the civil war of 1990s. Therefore, the present study was undertaken to determine the seroprevalence of camel brucellosis in selected districts of Banadir region of Somalia. Moreover, scarcity of camel brucellosis data, lack of awareness about brucellosis among the community together with the

prevailing tradition of raw camel milk consumption are the main encouraging points to the present study.

MATERIALS AND METHODS

Study Area:

The study was carried out in three districts of Banadir region of Somalia namely, Daynile, Yaqshid and Kahda districts. The region lies between latitude 2°2′59″N and longitude 45°15′44″E. Although by far the smallest administrative region in Somalia, it has the largest population estimated to be about 2.3 million and covers an area approximately 96,878 km [17, 18]. There is no information on Banadir camel population in particular**.** Therefore, these three districts were selected purposively due to their camel population. Samples were collected randomly from the nomadic herds and the dairy camel farms.

Study Population:

A total of 180 apparently healthy one-humped camels above two years old with no history of vaccination against brucellosis were randomly sampled from 16 camel herds. Two different camel production systems were tested in this study, including nomadic (extensive system) and dairy camel farms (semi-intensive system). These animals were sampled in the period between December 2015 and March 2016. Details of the study population from the selected districts screened for brucellosis are summarized in table (1).

Study design:

A cross-sectional study was carried out to investigate the seroprevalence of camel brucellosis in the selected districts of Banadir region of Somalia using four different serological tests varies in their sensitivity and specificity. Livestock farmers of the selected areas were informed about the survey. However, to encourage their participation in this study and facilitate the

process of sampling, the author administered anthelmintic and multivitamin injections to their animals during sampling period. At the time blood samples were collected, questionnaires were filled by the owner of each sampled herd.

Sample collection:

Blood Samples: Approximately, 8 ml of blood were collected aseptically from jugular vein of each camel using plain vaccutainers tubes. The samples were transported to the laboratory of Abrar Research and Training Centre (ARTC) in Abrar University, Mogadishu-Somalia. Samples were left to clot at room temperature (25°C). Sera were separated and decanted into eppendorf tubes in duplicate (four aliquots) and stored at –20°C until needed for serological examination.

Questionnaire Samples: A questionnaire survey was conducted among camel keepers to assess the knowledge-attitude-practice (KAP) among these herders and farmers towards the brucellosis. The questionnaire was administered to sixteen respondents (herders) whose camels were included in the study population. The information gathered relates to camel management (milking, herding, watering, feeding, and delivery and mating assistance) and milk consumption habits, in addition to their knowledge on brucellosis and its control.

Serological Techniques:

Four serological tests (RBPT, mRBPT, SAT & cELISA) were used in this study for detection of *Brucella* infection in Camels. The RBPT and mRBPT were done in the laboratory of Abrar Research and Training Centre (ARTC), Abrar University, Mogadishu-Somalia, whereas SAT and cELISA were performed in Central Veterinary Research Laboratory (CVRL), Khartoum-Sudan.

Rose Bengal Plate Test (RBPT): All serum samples were initially screened by RBPT using *Brucella abortus* strain 1119-3 (USDA) (S1119-3) antigen kindly donated by Central Veterinary Research Laboratory (CVRL), Khartoum-Sudan. The tested serum samples and antigen were taken to the room temperature before testing for half an hour. The test was performed according to the procedure described by **Alton *et al*., (1975) and OIE manual (2016)** [19, 20].

Modified Rose Bengal Plate Test (mRBPT): All camel sera were tested by mRBPT as described by **Blasco *et al*., (1994)** [21]**;** this test is similar to the RBPT and differ in the volume ratio of antigen and serum sample which is 1 to 3 respectively.

Serum Agglutination Test (SAT): A total of 69 serum samples were included for SAT examination. These were the RBPT and mRBPT positive sera (7 samples) and 62 serum samples selected randomly from the RBPT and mRBPT negative samples. This test was performed in microplates according to **Alton *et al*., (1975) and OIE manual (2016)** [19, 20] using *B. abortus* strain 1119-3 (USDA) (S1119-3). Serum samples showing 30 or more IU per ml were considered positive [20].

Competitive Enzyme Linked Immunosorbent Assay (cELISA): All serum samples tested by SAT were re-evaluated by cELISA. The competitive enzyme linked Immunosorbent assay (cELISA) was done and its results were interpreted according to the instructions of the manufacture manual (SVANOVIR® *Brucella*-Ab cELISA test kits, Svanova Biotech AB Uppsala, Sweden). Any serum sample which gave 30% or more percent inhibition (pi) was considered positive.

DATA MANAGEMENT AND STATISTICAL ANALYSIS:

The data obtained from the field were recorded in notebook and later stored in Microsoft Excel and analysed using software SPSS® version 20. Chi-square test (X^2) was used to identify the statistical differences between the different variables associated with seropositive camels. The agreement between different serological tests was calculated using Kappa analysis. The differences were considered statistically significant when $P < 0.05$.

RESULTS

Seroprevalence of Camel Brucellosis using different serological tests:

The overall seroprevalence rate of camel brucellosis at herd level was 18.8% and 31.2% using RBPT and modified RBPT respectively while at individual level was 1.7% and 3.9% respectively (table 2). In both individual and herd levels, the statistical difference between the three districts was insignificant.

Out of the 11 camel herds further examined using SAT and cELISA, the results revealed that 5 herds (45.5%) and 4 herds (36.4%) were seropositive to *Brucella* antibodies respectively. Whereas, at individual level the prevalence was 10.1% for both SAT and cELISA tests (table 3).The estimated overall survey adjusted true animal level seroprevalence was 3.9% (7/180) for both SAT and cELISA, Based on cELISA, the percent inhibition (pi) of the seroprevalence of camel brucellosis is ranged from 40% to 77% (table 4.12).

Comparative results between different production systems:

The seroprevalence of brucellosis in camels under extensive management system was 3.6% by mRBPT, 8.7% by SAT, and

8.7% by cELISA. No antibodies were detected from these nomadic camels using RBPT. The seroprevalence of brucellosis in camels under semi-intensive management system was 2.4%, 4%, 10.9% and 10.9% by using RBPT, mRBPT, SAT and cELISA respectively (table 5 and 6). However, the seroprevalence rates for all serological tests used in this study were not statistically significant in the semi-intensive managed camels as compared to the camels in the extensive management system ($P > 0.05$) as presented in table (5 and 6).

Level of agreement in the sensitivity between the four serological tests:

The lowest positivity rate was obtained by the RBPT (table 4). All serological tests were able to confirm the positivity of the 3 samples detected negative by RBPT. Four out of the 62 negative samples by RBPT were found positive by mRBPT, SAT and cELISA. Only one sample from these samples was resulted positive by both mRBPT and SAT, moreover it was found negative by cELISA and vice versa with another serum sample (table 7). Thus, eight out of 180 camel serum samples were positive to *Brucella* antibodies by at least one of the four serological tests used in this study (table 7). Therefore, the overall seroprevalence for the present study was 4.4% and 31.3% at individual and herd levels respectively. As shown in table (8), when compared RBPT to cELISA, (taking ELISA to be the gold standard in this study), the sensitivity of the RBPT is 42.9% .The level of agreement between RBPT and cELISA using kappa analysis was moderate agreement with a kappa value of (0.589) according to **Dohoo** [22]. When compared modified RBPT to cELISA the sensitivity (85.7%) was higher than that of the RBPT with slightly similar specificity of (98.4%). Perfect agreement between mRBPT and cELISA was proven by calculating the kappa value (0.841) (table 9). As delineated in table (10), the comparison between SAT and cELISA was similar to that of mRBPT. The sensitivity was

(85.7%) and the specificity was (98.4%). Thus the level of agreement appears perfect with a kappa value (0.841).

Questionnaire Results:

Although no statistically significant difference (P=0.78) was observed between camels in contact with small ruminants and unaccompanied camels, the present work revealed that nearly half (49%) of the respondents keep camels with small ruminants. All of the respondents (100%) consume raw camel milk. Moreover, 77% of the camel owners interviewed did not know brucellosis. Almost all camel herders handle the abortion material and other excreta with bare hands. The abortion cases in studied farms were (63%). The frequency of abortion of 13% of these abortion cases were occur repeatedly. The cases of retention of placenta were recorded in 63% of the interviewed herds. The rate of mastitis was (94%).The majority of respondents (72%) mentioned different causes of abortion. However, only 23% had stated that *Brucella* as one of the cause. The rest of interviewees who know the causes of abortion (48%) mentioned different causes including trypanosomiasis, tick paralysis and environmental stress. The majority of interviewed camel farmers (77%) send camels with proven reproductive problems to slaughterhouses. Only (22%) leave it within the herd without medication.

DISCUSSION

In the present study, the seroprevalence of camel brucellosis in Banadir region of Somalia was 1.7% by RBPT, 3.9% by mRBPT, 10.1% by SAT and 10.1% by cELISA. However, the estimated seroprevalence adjusted confirmed brucellosis infection was 3.9% using both SAT and cELISA.

The overall seroprevalence of brucellosis in this study was 4.4% (8 out 180). This is in agreement with the studies obtained from camels in UAE (4.4%) [23], Ethiopia (4.4%) [24]

and Sudan (4.9%) [25]. Lower seroprevalence was reported before in camels from Somalia (3.1%) [16] and (0.3%) [15], and Ethiopia (2.4%) [26]. Higher seroprevalence of camel brucellosis has been also reported in Somalia (10.4%) [14]. additionally, our result is higher than that reported in Egypt (2.3%) [27]. Higher prevalence rates were reported in Ethiopia (7.6%) [28], Kenya (10.5%) [29], Sudan (40.5%) [30], Yemen (11%) [31], Saudi Arabia (8%) [32] and Kuwait (14.8%) [33]. The low seroprevalence (4.4%) detected in the present study might be due to the low density of camel population kept in a widely extended grazing or/and farm land which reduce the concentration and close contact of camels. Moreover, the good practice of herders' timely culling of camels with proven reproductive problems from the herds might have contributed to the current low prevalence.

Our results revealed that modified RBPT detected more positive cases than the RBPT, actually RBPT is validated and its antigen standardized to screen bovine samples for brucellosis. Thus, mRBPT could be an alternative test to advantageously replace the RBPT for the screening of brucellosis in camels; and the test is recommended by the OIE for camel serum samples screening for brucellosis antibodies. On the other hand, there were no differences between mRBPT and SAT in terms of sensitivity. Four (6.5%) of the 62 RBPT-negative samples were positive on the cELISA, giving that the RBPT have missed 6.5% of seropositive (false negative) camels. Similar findings comparing different serological tests were reported by Omer *et al., (2010)* [30]. The later author found that cELISA detected 2.1% more positives than the RBPT. In the present study, a perfect agreement between mRBPT, SAT and cELISA was proven by calculating Kappa values (0.841) with high sensitivity of (85.7%) of all tests, while the RBPT have only a sensitivity of (42.9%) and Kappa value of (0.589) when compared to the cELISA. Thus, our results suggest combining

cELISA with either mRBPT or SAT for detection of *Brucella* antibodies in camels.

Although no statistically significant difference (P>0.05) observed between the two production systems, the present study agreed with many authors [11, 32, 34] that higher seroprevalence was found in semi-intensive camel farms (4.0%) than extensively managed camels (3.6%). However, both production systems in this study were in the range of the low prevalence rate (2-5%) as reported by Abbas [12]. This is might be contributed by the low concentration of camel population kept in both systems which reduces the chances of contact between animals which is one of the factors of likelihood of *Brucella* infection [35].

In contrary to the established fact, no significant difference was observed in the prevalence of brucellosis between camels co-herded with small ruminants and camels kept alone in this study. Even though Bekele (2004), [11] and Al-Majali *et al.* (2008) [34] have reported that contact of camel herds with small ruminants were a contributing risk factor to brucellosis at herd and individual levels [11, 34]. A high number (77%) of interviewees did not know brucellosis. Moreover, all respondents manage abortion materials and other excreta with bare hands. In addition to that, 100% of the participants consume raw camel milk. These findings can potentially play a major role of transmission of the disease in both animals and human.

In conclusion: The present study revealed that the seroprevalence of brucellosis in camels from Banadir region of Somalia was low (4.4%). Although the seroprevalence of camel brucellosis is low, the disease still poses a considerable risk that contributes to the occurrence of the disease in an unaffected animals and herds and to the public health because of its zoonotic nature as well as market value of the camels. Therefore, the study recommends further brucellosis

epidemiological studies in camels, other ruminants and human. Isolation and identification of the *Brucella* biovars in Somalia will leads to selection of the best option of control strategy suitable to country. This will lead to improvement of animal and human health.

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Table 1: Number of camels sampled in the study area

Region	Districts	Number of camels sampled			Number of herds	Production System	
		Total	Female	Male		Extensive	Semi-intensive
Banadir	Daynile	85	83	2	11	33	52
	Yaqshid	23	22	1	1	23	0
	Kahda	72	69	3	4	0	72
Total		**180**	**174**	**6**	**16**	**56**	**124**
Total						**180**	

Table 2: Seroprevalence of camel brucellosis at herd and individual levels using RBPT and mRBPT:

District	Number of herds	RBPT Positive (%)	P-value	mRBPT Positive (%)	P-value	Number of samples	RBPT Positive (%)	P-value	mRBPT Positive (%)	P-value
Daynile	11	2 (18.2%)	0.85	2 (18.2%)	0.16	85	2 (2.4%)	0.71	2 (2.4%)	0.37
Kahda	4	1 (25.0%)		2 (50.0%)		72	1 (1.4%)		3 (4.2%)	
Yaqshid	1	0 (0.0%)		1 (100%)		23	0 (0.0%)		2 (8.7%)	
Total	**16**	**3 (18.8%)**		**5 (31.2%)**		**180**	**3 (1.7%)**		**7 (3.9%)**	

Table 3: Seroprevalence of camel brucellosis at herd and individual levels using SAT and c-ELISA:

District	Number of herds	SAT Positive (%)	P-value	cELISA Positive (%)	P-value	Number of samples	SAT Positive (%)	P-value	cELISA Positive (%)	P-value
Daynile	7	2 (28.6%)	0.28	2 (28.6%)	0.38	40	2 (5.0%)	0.24	2 (5.0%)	0.24
Kahda	3	2 (66.7%)		1 (33.3%)		19	3 (15.8%)		3 (15.8%)	
Yaqshid	1	1 (100%)		1 (100%)		10	2 (20.0%)		2 (20.0%)	
Total	**11**	**5 (45.5%)**		**4 (36.4%)**		**69**	**7 (10.1%)**		**7 (10.1%)**	

Table 4: Serological test results of the serum samples from camels in Banadir

Number of samples	RBPT		mRBPT		SAT		cELISA	
	P+ve (%)	N-ve (%)	P+ve (%)	N-ve (%)	P+ve (%)	N-ve (%)	P+ve (%)	N-ve (%)
180 for RBPT & mRBPT 69 for SAT & cELISA	3(1.7)	177(98.3%)	7(3.9%)	173(96.1%)	7(10.1%)	62(89.9%)	7(10.1%)	62(89.9%)

Table 5: Prevalence of camel brucellosis in relation to the production systems using standard RBPT and modified RBPT

Description	Category	Total sample	RBPT positive (%)	P-value	mRBPT positive (%)	P-value
Production System	Extensive	56	0 (0.0%)	0.24	2 (3.6%)	0.88
	Semi-intensive	124	3 (2.4%)		5 (4.0%)	
Total		**180**	**3 (1.7%)**		**7 (3.9%)**	

Table 6: Prevalence of camel brucellosis in relation to the production systems using SAT and cELISA

Description	Category	Total sample	SAT positive (%)	P-value	cELISA positive (%)	P-value
Production System	Extensive	23	2 (8.7%)	0.78	2 (8.7%)	0.78
	Semi-intensive	46	5 (10.9%)		5 (10.9%)	
Total		**69**	**7 (10.1%)**		**7 (10.1%)**	

Table 7: Comparison of serological test results

P+ve Sample Identification	RBPT	mRBPT	SAT	cELISA
SOCM 34	–	+	+	+
SOCM 36	–	+	+	+
SOCM 53	+	+	+	+
SOCM 56	–	+	+	+
SOCM 83	+	+	+	+
SOCM 110	+	+	+	+
SOCM 129	–	–	–	+
SOCM 145	–	+	+	–

Table 8: Comparison of RBPT and cELISA test results

RBPT	cELISA		Total
	Positive	Negative	
Positive	3	0	**3**
Negative	4	62	**66**
Total	**7**	**62**	**69**
Sensitivity		42.9%	
Specificity		100.0%	
Overall agreement		94.2%	
Kappa value		0.589%	

Table 9: Comparison of mRBPT and cELISA test results

mRBPT	cELISA		Total
	Positive	Negative	
Positive	6	1	**7**
Negative	1	61	**62**
Total	**7**	**62**	**69**
Sensitivity		85.7%	
Specificity		98.4%	
Overall agreement		97.1%	
Kappa value		0.841	

Table 10: Comparison of SAT and cELISA test results

SAT	cELISA		Total
	Positive	Negative	
Positive	6	1	**7**
Negative	1	61	**62**
Total	**7**	**62**	**69**
Sensitivity		85.7%	
Specificity		98.4%	
Overall agreement		97.1%	
Kappa value		0.841	