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Research Article

Seroprevalence of Camel Brucellosis in Yabello District of Borena Zone, Southern Ethiopia

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Abstract

A cross-sectional study was carried from November 2014 to April 2015 to estimate the seroprevalence and assess associated risk factors of camel (*Camelus dromedarius*) brucellosis in Yabello district of Borena Zone, Southern Ethiopia. A multi-stage cluster sampling method was used to select pastoral associations and camel herds and a questionnaire survey was administered to 46 willing respondents whose camels were included in the sample unit. The sera obtained were initially screened with Rose Bengal Plate Test (RBPT) and those samples found positive by RBPT were further tested by Complement Fixation Test (CFT) for confirmation. Out of 384 sera 14 (3.6%) were positive using RBPT and 12 (3.1%, 95% CI: 1.3 to 4.9%) were positive using CFT. The study showed there was statistically significant difference ($P<0.05$) between age groups and those with history of abortion. However, no statistical significant difference ($P>0.05$) was observed among the pastoral associations, contact with other ruminant, parity, herd size and sexes of animals.The questionnaire survey showed that all owners have no awareness about zoonotic importance of the disease, drink raw milk and did not take care of retained fetal membranes and aborted fetuses. The current level of seroprevalence is enough to be a potential hazard for public health in the study area; therefore, public education about zoonotic importance of brucellosis, controlling the risk factors, proper hygienic practices and team work between veterinary and health personnel should be improved.

ABBREVIATIONS

CFT: Complement Fixation Test; RBPT: Rose Bengal Plate Test

INTRODUCTION

The camel (*Camelus dromedarius*) is an economically important livestock species uniquely adapted to hot arid environments. World camel population is estimated to be around 25.89 million across 47 countries. About 85% of the camel population inhabits mainly eastern and northern Africa and the rest in Indian subcontinent and Middle East countries [1]. According to Central Statistical Authority of Ethiopia (unpublished), camels represent a subset of major livestock resources with a population estimated at >2.4 million. The main ethnic groups owning camels in Ethiopia are the Beja, Rashaida, Afar, Somali and Borena [2]. Camels play a significant multi-purpose role in the low lands of Ethiopia that comprises 61% of the national land area [3]. Camel production is practiced by pastoral communities under diverse constraints in dry and marginal areas. Camels have been formerly considered as hardy animals and less susceptible to most of the diseases that affect other livestock in the same ecological zones. Pastoralists have herded dromedary camels for centuries in the arid and semiarid areas of Ethiopia and have repeatedly encountered and named various diseases. However, the exact causes of many of these illnesses, known by local vernacular names, remain unknown [4].

Brucellosis is a zoonotic infectious disease of animals caused by Gram negative intracellular bacteria of the genus *Brucella* [5]. Four species commonly infect man: *B. abortus, B. melitensis, B. suis* and *B. canis* [6]. The disease can affect almost all domestic species and cross transmission can occur between cattle, sheep, goat, camel and other species [7]. Camels are not known to be primary host for any of *Brucella* organisms but they are susceptible to both *B. abortus* and *B. melitensis* [8].

Besides its worldwide zoonotic importance, brucellosis is recognized as a major cause of heavy economic losses to the livestock industry. The World Health Organization (WHO) estimates that a quarter of human cases go unreported, even half a million cases per year are recorded [9]. The disease is characterized by febrile illness in humans and often difficult to diagnose solely from the clinical picture, due to its similarities to other febrile diseases, such as malaria or typhoid fever [10]. The transmission of brucellosis from animals to humans is effected by ingestion of raw milk, milk products and raw liver, and contact with or handling of materials from infected animals [11]. Though the main risk of transmission to humans in larger industrial settings considered to be occupational, in smaller-scale farming systems, such as pastoral production, are also affected, due to the proximity between the animals and their owners, their mobile lifestyle and the traditional marketing of unpasteurized milk and milk products [12]. Brucellosis also cause significant loss of productivity in camels through late first calving age, long calving interval time, low herd fertility and comparatively low milk production [13].

In Ethiopia, the first report of camel brucellosis was revealed in the provinces of Sidamo, Harar and Tigray with seroprevalence of 4.4% (n=977) [14]. Teshome and his colleagues [15] also investigated seroprevalence of brucellosis in 1442 camels in arid and semi-arid camel-rearing regions (Afar, Somali and Borena) of Ethiopia. In their study, seroprevalences of 5.7% and 4.2% were obtained using RBPT and CFT, respectively. In addition, camel brucellosis was investigated In Borena lowland with seroprevalence of 1.8% (58/3218) [16]. Birhanu [17] reported an individual animal and herd seroprevalence of 2.43% (n=822) and 10.3% (n=185), respectively, in camels in southeast lowland areas of the Somali Region. However, the study of camel brucellosis in other areas of Ethiopia was so scanty and did not provide detail epidemiological information of the disease. In addition there was no previous study conducted on camel brucellosis in Yabello district. Therefore, the objective of the present study was to estimate the seroprevalence and to assess associated risk factors of camel (*Camelus dromedarius*) brucellosis in Yabello district of Borena Zone, Southern Ethiopia.

MATERIAL AND METHODS

Study area

The study was conducted from November 2014 to April 2015 in Yabello district of Borana Zone, southern Ethiopia. The district comprises about 23 pastoral associations (PAs), in which 11 (48%) PAs and 12 (52%) PA's of the peoples dwelling in and around the district practicing pastoral and agro-pastoral activities, respectively [3]. Pastoral societies mainly rear and derive most of their income from livestock; whereas, agro-pastoralists are segments of pastoral society who promote opportunistic crop farming integrated to their livestock husbandry practices [18]. Yabello district is located at the southern part of Ethiopia in Oromia Regional State at about 570 km away from Addis Ababa in southern direction [19]. Geographically the district is located at latitude of about 5°23'49" N and longitude of about 39°31'52" E and at elevation ranging 1000-1500 meter above sea level. The mean annual minimum and maximum temperatures are 24 and 29°C, respectively. The climate is generally semi-arid with annual average rainfall ranging from 300 mm in the south to >700 mm in the north. According to Borana zone department of planning and economic development bureau (unpublished), the total camel population of Borena zone and Yabello district were estimated to be about 232,589 and 44,042, respectively.

Study population and design

The sources of study population were all camels in Yabello district. The total camel population of the district is estimated to be about 44,042 and a cross-sectional study design was used for the study.

Sampling Methods

Multi-stage cluster sampling technique was used in the study by considering PAs as primary units, camel herds found in each PAs as secondary units and selected camel herds as tertiary units. Cluster sampling was the suitable method for this study as constructing sample frame for random sampling was not possible in pastoral production system. The clustering of the PAs was based on accessibility to villages by vehicle or proximity to road and camel population. The study animal selection strategy was by categorizing animals in the herds into adult and young animals. Herds were visited and sampled early in the morning before released to the field. Finally, for the prevalence study, a total of 384 animals of above 1 year of age with no history of vaccination against brucellosis and both sex (92 from Dharito, 162 from Surupha, 46 from Dida Hara and 84 from Danbala Sadin) were selected from 46 different herds.

Determination of sample size

The study sample size was determined according to Thrusfield [20] formula for an infinite population with 95% confidence level, 5% desired absolute precision by considering expected prevalence of camel brucellosis in the area. Accordingly, 384 camels were sampled.

$$N = \frac{(1.96)^2 * Pexp*(1- Pexp)}{d^2} = 384$$

Where:

n = required sample size

P_{exp} = Expected prevalence

d = desired absolute precision

Data Collection

Relevant data on the animals such as sex, age and other potential risk factors (herd size, abortion, parity and contact with other ruminants) associated with brucellosis was recorded during each sample collection. In addition, a semi-structured questionnaire survey containing open and closed ended questions were administered to 46 willing respondents whose camels were included in the sample unit to collect information regarding history and period of abortion, herd size, production type, use of camel, source of new camel, awareness of brucellosis, contact with other camel herds, contact with other ruminants, source of breeding camel and reproductive disorders (retention of fetal membranes, stillbirth and infertility) and presence of testicular and joint swellings.

The determination of age groups for this study was based on findings of researchers in extensive production systems. Camels produced under extensive production system reach maturity at 3 to 4 years of age [21]. Age at puberty and first calving in camel were 4 and 5 years, respectively for females whereas males had age of 5 years at puberty [22]. Age was classified as '<4 years and >4 years' in order to see the distribution of the disease in immatured and sexually matured camels and camels of 4 years and above are considered matured (at age of puberty) and less than 4 years considered sexually immature for this study [23].

Sample collection

Approximately about 10 ml of whole blood sample was collected from the jugular vein of each camel included in the study using plain vacutainer tubes and needles. Each sample tube was labeled using codes specific to the individual sample. The collected blood samples were allowed to clot at room

temperature and serum was separated from clotted blood by decanting to plastic criovials. Separated sera were stored at -20°C for further serological testing.

Examination of blood specimens

In Yabello Regional Veterinary Laboratory, all sera samples collected were initially screened by Rose Bengal Plate Test (RBPT) using RBPT antigen (IVRI, Indian Veterinary Research Institute, Izatnagar, U.P., India). Sera and antigen were taken from refrigerator and left at room temperature for half an hour and processed following the test procedure recommended by Alton and his colleagues [24] and OIE [25]. Briefly, it was recorded as ++++ (coarse clumping and clearing), +++ (clumping and some clearing), ++ (visible fine agglutination), + (weak fine agglutinations using magnifying glass) in case of positive reactions, and 0 (no agglutinations) in negative reactions.

Sera that tested positive to the RBPT were further tested using Complement Fixation Test (CFT) for confirmation using Standard *B. abortus* antigen S99 (CVL, New Haw Weybridge, and Surry KT15 3NB, UK) at National Veterinary Institute, Debre-Zeit. Preparation of the reagent was evaluated by titration and performed according to protocols recommended by World Organization for Animal Health [26]. 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. Samples were considered positive for brucellosis if they were positive for both RBPT and CFT.

Data Analysis

All data collected during the study were carefully entered into Microsoft Excel Spread Sheet and then imported to SPSS Version 16. Descriptive and analytic statistics were computed using software SPSS Version 16. Logistic regression and Chi-square test (χ^2) were employed to identify possible risk factors associated with seropositive camels. The degree of association was computed using odds ratio (OR) signified by 95% confidence intervals [20].

RESULTS

Out of 384 tested samples, only 14 (3.6%) were found positive by RBPT and further confirmation with CFT showed that 12 (3.1%, 95% CI: 1.3 to 4.9%) were positive out of the 14 RBPT reactors. The seroprevalence was found to vary insignificantly in different PAs (P>0.05). The highest prevalence was found in Danbala Sadin (4.7%) followed by Surupha (3.1%) then Dida Hara (2.2%) and Dharito (2.2%), respectively. The overall and district level differences in seroprevalence were not statistically significant (P>0.05). The present study also showed herd seroprevalence of brucellosis of 26% (12 out of the 46 contained infected cases) and the infection rate within them ranged from 3.8 to 16% per herd.

Prevalence of camel brucellosis in the study area was 0.6% in the younger stock which was increasing with the advance of age and reached as much as 4.8% in animals older than 4 years. The differences in seroprevalence of brucellosis between age groups were statistically significant (P<0.05) (Table 1). Variation in the seroprevalence was also observed with in sex. Female maintained a comparatively lower seroprevalence than the male. The prevalence of brucellosis, regardless of the PAs, out of the total 384 camels examined, 293 were she-camels in which 8

Table 1: Risk factors with dependent *Brucella* seropositivity in camels of Yabello district of Borena zone, southern Ethiopia.

Risk factors	Category	Number Tested	CFT Positive (%)	P-value	OR (95% CI)
PAs	Dharito	92	2(2.2%)		
	Surupha	162	5(3.1%)	0.763	2.250 (0.401-12.614)
	Dida Hara	46	1(2.2%)		
	Danbala Sadin	84	4(4.7%)		
Age	< 4 years	157	1 (0.6%)	0.020*	7.944(1.015-62.173)
	> 4 years	227	11 (4.8%)		
Sex	Male	91	4 (4.4%)	0.425	1.638 (0.482-5.570)
	Female	293	8 (2.7%)		
Abortion	Yes	91	8 (8.8%)	0.000*	0.477 (0.138-1.644)
	No	202	0 (0.0%)		
Contact	Yes	171	6 (3.5%)	0.699	1.255 (0.397-3.967)
	No	213	6 (2.8%)		
Parity	No	91	0 (0.0%)	0.104	0.963 (0.897-1.035)
	Single	36	0(0.0%)		
	More than one	166	8 (4.8%)		
Herd size	1-9	69	2 (2.9%)	0.987	0.934(0.405-2.157)
	10-20	194	6 (3.1%)		
	> 20	121	4 (3.3%)		

PAs: Pastoral Associations; CFT: Complement Fixation Test; OR: Odds Ratio; CI: Confidence Interval; * statistically significant (*P < 0.05*).

(2.7%) of them seropositive to *Brucella* infection. Similarly, out of 91 male camels 4 (4.4%) were seropositive. The variation in seroprevalence of brucellosis with in sex showed no statistically significant difference (P>0.05). The study showed seroprevalence of brucellosis between camels in contact with other ruminants were from 171 camels 6 (3.5%) of them seropositive to *Brucella* infection and 6 (2.7%) camels from 213 camels not get contact with other ruminants were seropositive to *Brucella* infection. Camels in contact with other ruminants and those not get contact with other ruminants showed no statistically significant difference (P>0.05) (Table 1).

Comparison was made on the seroprevalence of brucellosis in females with history of abortion and females without history of abortion to observe the effect of abortion in the abundance of the disease, the result of the current study showed that seropositivity was higher (8.8%) in females with history of abortion than in females without history of abortion (0.0%). However, the observed difference was statically significant (P<0.05). According to the result of the present study, 2.9% was recorded in herds with 1-9 animals, 3.1% was recorded in herds with 10-20 animals and 3.3% was recorded in herds with >20 animals. However, there was no statistically significant variation between the herds (P>0.05).The present study attempted to identify the existence of an association between the seroprevalence of brucellosis and parity. Thus, the result showed that the seroprevalence of camel brucellosis in animals with no parity was 0.0%, with single parity 0.0% and 4.8% in animals with more than one parity (Table 1). Although seropositivity was higher in camels with more than one parity, the difference in prevalence of brucellosis between the camels considering parity as a risk factor was not statistically significant (P>0.05).

The Chi-square analysis revealed that it was only age (P=0.020) and abortion (P=0.000) that showed statistically significant (P<0.05) association with seropositivity of camel brucellosis than the other risk factors which were not statistically significant (P>0.05). When these two major risk factors are compared, abortion was highly associated (P=0.000) with the occurrence of seropositivity of the disease in camels than age (Table 1).

The questionnaire survey revealed that extensive management system was exercised in the area and managed by non-educated persons; camels are kept alone as well as together with other species of animals mainly for milk production, and other functions including transport and social security. The highest proportion of camel herds (65.2%) kept alone and the remaining camel herds (34.8%) were kept with other ruminants (cattle, sheep and goats). According to the respondents, 60.9% of the camel herds got contact with other camel herds while 39.1% was not got contact with other camel herds. The highest proportion of the respondents (69.4%) kept mixed (males and females) camels together, while 30.6% of them kept herd containing female camels only. Most of camel herders (56.5%) use herd as source of new camel and 43.5% of them obtained new camel by purchasing. Most of the herders (65.2%) used breeding bull from communal village while 34.8% of them used their own herd bull.

According to the respondents, 47.8% of the camel herds had history of abortion and 52.2% did not show abortion. None of the herders (100%) practice milking hygiene, all consumed fresh raw milk without any heat treatment, and none of the respondents have awareness about camel brucellosis. Furthermore, camel herders do not practice disposal of aborted fetus, placenta and discharges and left them on the ground. Camel owners use traditional wells and ponds (89.1%) as the main water sources during dry season whereas 10.9% of them use tape water as the source of water for their herd.

DISCUSSION

In the present study the overall seroprevalence of camel brucellosis using CFT was 3.1% (95% CI: 1.3 to 4.9%). As per the study by Abbas and Agab [26], the seroprevalence of brucellosis in camels appears to follow two distinct patterns: low (2-5%) prevalence in nomadic camels and high (8-15%) prevalence in camels kept intensively or semi-intensively. The present study agrees with the low prevalence as nomadic people keep most camels in this district. This finding is in agreement with the study conducted in Ethiopia (Tigray) [27], Iraq [28], Somalia [7], Ethiopia (Afar, Somali and Borena) [15] and Eritrea [29] with prevalence of 3.67%, 3.03%, 3.1%, 2.8% and 3.1%, respectively. This could be attributed to the similarity in agro-ecological conditions and livestock management system in the areas.

However, the result of this study is lower than the observations recorded in Borena/Ethiopia [14], Libya [Gameel et al., 1993], Sudan [31], Egypt [8] and Afar region of Ethiopia [32] with prevalence rates of 4.4%, 4.1% , 5.5% , 7.3% and 7.6%, respectively. It is also much lower than the findings in Kenya (6.0 to 38.0%) [33], Jordan (19.4%) [34], and Sudan (8.0, 30.5 and 23.8%) [11,35,36].

The differences could be due to variations in animal management and production systems. Kenya and Sudan are characterized by mixed farming in which fewer animals are raised and they are kept separately [4,33], whereas in the camel rearing areas of Ethiopia, large numbers of different species of animals are raised on communal pastures and watering areas [37].

In contrast, the observation of current investigation is higher than prevalence rates of 0.4 to 2.5% reported in Borena/Ethiopia [38], 2.43% in Jigjiga and Babile districts of Somali Region/ Ethiopia [37], 0.3 to 1.9% in Somalia [39], 1.8 and 1.5% in other areas of Ethiopia [40, 41], and 1.4% in Saudi Arabia [42]. These all variation in seroprevalence could be due to the difference in sample size used and agro-ecology. Since brucellosis is considered as disease of herd importance, in this study higher herd level seropositivity of 26.0% was found than 16% in Borena [38] and 10.27% from Jigjiga and Babile districts of Somali Region of Ethiopia [37]. This could be due to the presence of high number of camels in the herds and mixing of aborting camels with normally parturient camels and difference in number of herds involved in sample unit. Even though, brucellosis was detected in all the four PAs with slight variation in prevalence but with no statistically significance (P>0.05). This could be attributed to the similarity in susceptibility of the animals, virulence of the organisms, presence of reactor animals in the area and pastoralists' movement from place to place.

Although statistically significant difference (P<0.05) was observed in seroprevalence of brucellosis between the young and mature age groups, higher seroprevalence was found in mature camels (4.8%) than young camels (0.6%). This finding was in line with the study conducted in Dire Dawa with seroprevalence of 1.8% in mature camels and 0.7% in young camels [41]. Sexually matured animals are more prone to *Brucella* infection than sexually immatured animals of either sex. This might be due to the fact that as sex hormones and erythritol tend to increase in concentration with age and sexual maturity and favor growth and multiplication of brucellae organisms [43]. On the other hand, it is also true that younger animals tend to be more resistant to infection and frequently clear an established infection [44] although latent infections can occur [45].

There was no statistically significant association (P>0.05) between parity and the seroprevalence of the disease. The seropositivity of she-camels with the history of no parity, single parity and more than one parity were 0.0%, 0.0% and 4.8%, respectively. Higher seropositivity was recorded in she-camels which gave birth to more than one calf than those with single parity. This is therefore, in consistent with the previous studies [32,38], in which higher reactors were recorded in camels with more than one parity, compared to other group of camels. This might be due to repeated exposure of the she-camels to parturition and other physiological stress increases the probability of acquiring *Brucella* infection.

The analysis result also revealed that the prevalence of brucellosis between sexes did not show significant association (P>0.05). The prevalence was higher in males (4.4%) compared to prevalence in females (2.7%). The present finding was in agreement with the records obtained from Jigjiga and Babile districts of Somali Region/Ethiopia with seroprevalence of 2.76% in males and 2.34% in females [37]. On the contrary, in Ethiopia [38], Sudan [46,47] and Nigeria [48], the likelihood of occurrence of infection is higher in female than male animals. Relatively higher susceptibility of she-camels could be due to the fact that they have more physiological stresses than the males [45]. In addition, Hirsh and Zee [49] have reported that male animals are less susceptible to *Brucella* infection due to the absence of erythritol, other researchers [47,50] reported equal distribution of *Brucella* antibodies between both sexes. But the current finding might be due to the number of breeding males kept by the pastoralists in the camel herds of the present study was very small on which random sampling method was applied and this predictably bias the statistical analysis.

High number of camels, cattle and small ruminant diversification were noticed in the study district. Such animal species distribution and diversification is common to other areas and has economic and ecological advantages [33]. However, it increases the chance of brucellosis and other disease transmission from other infected ruminants to dromedaries [51,52]. In the present study, seroprevalence in camel made contact with other ruminants and without contact with other ruminants were 3.5 and 2.8%, respectively. However, no significant difference (P>0.05) was observed between these two camel groups. The present finding was in line with the observation from Somalia [51] and Saudi Arabia [53]. A contributing factor to the spread of the disease may be the movement of animals for grazing and watering during the dry season as aggregating the animals around watering point might increase the contact between infected and healthy animals and thereby facilitate the spread of the disease [43].

The results obtained in this study revealed that, abortion appears to be a major risk factor for brucellosis compared with other risk factors (p=0.000). Higher seroprevalence of Brucellosis observed in adult female camels which had history of abortion (8.8%) compared to that of adult female camels which had no history of abortion (0.0%). This result is in agreement with the findings obtained from different regions of Sudan with the seroprevalence ranging from 3.1-72.7% in camels with reproductive disorders [52]. This result supports the truth that reproductive problems like abortion in camels can be caused by brucellosis [45]. However, the current finding was opposed by Megersa and his colleagues [40] who reported the absence of association between camel brucellosis and abortion.

Stocking densities are important potential determinants for brucellosis transmission [29]. This concept coincides with the current study that the seroprevalence of brucellosis among three categorized herd sizes (1-9, 10-20 and >20) showed higher seroprevalence recorded in the large herd sizes of camels. Herds with more than 20 camels were more frequently affected. Seroprevelance was 3.3% in large herds, 3.1% in herds with 10-20 camels and 2.9% in small herds (1-9 camels). This result was in agreement with the previous reports in Afar [32] and Borena [38] regions of Ethiopia. As herd size increases, the chance of contact between animals' increases leading to more chances of infection, which is particularly more important during calving or abortion when most of the *Brucella* contamination occur [26] Thus, herd size and density of animal population together with poor management are directly related to infection rate [13].

CONCLUSIONS

The data obtained in the present study revealed that brucellosis in camels is a widespread disease in Yabello District of Borena Zone, Southern Ethiopia. The main risk factors identified for the presence and transmission of the disease from animal to animal were age and abortion. In general, traditional husbandry and poor management practices, mixing with other animals and sharing of breeding male camels were thought to support spread of the disease from animal to animal in the study area. Lack of awareness about the zoonotic nature of brucellosis, together with an existing habit of raw milk consumption and close contact with animals, can serve as means of infection to human beings. Therefore, public health education on modern animal husbandry, disease prevention techniques and risk of zoonotic diseases should be imparted in the study area continuously.

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