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Sero-epidemiology of camel brucellosis in the Afar region of Northeast Ethiopia

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Camel brucellosis represents a major public health concern, which affects social and economic development in developing countries. A cross-sectional study was conducted in three selected districts of Afar region of Ethiopia to determine seroprevalence of camel brucellosis. A total of 1152 camels from 168 camel herds were included in the study. All serum samples were consequently tested and confirmed serologically using Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT). Risk factors analysis was also conducted using multivariable and univariate logistic regression analysis. As a result, 58 (5.0%) were RBPT reactors in which 47 (4.1%, 95% CI: 2.9 to 5.3%) were confirmed to be positive using CFT and at least one reactor camel was found in 37 (22.0%) of the total herds sampled. The statistical analysis indicated that herd size (OR=0.64; 95% CI: 0.42 to 0.98, P=0.04) and contact with other ruminants (OR=0.62; 95% CI: 0.47 to 0.82, P=0.001) were the major risk factors for the presence and transmission of the disease between animals. In addition, pluriparous (4.7%), abortive (5.7%), pregnant (6.6%) and lactating (4.1%) camels were found with higher seropositivity which contributed in transmission of the disease to calves, other ruminants as well as to humans, but this was not a statistically significant association (P>0.05). In conclusion, camel brucellosis is prevalent in this 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 in the area.

Key words: Camel brucellosis, complement fixation test (CFT), Ethiopia, Rose Bengal plate test (RBPT), risk factors, seroprevalence.

INTRODUCTION

Brucellosis is an infectious disease of domestic and wild animals with serious zoonotic and economic implication in humans. The disease is an important public health problem in many parts of the world (Pal, 2007; Hadush and Pal, 2013). The disease in dromedary camels can be caused by *Brucella abortus, Brucella melitensis* and *Brucella ovis* (Seifert, 1996). Different studies showed that *B. abortus* and *B. melitensis* are the most frequently isolated from milk, aborted fetus and vaginal swabs of diseased camels (Radwan et al., 1992; Gameel et al., 1993; Agab et al., 1994; Abou-Eisha, 2000; Hamdy and Amin, 2002) and the transmission of brucellosis depends on the *Brucella* species being prevalent in other animals sharing their habitat and on husbandry (Musa et al., 2008).

Camels are not known to be primary hosts of *Brucella*, but they are susceptible to both *B. abortus* and *B. melitensis*. Consequently, the prevalence depends upon

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the infection rate in primary hosts being in contact with them. Brucellosis may spread from camels to humans, especially via milk. Therefore, the zoonotic risks from camel milk must be considered in view of the traditional African and Arabian preference for raw milk consumption (Cooper, 1991). Groups at high risk for brucellosis are animal health workers, butchers, farmers, and those who habitually consume raw camel milk and come in contact with these animals (Chukwu, 1987).

The uncontrolled movement of camel from infected herds or area to *Brucella* free herds or areas is the major obstacles in brucellosis eradication program (Radostits et al., 2007). Other management factors influencing inter-herd transmission are proximity to infected herds, water ways, and scavengers. Vaccination level, herd size, population density, methods of housing, and use of maternity pens also influence the probability of exposure to the infection (Crawford et al., 1990).

The disease can generally cause significant loss of productivity through late first calving age, long calving interval time, low herd fertility and comparatively low milk production, as in cattle may also happen in camels. The disease can also have an impact on export and import of animals constraining livestock trade (Radostits et al., 2007).

Africa hosts 80% of the world population of dromedary (16.5 million) of which 63% are attributed to East Africa (Wilson, 1998). Camels are a subset of huge livestock resources in Ethiopia with the population estimated to be over one million. This number ranks the country third in Africa after Somalia and Sudan and fourth in the world. The arid and semi-arid areas of the country that constitutes more than 60% of the total area are suitable for camel production. The eastern and southern parts of the country, namely, Afar, Somali and Borena are the major areas where camel husbandry is widely practiced. In these areas, the livelihood of the pastoral communities is certainly ensured by dromedaries (Teka, 1991; Wossene, 1991).

Therefore, the present study was contemplated to determine the seroprevalence and associated risk factors of camel brucellosis in selected districts of Afar regional state of Ethiopia.

MATERIALS AND METHODS

Study areas

Afar regional state is located in the Great Rift Valley, comprising semi-arid range land in Northeastern Ethiopia. According to regional estimates, the livestock population of Afar is about 10.12 million Trap Logic Unit (TLU) and out of this, about 859,580 (8.5%) are camels. The Afar Regional State has five administrative zones, which are further subdivided into 32 districts. Pastoralism and agro-pastoralism are the two major livelihood ways practiced in the region. The population of the region is estimated to be about 1.2 million of which 90% are pastoralists and 10% agro-pastoral (CSA, 2007). This study was conducted in three purposively selected districts of zone one namely, Mille, Chifra and Dubti. This study was conducted in the pastoral areas of the districts. Pastoralist association (PA) is the lowest administrative unit within the districts considered during the study. Accordingly, six PAs each from Mille and Chifra and five PAs from Dubti district were randomly selected.

Study design

A cross-sectional study design was conducted to determine the prevalence of *Brucella* infection in camels in the selected districts and to identify the potential risk factors associated with the sero-positivity. Camels above six months of age with no history of vaccination against brucellosis were selected. Camel's history such as sex, age, herd size, body condition score and contact with other ruminants was recorded.

Sampling methods

About 30% of the PAs in each of the districts were considered representative to the districts and included in the study on the basis of feasibility and affordability or cost. Hence, six PAs each from Mille and Chifra, and five PAs from Dubti were selected randomly. Multi-stage cluster sampling technique was used in this 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. Since there was no previous year's prevalence of brucellosis in the districts, the average expected prevalence was assumed to be 50% for the areas within 95% confidence interval (CI) at 5% desired accuracy and the total sample is 384 (Thrusfield, 2005). However, cluster sampling can lead to an increased sampling variance and a large sample size would be required to reduce variance to acceptable levels. Therefore, the sample size was increased by three folds and a total of 1152 camels from 168 herds were considered for the study.

Samples collection

Approximately 6 to 8 ml of blood sample was collected from jugular vein of each camel using plain vacutainer tubes. 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.

Serological tests

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 before the test to maintain to room temperature and processed following the test procedure recommended by Alton et al. (1975) and OIE (2004). The result 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). Preparation of the reagent was evaluated by titration and performed according to protocols recommended by World Organisation 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. Samples were considered positive for brucellosis if they were positive for both RBPT and CFT.

Data management and statistical analysis

Descriptive and analytic statistics were computed using software SPSS® Version 20. 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 (Thrusfield, 2005).

RESULTS

A total of 1152 sera sample were collected from 168 camel herds with no previous history of vaccination against brucellosis from three districts. Out of 1152 tested samples, only 58 (5.0%) were found positive by RBPT and further confirmation with CFT showed that 47 (4.1%, 95% CI: 2.9 to 5.3%) were positive out of the 58 RBPT reactors. Further analysis of the confirmed positive samples (n=47) revealed that Mille district had slightly highest prevalence for brucellosis (n=20) (5.2%, 95% CI: 3.0 to 7.4%) followed by Dubti (n=15) (3.9%, 95% CI: 1.9 to 5.9%) and Chifra (n=12) (3.1%, 95% CI: 1.3 to 4.9%), respectively.

The Chi-square analysis revealed that it was only herd size (χ^2=8.043, P=0.018) and contact with other ruminants (χ^2=13.397, P=0.004) that showed statistically significant (P<0.05) association with seropositivity of camel brucellosis than the other risk factors considered during the study.

The univariable logistic regression analysis of the putative risk factors indicated statistically significant difference on seroprevalence of brucellosis between camels in contact with cattle (OR=3.546, 95% CI: 1.358 to 9.259, P<0.05) and camels in contact with small ruminants (OR=2.324, 95% CI: 1.221 to 4.424, P<0.05) than camels with no contact with any other ruminant. Camels in contact with both cattle and small ruminant (OR=1.854, 95% CI: 0.862 to 3.989, P>0.05) showed no statistically significant difference (Table 1).

Multivariate logistic regression analysis of risk factors determined herd size and contact with other ruminants (P<0.05) as the major risk factor for the occurrence of camel brucellosis seropositivity when compared with the rest risk factors considered in the analysis. When these two major risk factors are compared, contact with other ruminants was highly associated with the occurrence of seropositivity of the disease in camels than herd size with statistically highly significant association (P=0.001) in the study areas (Table 2).

Advance in herd size and contact with other ruminants were significantly associated with the infection (P<0.05) and have an effect on seropositivity when other factors which were not statistically significant (P>0.05) were removed (Table 3).

Out of the total 1152 camels examined, 810 were she-camels in which 598 were at the age of puberty with 28 (4.7%) of them seropositive to *Brucella* infection. Similarly, out of 256 male camels which were at age of puberty, 12 (4.7%) were seropositive (Table 4).

DISCUSSION

Previous serological surveys in Ethiopia showed that the disease is prevalent in different camel rearing areas of the country (Domenech, 1977; Richard, 1980; Teshome et al., 2003; Zewolda and Wereta, 2012). A study on camel husbandry practice in eastern part of the country by Getahun and Kassa (2000) indicated abortion rates and stillbirths of 9 and 4.3%, respectively, for which brucellosis is more likely to be incriminated. Hence, a cross-sectional study was conducted in three selected districts of Afar region to determine the prevalence of brucellosis in camels and to assess the associated risk factors in these areas.

In the present study, an overall seroprevalence of 4.1% (95% CI: 2.9 to 5.3%) was recorded in camels using both RBPT and CFT. This finding is in agreement with the results recorded by Teshome et al. (2003) and Domenech (1977) in Borena with prevalence of 4.2 and 4.4%, respectively and with Gameel et al. (1993) who recorded a prevalence of 4.1% in Libya. However, the result of this study is lower than the observation recorded by Richard (1980), Teshome et al. (2003) and Zewolda and Wereta (2012) with prevalence of 5.5, 5.7 and 7.6%, respectively in Afar region. It is also much lower than 6.0 to 38.0% reported by Wilson et al. (1990) in Kenya and 8.0% by Osman and Adlam (1987) in Sudan. But the observation of current investigation is higher than 0.4 to 2.5% reported by Bekele (2004) in Borena in which the variation could be due to the difference in sample size used and agro-ecology. The differences could also be due to variations in animal management and production systems. Kenya and Sudan are characterized by mixed farming (Wilson et al., 1990; Schwartz and Dioli, 1992) in which fewer animals are raised and they are kept separately, whereas in the camel-rearing areas of Ethiopia, large numbers of different species of animals are raised on communal pastures and watering areas.

Since brucellosis is considered as disease of herd importance, in this study higher herd level seropositivity of 22.0% was found than 16% recorded by Bekele (2004) in Borena. This could be due to the presence of high number of camels in the herds and mixing of aborting camels with normally parturient camels. Even though, brucellosis was detected in all the three districts with slight variation in prevalence, it was not statistically significant difference (P>0.05). This could be attributed to the similarity in agro-ecological conditions and livestock management system in the districts.

Table 1. Univariable logistic regression analysis of the putative risk factors.

Risk factor	Category	OR	95% CI	P value
Districts	Mille*	-	-	-
	Chifra	1.175	0.620-2.226	0.621
	Dubti	0.786	0.394-1.569	0.495
Sex	Male*	1.122	0.626-2.009	0.699
	Female			
Age	≤4 years*	1.041	0.597-1.815	0.888
	>4 years			
Body condition score	Good*	-	-	-
	Fair	2.075	0.888-4.853	0.092
	Poor	1.430	0.633-3.233	0.390
Herd size	≤10 camels*	-	-	-
	11-20 camels	1.948	0.989-3.837	0.054
	>20 camels	0.608	0.294-1.259	0.180
Contact with other ruminants	No contact*	-	-	-
	With cattle	3.546	1.358-9.259	0.010
	With cattle and SR	1.854	0.862-3.989	0.114
	With SR	2.324	1.221-4.424	0.010
Parity	No parturition*	-	-	-
	Primiparous	0.477	0.095-2.390	0.368
	Pluriparous	0.834	0.382-1.817	0.647
Reproductive problems	No RP*	-	-	-
	Abortion	0.837	0.244-2.868	0.777
	Still birth	1.444	0.576-3.621	0.433
	RFM	0.897	0.286-2.809	0.852
Physiological status	Heifer*	-	-	-
	Dry	0.477	0.095-2.390	0.368
	Lactating	0.545	0.191-1.555	0.256
	Pregnant	0.594	0.233-1.516	0.276

SR: Small ruminant; RP: Reproductive problems; RFM: Retained fetal membrane. *Reference category; OR: Odds ratio; CI: Confidence interval.

In contrary to the established fact, no significant difference was observed in the prevalence of brucellosis between sexes. 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. Even though Hirsh and Zee (1999) have reported that male animals are less susceptible to *Brucella* infection due to the absence of erythritol, other authors (Waghela et al., 1978; Abu-Damir et al., 1984; Abbas et al., 1987) reported equal distribution of *Brucella* antibodies between both sexes. On the contrary, Bekele (2004) from Ethiopia, Yagoub et al. *(*1990) and Agab et al. (1994) from Sudan, and Ajogi and Adamu (1998) from Nigeria revealed 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 (Walker, 1999).

Camels produced under extensive production system reach maturity at 3 to 4 years of age (Wilson, 1998). Tefera and Gebreab (2001) recorded age at puberty and first calving to be 4 and 5 years, respectively for females

Table 2. Multivariate logistic regression analysis of risk factors.

Risk factor	OR	95% CI	P value
Districts	0.854	0.593-1.231	0.398
Sex	0.961	0.503-1.836	0.904
Age	1.146	0.615-2.136	0.668
Body condition score	0.724	0.466-1.126	0.152
Herd size	0.640	0.421-0.975	0.038*
Contact with other ruminants	0.619	0.470-0.815	0.001*
Parity	1.126	0.602-2.104	0.711
Reproductive problems	0.952	0.680-1.332	0.733
Physiological status	1.285	0.802-2.060	0.297

OR: Odds ratio; CI= Confidence interval.*Herd size and contact with other ruminants were statistically found the risk factors for the occurrence of camel brucellosis (P<0.05) at 95% level of confidence.

Table 3. Putative effects of advance in herd size and contact with other ruminants on seroprevalence.

Risk factors	OR	95% CI	P value
Herd size	0.656	0.450-0.957	0.029
Contact with other ruminants	0.681	0.527-0.879	0.003

OR: Odds ratio; CI: Confidence interval.

Table 4. Status of seropositivity to camel brucellosis before and after age of puberty

Age	No. examined			CFT positive (% per sex)		
	Male	Female	Total	Male	Female	Total
Before puberty (<3 years)	86	212	298	2 (2.3)	5 (2.4)	7 (2.4)
After puberty (≥3 years)	256	598	854	12 (4.7)	28 (4.7)	40 (4.7)
Total	342	810	1152	14 (4.1)	33 (4.1)	47 (4.1)

whereas males had age of 5 years at puberty in Eastern Ethiopia. Wossene (1991) also reported the same age for puberty and first calving in Ogaden female dromedaries. Accordingly, age was classified as 'before and after puberty' in order to see the distribution of the disease in immatured and sexually matured camels and camels of 3 years and above are considered matured (at age of puberty) and less than 3 years considered sexually immatured for this study. Accordingly, out of the she-camels examined, 598 with seropositivity of 28 (4.7%) were at age of puberty (that is three years and above) and 212 with seropositivity of 5 (2.4%) were immatured (less than three years of age). Likewise, out of male camels examined, 86 with seropositivity of 2 (2.3%) were immatured and 256 with seropositivity of 12 (4.7%) were at age of puberty in which they can mate and used for breeding in the herds. This indicated that more seropositivity to camel brucellosis was seen in adults than in young camels as it is a disease of sexually matured animals. Hence, the presence of seropositive breeding males and she-camels were considered as risk factors playing a role in the transmission of the disease to other animals in the study districts.

Although no statistically significant difference (P>0.05) was observed between the two age groups, slightly higher seroprevalence was found in those groups with age of greater than 4 years (4.4%) than those groups with age of less than and equal to 4 years (3.6%). Sexually matured animals are more prone to *Brucella* infection than sexually immatured animals of either sex (Radostits et al., 2007). On the other hand, it is also true that younger animals tend to be more resistant to infection and frequently clear an established infection, although latent infections can occur (Walker, 1999; Quinn et al., 2004). This may be due to the fact that sex hormones and erythritol, which stimulate the growth and multiplication of *Brucella* organisms, tend to increase in concentration with age and sexual maturity (Radostits et al., 2007).

Immunity against various infections can be depressed

due to different reasons in which stress and feed play a greater role. Underfed animals are expected to have a decreased immunity that is manifested by poor body condition (Faye and Bengoumi, 2006; Radostits et al., 2007). Therefore, body condition of the camels was considered during the study to see the distribution of the infection in different body condition scores. But, high seropositivity was found in camels with good (5.7%) and fair (3.6%) body condition score than camels with poor (3.3%) body condition score and the difference was statistically not significant (P>0.05). This illogical finding could be due to the condition that majority of the camels sampled (81.4%) were with good and fair body condition score and only 18.6% of the total samples were with poor body condition.

This study revealed that herd size was significantly associated with brucellosis in camels (P<0.05). Consequently, herd size was statistically identified to be the second major risk factor for brucellosis to occur in relation to other factors (P=0.04). This is in accordance with the findings of Bekele (2004) and Zewolda and Wereta (2012) in Borena and Afar, respectively.

As herd size increases, the chance of contact between animals increases leading to more chances of infection (Abbas and Agab, 2002), which is particularly more important during calving or abortion when most of the *Brucella* contamination occur (Gameel et al., 1993; Agab et al., 1994). Thus, herd size and density of animal population together with poor management are directly related to infection rate (Abbas et al., 1987; Abou-Eisha, 2000; Wernery and Kaaden, 2002).

High number of camels, cattle and small ruminant diversification were noticed in the study districts. Such animal species distribution and diversification is common to other areas and has economic and ecological advantages (Wilson et al., 1990; Getahun and Kassa, 2000). However, it increases the chance of brucellosis and other disease transmission from other infected ruminants to dromedaries (Andreani et al., 1982; Radwan et al., 1992). In the present study, there was highly statistically significant difference (P<0.05) in the prevalence of the disease in the camel population which had contact with other ruminants. It is considered as the first major risk factor (P=0.001) for the occurrence of brucellosis when compared with other factors and even when compared with herd size. Those camel herds which usually made close contact on pasture with cattle were 3.6 times more at risk of being seropositive to the disease than those with no contact and those mixed with small ruminant were 2.3 times more at risk than those camels not mixed with other ruminants.

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 single parity and more than one parity were 3.9 and 4.7%, respectively which is slightly higher than those with no parturition (2.7%). 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 study by Bekele (2004) and Zewolda and Wereta (2012) where higher reactors were recorded in camels with more than one parity, compared to other group of camels. The possible explanation for this is that because the repeated exposure of the she-camels to parturition and other physiological stress increases the probability of acquiring *Brucella* infection.

This study also illustrated that there was no statistically significant difference (P>0.05) in distribution of *Brucella* infection among the different reproductive problems and physiological statuses of the she-camels considered. Among the she-camels with the history of reproductive problems, abortion (5.7%) and retained fetal membrane (4.1%) were found with slightly higher seropositivity to *Brucella* infection. Moreover, the pregnant (6.6%) and lactating (4.1%) she-camels showed higher seroprevalence of brucellosis. Therefore, the pregnant she-camels during delivery time and the lactating she-camels excreting the organisms through milk were the risk factors for transmission of the infection to calves, other animals and even to human beings (Abbas and Agab, 2002).

Conclusion

The seroprevalence recorded in the present study revealed that brucellosis is a widespread and established disease in the three camel rearing districts. The risk factors identified for the presence and transmission of the disease from animal to animal were sex, age, body condition, herd size, contact with other ruminants, parity, reproductive problems and physiological status. However, according to the statistical analysis, advance in herd size and contact with other ruminants were found to be the major risk factors for the transmission of the disease from camel to camel as well as from area to area. Moreover, higher seropositivity was recorded in female, matured, pluriparous, pregnant, abortive and lactating camels which contributed for transmission of the disease. Traditional husbandry and poor management practices, mixing with other animals and unrestricted movement of camels were thought to support spread of the disease in the study area. Therefore, a strategic plan should be developed to support in decreasing the chance of contact of animals at different situations and to keep only few healthy and fertile camels per herd together with immunization campaigns, and public health education on modern animal husbandry and disease prevention techniques should be imparted continuously.

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