

Prevalence and Risk Factor of Brucellosis in Dromedaries in Selected Pastoral Districts of Afar, Northeastern Ethiopia

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Abstract

A cross-sectional study was conducted on 813 camels from 63 herds in selected Afar pastoral areas from May 2012 to February 2013 to determine the prevalence and risk factors associated with camel brucellosis. Serum samples were collected and screened for brucellosis using Rose Bengal Plate Test followed by Complement Fixation Test of positive samples for confirmation. The herd level seroprevalence was 17.46% and varied significantly among different herd sizes ($\chi^2=8.84$; $P<0.05$) and contact with small ruminants ($\chi^2=3.91$; $P<0.05$). Camel herds in contact with small ruminants were 6.64 times (OR=6.64; 95% CI: 1.30-33.88) more at risk for brucellosis infection than those herds which had no contact with small ruminants. Animal level seroprevalence was 2.09% and varied significantly among different herd size ($\chi^2=8.079$; $P=0.018$). The prevalence of brucellosis was significantly higher in camels with history of abortion (6.67%) ($\chi^2=10.534$; $P=0.032$). Sex, age, body condition, physiological status and parity were insignificantly associated ($P>0.05$) with the prevalence of brucellosis. The present study suggests that *Brucella* infection is the likely cause of abortion in camel and small ruminants were the probable source of *Brucella* infection for camels in the study. On the other hand, based on the observation of the author, none of the camel herdsmen knew the disease and the prevailing habit of consumption of raw camel milk and the man-animal close contact in Afar pastoral area demonstrate the potential role of brucellosis as a zoonosis in the area. Therefore, improving management practices, public awareness, economic and zoonotic importance of the disease can assist disease prevention.

Keywords: Afar pastoral, Brucellosis, Camels, Prevalence, Risk factors, zoonosis

INTRODUCTION

Brucellosis is a disease of high economic and public health importance and has a worldwide distribution (Al-Majali et al., 2008; Saegerman et al., 2008). *Brucella* infection is still endemic in countries of the Mediterranean basin, the Middle East and Central Asia (Radostits et al., 2007; Saegerman et al., 2008). Brucellosis is a widely spread disease in camel producing horn of African countries such as Ethiopia, Eritrea, Somalia and Sudan. In Ethiopia, few field surveys have been carried out to determine the magnitude of camel brucellosis in pastoral areas. Generally the previous serological surveys showed that sero-prevalence rates of 4.4% (Domenech, 1997) and 5.5% (Richard, 1980) in Somali pastoral region. The studies have also shown that the prevalence of camel brucellosis was 1.8% in Borana (Bekele, 2004). A recent study in DireDawa city administration also reported a 1.6% prevalence of camel brucellosis (Mohammed et al., 2011).

Cross-transmission of brucellosis can occur between cattle, sheep, goats, camels and other species (Radostits et al. 2007). Camels are frequently infected with *Brucella* organisms, especially when they are in contact with infected large and small ruminants (Radwan et al., 1992; Radostits et al., 2007). Camel brucellosis can generally cause significant loss of productivity in camels through late first calving age, long calving interval time, low herd fertility and comparatively low milk production (Wernery and Kaaden, 2002; Radostits et al., 2007). Moreover, brucellosis in human represents a major public health hazard, which affects social and economic development in various countries. Brucellosis in humans is known as "undulant fever" or "Mediterranean fever", "Malta fever" or "Bangs disease", causing a debilitating illness. The annual occurrence of human brucellosis in the world is more than 500,000 cases (Donev et al, 2010). Infection of human occurs through direct or indirect contact with infected animals or their products. It affects people of all age groups and of both sexes (Corbel, 2006). Human infection due to *Brucella* from camels is known to occur mostly through the consumption of raw milk (Radostits et al., 2007). Camel milk as well as camel liver are consumed without heat treatment by camel keepers (pastoralists) and even considered as delicacy (Gameel et al., 1993). The isolation of *B. abortus* and *B. melitensis* (Radwan et al., 1992; Gameel et al., 1993; Agab et al., 1994; Abou-Eisha, 2000) has certainly demonstrated the danger of camel milk to public health.

In Ethiopia, brucellosis is endemic and the disease is highly prevalent in cattle, camels and small ruminants in pastoral and agro-pastoral areas (Yimer et al., 2008). Despite endemic nature of brucellosis in many developing countries the disease remains under diagnosed and under-reported (Donev et al., 2010). The disease in Ethiopian camels has not been extensively investigated in comparison with those of other domesticated

species. Even though few study tried to address the prevalence of camel brucellosis in pastoral and agro-pastoral area of Ethiopia but the magnitude of the disease has not been fully investigated in Afar. Therefore, the objectives of this study were to investigate the seroprevalence of camel brucellosis and to identify risk factors associated with camel *Brucella* infection in selected pastoral area of Afar

MATERIALS AND METHODS

Study Area: The study was conducted in two zones (Zone 1 and 3) of Afar region state. The Afar National Regional State is located in the Great Rift Valley, comprising rangeland in northeast Ethiopia with an estimated area of 95958 Km². There is a great potential resource of livestock resources comprising of 2336488 heads of cattle, 4267969 goats, 2463632 sheep, 852016 camels and 187287 equines that support the region and contributes to the national economy (CSA, 2010). The annual temperature and rainfall in the region is 30-50°C and 200-600mm, respectively. The altitude of the region ranges from 100-1000 meter above sea level. It is located at 8° 40' 13" to 14° 27' 29" N and 39° 51' 13" to 42° 23' 03" E latitude and longitude, respectively. The study areas constituted (Figure. 1) Aysaita and Mille from Zone 1 and Awash-Fentale and Amibera from Zone 3.

Study Animals: 813 dromedary type (*Camelus dromedaries*) camels were included in this study.

Study Design: A cross-sectional study was carried out on 813 camels, from May 2012 to February 2013 in selected districts of the two Zones of Afar Regional State, using serological tests. Information of each sampled camel including herd size, sex, age, physiological status, parity, reproductive disorder, co-existence with other ruminants among other things was recorded using structured questionnaire.

Sampling Method and Size: The sampling method was supposed to be a multi-stage cluster sampling approach. However, due to the absence of between cluster variance and sampling frame, the sample size was not able to determine using cluster sample formula. Therefore, the representative zones, kebeles (Peasant Associations) and herds were selected purposely based on camel population, willingness of pastoralists and accessibility to vehicles. The primary and second stage was sampling of zones and woredas, respectively. Selection of kebelles, herds and individual camels within the herds was the 3rd, 4th and 5th stages, respectively. From the five administrative zones of Afar region, two zones (Zone 1 and 3) were selected. Four woredas (Aysaita and Mille from Zone 1, and Awash- Fentale and Amibera from zone 3) were selected based on convenience and camel population. Proportional allocation (30%) have been used to distribute the sampling unit in each sampling stage. Accordingly, a total of 813 camels (208 camels from Amibera, 201 camels from Awash-Fentale, 215 camels from Mille 189 camels from Aysaita) were included in this study. Every camel was selected from a given herd using the systematic random sampling.

Blood Collection: About 10 ml of whole blood sample was collected from the jugular vein, using plain vacutainer tubes and needles, from each camel aged six months and above. There was no history of vaccination for brucellosis in the region in general and in the study area in particular. Each sample was labeled using codes specific to the individual animal and herd information. The tubes were tilted on a table overnight at room temperature to allow clotting. Serum was collected either passively by decanting or using centrifuges at 2500 revolutions per minute for five minutes. The serum was stored at -20°C in Semera Regional Veterinary Laboratory.

Serological Testing: Collected sera were initially screened using Rose Bengal Plate Test (RBPT) for the presence of antibodies against *Brucella*. Positive and inconclusive serum samples were further tested using complement fixation test (CFT) (Al-Majali et al., 2008).

Rose Bengal Plate Test (RBPT): The RBPT was conducted at Semera Regional Veterinary Laboratory using the procedure described for RBPT by Staak et al. (2000). Briefly, 30ml of the sera samples were dispensed onto the plate and 30ml of RBPT antigen was dropped alongside the sera. The plate was rocked by hand for 4 min and the test was read by comparing with the positive and negative control sera by examining for agglutination in natural light. Magnifying glass was used to detect micro-agglutination. Results of RBPT were interpreted as 0, +, ++ and +++ as described by Staak et al. (2000). 0 = no agglutination; + = barely visible agglutination (seen by using magnifying glass); ++ = fine agglutination and +++ = coarse agglutination. Samples with no agglutination (0) were recorded as negative while those with +, ++ and +++ were recorded as positive.

Complement Fixation Test (CFT): The CFT procedure was undertaken at the National Veterinary Institute, Department of Immunology at Debre-Zeit, Ethiopia. Preparation of the reagents was performed according to OIE protocols (2004). A titration of hemolysin and antigen was performed before the test. The minimum hemolytic dose was also estimated for each run. As for the interpretation of test results, positive reactions were indicated by sedimentation of Sheep Red Blood Cells (SRBC) and absence of hemolysis. Negative reactions were revealed by hemolysis of SRBC. According to OIE protocol (2004) sera with strong reaction, more than 75% fixation of complement at a dilution of 1:10 and at least with 50% fixation of complement at a working dilution (1:5) was classified.

Data management and analysis: Data collected from field and serological test were coded and stored in Microsoft Office Excel spread sheet and transferred to STATA version 11 for statistical analysis. The

seroprevalence for animal level was calculated on the basis of RBPT and CFT positivity, dividing the number of *Brucella* reactors by total number of tested animals. Similarly, herd level prevalence was computed as the number of herds with at least one positive animal divided by the total number of herds tested. Descriptive and analytic statistics were computed and Logistic regression was employed to see the association of risk factors with that of seropositivity to *Brucella* antibody; the degree of association was computed using Odds ratio (OR) and 95% confidence interval (CI) (Thrusfield, 2005).

RESULTS

Herd Level Seroprevalence of Camel Brucellosis and Risk Factors

Based on the CFT positivity out of the 63 herds tested 11 herds were positive for *Brucella* infection and hence a herd prevalence of 17.46% was recorded. Herds with at least one positive animal were considered as seropositive herd. The herd level risk factors such as herd size, camels in contact with ruminants (both cattle and small ruminant), with cattle only or with only small ruminants are shown in Table 1. Table 1 showed that herds with larger size (>50 camels) had highest prevalence (36.84%) than medium (15.38%) and small sizes i.e. *Brucella* seropositivity increased with increase in herd size. Camel herds living in association with small ruminants had higher prevalence (30%) than herds had no contact with small ruminants (6.06%). Camel herds had close contact with small ruminant were 6.64 times (OR=6.64; 95% CI: 1.30-33.88) more at risk for brucellosis infection than those herds which had no contact with small ruminants.

Animal Level Seroprevalence of Camel Brucellosis and Risk Factors

Out of 813 camels examined 37 (4.55%) were positive on RBPT screening. However, on further confirmation by CFT only 15 camels (2.09%) showed *Brucella* infection. Therefore, the true prevalence of camel brucellosis in the selected pastoral area was 2.09%.

The prevalence of brucellosis seems higher in Amibera (4.33%) than Mille (1.86%), Assyita (1.06%) and Awash-Fentale (1%) but the difference was statistically insignificant ($\chi^2=7.2990$; $P=0.063$) (figure 2).

Table 2 shows that none of the risk factor at animal level except herd size was found statistically significant. Animal level seroprevalence varied significantly among different herd sizes ($\chi^2=8.079$; $P=0.018$). On univariate logistic analyses (Table 2), camels kept in herds which had >50 camels were eleven times (OR=11; 95% CI: 1.38-87.45) higher at risk for *Brucella* infection than herds size of 10-30 camels.

According to camel herders information 81.1% (443) she-camels had no history of reproductive disorder, 15.96% (90) had aborted at least once, 0.36% (2) camels gave still births and 2.01% (11) camels gave birth to weak calves (table 3). Higher prevalence of camel brucellosis was recorded significantly in camels with history of abortion (6.67%) than with history of no reproductive disorder (1.80%) (Table 4). Camels with history of abortion were 4.50 folds at risk (OR=4.50; 95% CI=1.62-12.53) to brucella infection than camels with no history of reproductive disorder.

DISCUSSION

Several serological surveys have shown that brucellosis is an endemic and widespread disease in Ethiopia (Domenech, 1997; Teshome et al., 2003; Bekele et al., 2005). In this study, the overall seroprevalence of camel brucellosis in various districts recorded was 4.55% by the RBPT and 2.09% by CFT. The true prevalence of 2.09% recorded in the present study is in agreement with earlier reports of Mohammed et al. (2011) in DireDawa (1.6%), Bekele et al. (2005), in Borona pastoral area (1.8%), Teshome et al. (2003) in Borena Zone of southern Ethiopia (1.2%), Domenech (1977) in Tigray (1.7%) and Hararge region of Ethiopia (1.7%). Other serological surveys which are in accordance with this study include 1.95% prevalence in Somalia by Baumann and Zessin (1992) and 1.4% prevalence in Saudi Arabia by Hashim et al. (1987). In contrast, it was slightly lower than the previous serological surveys showing seroprevalence of 5.5% (Richard, 1980) and 5.22% in Afar (Teshome et al., 2003). Generally, the results of the present study fall in between the range of previous reports ranging from 1.2% (Teshome et al., 2003) to 5.5% (Richard, 1980) in Ethiopian camels.

The seroprevalence of brucellosis is low in camels kept extensively by pastoralists. Thus, prevalence ranging between 2% and 5% was reported from most countries where camels are produced by pastoralists (Abbas and Agab, 2002; Wernery and Kaaden, 2002). On the other hand, it was reported as high as 8 to 15% in intensively kept camels especially in Saudi Arabia (Radwan et al., 1992) and Kuwait (Al-Khalaf and El-Khaladi, 1989). In such a production system, the large herds overcrowded in restricted area provide more chances of contact between animals leading to increased likelihood of infection.

The herd prevalence recorded in the present study 17.46% (11/63), is in concurrence with the herd prevalence of 16% (40/250) reported by Bekele (2004) in pastoral area of Borena Zone of southern Ethiopia whereas higher herd prevalence (35.1%) was reported from Jordan by Al-Majali et al. (2008) and Sudan ranging from 26.5 to 30% (Abbas and Agab (2002) in camel herds. Majid et al. (1999) also reported a seroprevalence rate which ranged from 14% to 43.9% from the same country.

In this study, larger herd size was identified as a major risk factor of brucellosis in camels both at the

herd and individual animal level. Similar observations have been reported previously (Abbas and Agab, 2002, Al-Majali et al., 2008, Mohammed et al., 2011). As herd size increases, the chances of contact between animals increases, leading to more chances of infection (Abbas and Agab, 2002; Radostits et al., 2007; Al-Majali et al., 2008), which is particularly more important during calving or abortion when maximum brucellosis contamination occurs (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; Abu-Eisha, 2000; Wernery and Kaaden, 2002).

Herders in Afar pastoral area keep small ruminants and cattle alongside with camels. In the present study camel herd kept in close contact with small ruminants had higher prevalence (30%). Abou-Eisha (2000) also observed higher seroprevalence in camels that were in contact with sheep and goat. There are higher chances of brucellosis transmission from these ruminants to dromedaries as they live in free range in promiscuity in the bush and at water points (Andreani et al., 1982). Specially, contact between dromedaries and small ruminants were more incriminated for the transmission of brucellosis to camels (Ismaily et al., 1988; Radwan et al., 1992; Abbas et al., 2000).

Brucellosis can generally cause significant economic loss through abortion, prolonged first calving age, long calving interval time, low herd fertility, culling and comparatively low milk production (Wernery and Kaaden, 2002; Radostits et al., 2007). In this study, percent of abortion, stillbirth and birth to weak calves observed was 15.96%, 0.36% and 2.01%, respectively. Getahun and Kassa (2000) reported that annual abortion and stillbirth rates of 9% and 4.3%, respectively in camels kept under similar production systems in eastern Ethiopia. Brucellosis in camelids occurs in all known farms whereby abortion is the most obvious manifestations (Wernery and Kaaden 2002; Radostits et al. 2007). In this study abortion rate of 15.96%, with 6.67% seroprevalence was observed. Findings of Radwan et al. (1992) who recorded an abortion rate of 12% with 8% seroprevalence are in accordance with the present study.

In conclusion, the results of the present study revealed the status of camel brucellosis in pastoral area of Afar. Although prevalence rate of camel brucellosis recorded was low but it is enough to affect animal and human health and economy of the country as it bans international animal trade. Univariate analysis of herd size and contact with small ruminants were found important risk factors associated with *Brucella* seroreactors. Lack of awareness about brucellosis together with existing habit of raw milk consumption and close contact with animals may exacerbate the zoonotic importance. The low prevalence rate of camel brucellosis observed in the study area may suggest the implementation of a test-and-slaughter policy. However, this is not feasible for the time being due to the free movement of herds in the pastoral areas and unaffordable compensation to the owners. Therefore; improving management practices, public awareness on the economic and zoonotic importance of the disease can assist disease prevention. Finally, further investigation on the epidemiology, zoonotic importance and potential etiology of the disease should be conducted.

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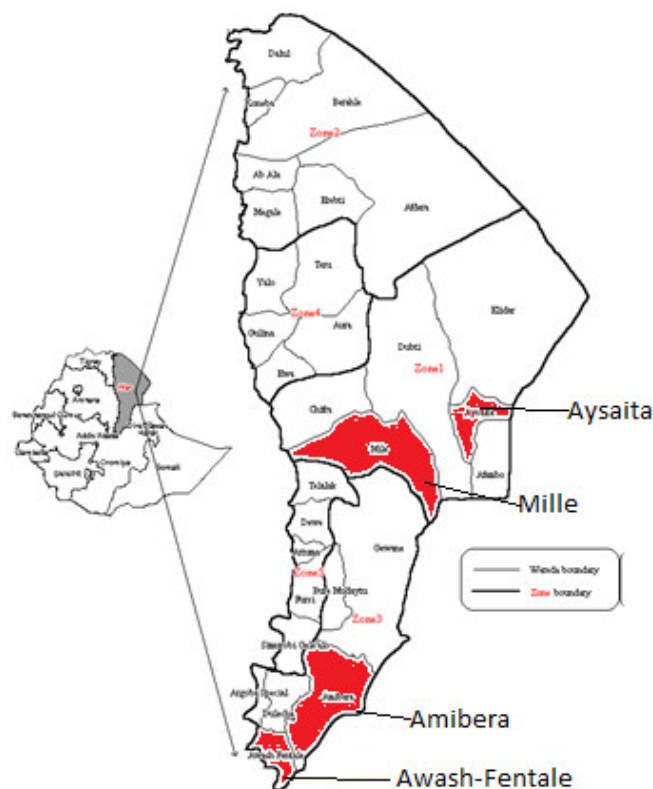


Figure 1. Administrative location of Afar region showing the study Woredas

Table1: Risk factors for the occurrence of seropositivity to Brucellosis at herd level

Risk factors		No. of herd		P-value	OR(95%CI)
		Tested	Positive (%)		
Contact with small ruminant only	Yes	30	9(30%)	0.023	6.64(1.30-33.88)
	No	33	2(6.06%)		
Contact with Cattle only	Yes	20	4(36.36%)	0.718	1.28(0.33-5.02)
	No	43	7(63.64%)		
Contact with small and large ruminant	Yes	14	4(28.57%)	0.223	2.4(0.58-9.82)
	No	49	7(14.29%)		
Herd size					
	10-30	18	-	-	-
	31-50	26	4 (15.38%)	-	2.83e+07
	>50	19	7 (36.84%)	0.007	4.89(1.53-15.62)

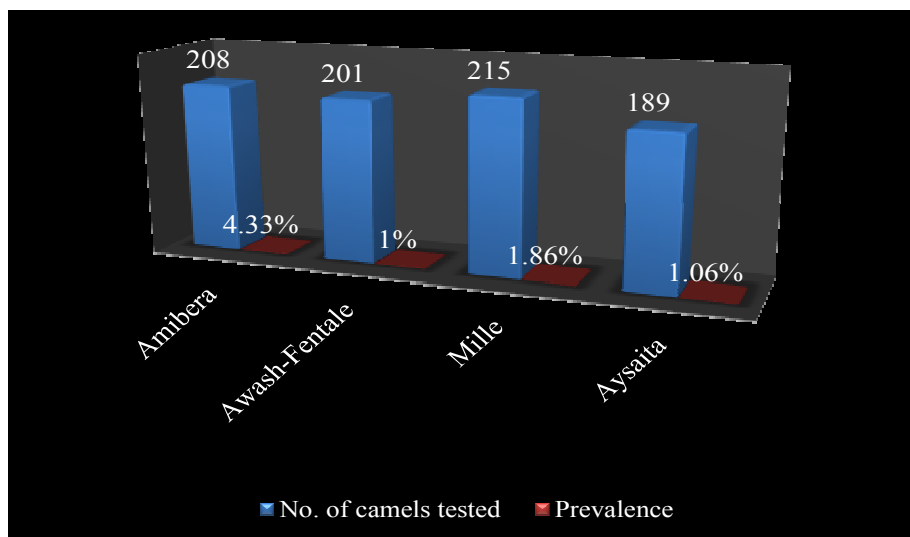


Figure 2: Prevalence of brucellosis based on selected districts (woredas)

Table 2: Association of different recorded variables and *Brucella* seropositivity at animal level

Variable	No. of camels		χ^2 -value	P-value	OR (95% CI)
	Tested	Positive (%)			
Sex			0.000	0.996	
Male	96	2(2.08)			
Female	717	15(2.09)			
Age			1.071	0.301	
Young (6mon. -4yrs)	235	3(1.28)			
Adult (>4yrs)	578	14(2.42)			
Herd size			8.079	0.018	
10-30	255	1(0.39)			1
31-50	341	7(2.05)			5.32 (0.65-43.54)
>50	217	9(4.15)			11(1.38-87.45)
Body condition			1.758	0.415	
Poor	142	2(1.41)			
Medium	391	11(2.81)			
Good	268	4(1.49)			
Physiological status			2.1944	0.700	
Bull	96	2(2.08)			
Heifer	143	4(2.80)			
Pregnant	69	-			
Lactating	361	7(1.94)			
Dry she-camel	144	4(2.78)			
Parity			2.3986	0.301	
No parturition	168	1(0.6)			
Single parity	118	3(2.46)			
More than once parity	428	11(2.57)			

Table 3: Seroprevalence of camel brucellosis in adult female in relation to reproductive disorders

Reproductive disorder	No. of camels		χ^2 -value	P-value	OR (95% CI)
	Tested	Positive (%)			
No reproductive disorder	443	8(1.80)			1
Abortion	90	6(6.67)			4.50 (1.62-12.53)
Still birth	2	-			-
Weak calve	11	-			-
			10.5345	0.032	