



Research Article

## Seroprevalence of Brucellosis and Q-Fever in Southeast Ethiopian Pastoral Livestock

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### Abstract

To assess seroprevalences of *Brucella* and *C. burnetii* in pastoral livestock in southeast Ethiopia, a cross-sectional study was carried out in three livestock species (cattle, camels and goats). The study was conducted from July 2008 to August 2010, and eight pastoral associations (PAs) from the selected districts were included in the study. Sera from a total of 1830 animals, comprising 862 cattle, 458 camels and 510 goats were screened initially with Rose Bengal plate test (RBPT) for *Brucella*. All RBPT positive and 25% of randomly selected negative sera were further tested by ELISA. These comprise a total of 460 animals (211 cattle, 102 camels and 147 goats). Out of sera from total of 1830 animals, 20% were randomly selected (180 cattle, 90 camels and 98 goats) and tested for *C. burnetii* using ELISA. The seroprevalences of *Brucella* was 1.4% (95% confidence interval (CI), 0.8-2.6), 0.9% (95% CI, 0.3-2.7) and 9.6% (95% CI, 5.2-17.1) in cattle, camels and goats, respectively. Goats and older animals were at higher risk of infection (OR=7.3, 95% CI, 2.8-19.1) and (OR=1.7 95% CI, 0.9-2.9), respectively. Out of 98 RBPT negative camel sera, 12.0% were positive for ELISA. The seroprevalences of *C. burnetii* were 31.6% (95% CI, 24.7-39.5), 90.0% (95% CI, 81.8-94.7) and 54.2% (95% CI, 46.1-62.1) in cattle, camels and goats, respectively. We found positive animals for *C. burnetii* test in all tested PAs for all animal species. Being camel and older animal was a risk factor for infection (OR=19.0, 95% CI, 8.9-41.2) and (OR=3.6, 95% CI, 2.0-6.6), respectively. High seropositivity of *C. burnetii* in all livestock species tested and higher seropositive in goats for *Brucella*, implies risks of human infection by both diseases. Thus, merit necessity of further study of both diseases in animals and humans in the area.

### Keywords

Brucellosis; Q-fever; Seroprevalence; Pastoral livestock; Southeast Ethiopia

### Introduction

Brucellosis is a disease of animals, especially livestock (cattle, goats, sheep, camels and pigs), but also wild animals. It is caused by bacteria of the genus *Brucella* spp. In livestock, it is primarily a reproductive disease characterized by late abortion, retained foetal membranes, orchitis and impaired fertility [1]. *B. melitensis* is considered to have the highest zoonotic potential, followed by *B.*

*abortus*, and *B. suis*. Brucellosis remains one of the most common zoonotic diseases worldwide, with more than 500,000 human cases reported annually, particularly from developing countries [2-4].

The economic and public health impact of brucellosis remains of concern in developing countries [5]. The disease poses a barrier to trade of animals and animal products, causes a public health hazard, and is an impediment to free animal movement [1]. In Africa and Central Asia, the incidence of brucellosis is generally considered higher in livestock raised in pastoral production systems [6], however, increasing intensified peri-urban production leads nowadays often to higher prevalence than in pastoral production systems [7]. Brucellosis is endemic in humans and livestock in the Mediterranean region, Africa, the Near East, Central Asia and Central America [8]. Brucellosis in livestock and humans is re-emerging as a major epidemic in countries of the former Soviet Union [5].

In Ethiopia, serological studies of brucellosis have been carried out in farm animals. The presence in livestock varies between different parts of the country [9-12]. Only few serological studies of brucellosis have demonstrated the occurrence of the disease among Borana and Hamar pastoralists; however these have highlighted the public health significance [13].

Q-fever is a zoonotic disease caused by *Coxiella burnetii*. Livestock (cattle, sheep, camels and goats) are the main reservoirs of infection to humans [14,15]. It is also known as an occupational disease of veterinarians, farmers and abattoir workers [16]. *Coxiella burnetii*, the causative agent has been isolated from ticks. Infection in humans is often asymptomatic, but it can manifest as an acute disease (usually a self-limited flu-like illness, pneumonia or hepatitis), or as a chronic form (mainly endocarditis, but also hepatitis and chronic-fatigue syndrome). Q-fever is frequently misdiagnosed by physicians [14]. It is endemic, both in livestock and humans in North and Sub-Saharan Africa [17-20].

In Ethiopia, the existence of antibody against *C. burnetii* was reported in goats and sheep slaughtered at Addis Ababa abattoir, and its peri-urban zone [21]. A seroprevalence of 6.5% was also reported in Addis Ababa abattoir workers [22]. To our knowledge, there was no study on Q-fever in Ethiopian herds or in pastoral zones, where people live in very close contact to their livestock. Information on both diseases is scarce in the study zones. The objective of the present study was to assess the seroprevalences of *Brucella* and *C. burnetii* in pastoral livestock in southern Ethiopia, and factors associated with seropositivity.

### Materials and Methods

#### Study areas

A cross-sectional study with a cluster sampling design was conducted from July 2008 to August 2010 in South Eastern Ethiopian pastoral zones of the Somali and Oromia regional states (Figure 1).

Extensive pastoral livestock production is the main system and the basis of livelihood for millions of pastoralists in the study area. Climatic condition of the selected study areas is characterized by arid and semi-arid climate, with bimodal rainfall pattern. Two districts/woredas were conveniently selected based on accessibility and

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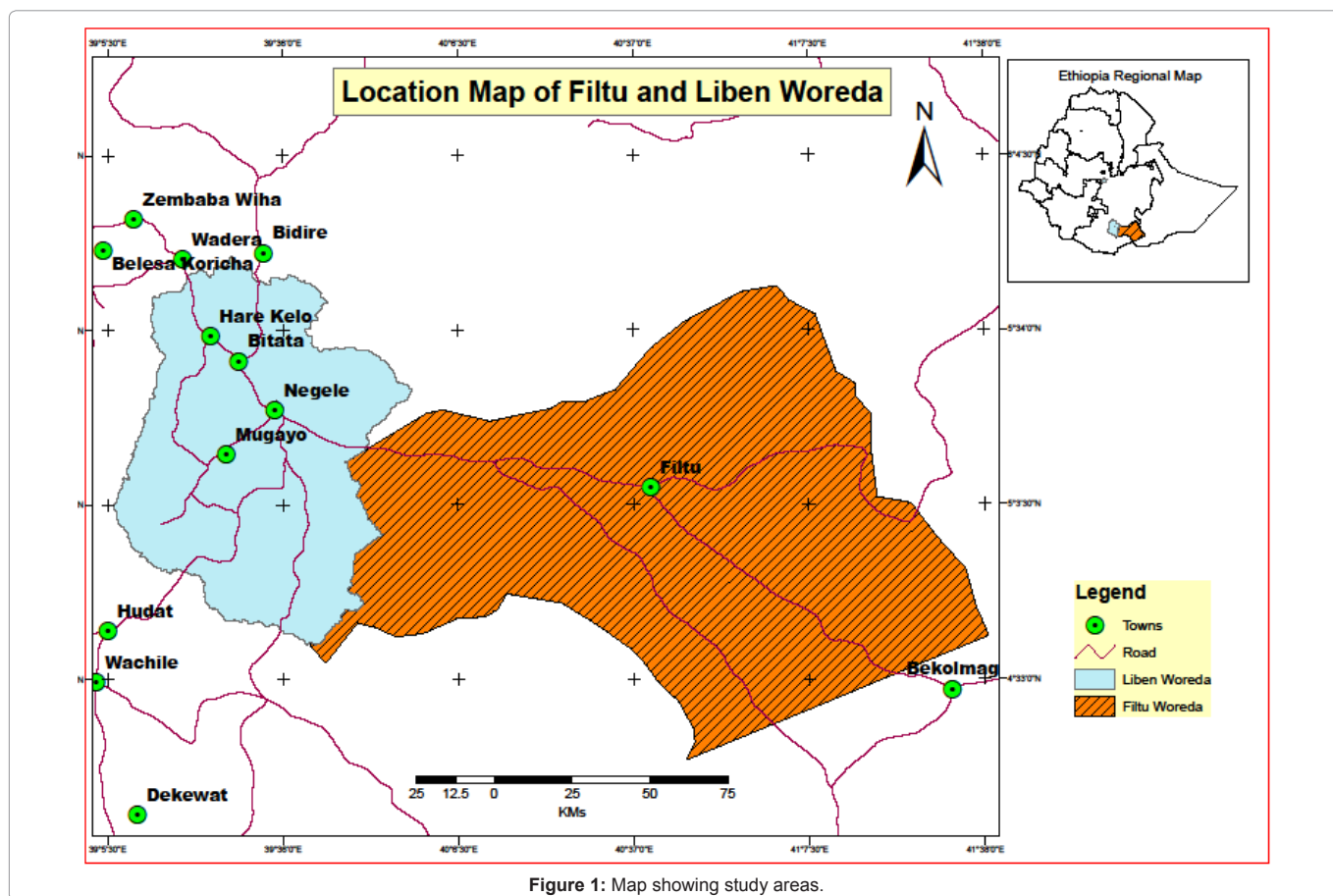


Figure 1: Map showing study areas.

security reasons, from each of the two regional states. Liben and Filtu districts from Oromia and Somali Regional States were included. Eight pastoral associations (PAs)—each one of the cardinal directions per zonal capital were included in the study. These were Dhuko, Sirba, Arda-Bururi and Siminto PA’s from Oromia and Bifatu, Melkalibe, Hayadimtu and Bakaka PAs from Somali Regional State.

### Sample size

The sample size estimation considered clustering of animals within herds [23], the sought precision of  $\pm 3\%$  (standard error of 1.5%), assumed an intra class correlation coefficient ( $\rho$ ) of 0.2, and an expected *Brucella* seroprevalence of 3%. The total sample size calculated was 480 per species of animals per study site, and a total of 128 herds. In Oromia, only cattle were sampled, whereas in Somali region, cattle, camels and goats were present for sampling. Twenty percent of the total sample was tested for *C. burnetii*.

### Selection of pastoral households within PAs

In each of the 8 PAs, fifteen animals per herd and species were selected randomly in eight herds. After discussion and agreement on procedures at the general PA meeting of each site, interested households were asked to register for participation. Using a list of registered households per PA as sampling frame, 8 households were selected with random numbers.

### Sample collection

About 10 ml of blood sample was collected from the jugular vein of each animal, using plain vacutainer tubes and needles. Each sample

was labeled with unique identification number. The tubes were kept overnight at room temperature, to allow clotting of blood. The next morning sera was removed from the clot and stored in cryotubes at  $-20^{\circ}\text{C}$ , until analyses in the laboratory.

### Serology

All sera were initially tested by Rose Bengal Test (RBPT). For RBPT, 30  $\mu\text{l}$  of serum and 30  $\mu\text{l}$  of antigen (Rose Bengal stained *B. abortus* antigen obtained from BIO-RAD, Marnes-la-Coquette, France) were mixed and rotated on a glass plate for 4 minutes. Sera with no visible agglutination were recorded as negative, while sera showing agglutination were considered positive. For further analysis, all RBPT-positive and randomly selected 25% RBPT-negative sera were tested by using ELISA kits for *Brucella abortus*. In addition, another randomly selected 20% of sera from 862 cattle, 458 camels and 510 were tested for *Coxiella burnetii*. ELISA kit was obtained from IDEXX, Liebfeld-Bern Switzerland, and tests were performed according to manufacturer’s instructions. All samples and controls in the *Brucella* and *C. burnetii* ELISA were tested in duplicate, and the mean OD values were used. Results were expressed as the percentage of the ratio between the sample OD and positive control OD (S/P-ratio), and were calculated as follows:

$$\frac{S}{P} = \frac{\text{mean OD sample} - \text{mean OD negative control}}{\text{mean OD positive control} - \text{mean OD negative control}} \times 100\%$$

The samples were considered seropositive for *Brucella* if the percentage of the ratio was  $\geq 80\%$  and negative if lower; and for *C. burnetii* seropositive if  $\geq 40\%$ ; doubtful for values between 30% and

40% and negative if <30 %. These threshold-values for both tests were recommended by the manufacturers.

### Data analysis

The data was double-entered in Microsoft Access 2002 (Microsoft Corp., USA), and validated with EpiInfo version 3.3.2, before being imported to STATA 10/SE (STATA Corp., College Station, TX) for analysis. We have used the xtgee model to determine the seroprevalence for each animal species, while considering clustering within herds, and to see if species, age category and sex were associated with sero-status. The xtgee model fits population-averaged panel-data models using generalized estimating equations.

Age categories were for cattle and goats <= 1 years=1 (young animal), 1- <= 3 years=2 (juveniles) and >3 years=3(mature/old), and for camels <= 2 years=1 (young animal), 2- <= 4 years =2 (juveniles) and >4 years=3(mature/old). The different age cut off for different animal species was used, because the age at which they become matured and old age are different. This is to make easy for mixed analysis, using the same codes for all animal species. Since not all sera were tested with the *Brucella* ELISA, we present only results with the outcome of the RBPT- binarily classified sero-status.

Kappa statistics was used to determine the level of agreement between RBPT and i-ELISA in detecting positive animals.

## Results

### Brucella seroprevalence

A total of 862 cattle, 458 camels and 510 goats were tested for *Brucella* anti-body, from 59, 32 and 34 herds, respectively. The seroprevalences per species were 1.4% (95% CI, 0.8-2.6), 0.9% (95% CI, 0.3-2.7%) and 9.6% (95% CI, 5.2-17.1%) in cattle, camels and goats, respectively (Table 1). In 10 out of 59 cattle herds and 5 of the 8 PAs, there was at least one positive animal. The three out of 32 camel herds and 2 of the 4 sampled PAs and 12 in 34 goat herds in all 4 sampled PAs were seropositive. Out of 98 RBPT negative camel sera, 12.0% was positive for ELISA. Univariable analysis of RBPT showed that goats and older animals were at higher risk of infection (OR=7.3, 95% CI, 2.8-19.1) and (OR=1.7 95% CI, 0.9-2.9), respectively.

The kappa statistics showed that there was substantial agreement between RBPT and i-ELISA for cattle. Only fair agreement was observed between the two serological tests in camels, while a perfect agreement was observed between them for goats (Table 2). The overall agreement observed was substantial.

**Table 1:** Associations with risk factors for *Brucella* seropositivity.

Risk factors		Number of test negative	Number test positive (%)	Univariable OR (95% CI)
Species	Cattle	850	12 (1.4)	1
	Camel	454	4 (0.9)	0.7 (0.2-2.1)
	Goat	462	48 (9.6)	7.3(2.8-19.1)***
Age class	1 <sup>a</sup>	295	4 (1.2)	1
	2 <sup>b</sup>	772	32 (3.8)	1.4 (0.9-2.2)
	3 <sup>c</sup>	699	28 (4.0)	1.7(0.9-2.9)*
Sex	Female	1408	63 (4.0)	1
	Male	358	1 (0.3)	0.3(0.2-0.4)***

\*p<0.05; \*\*\*p<0.001

<sup>a</sup>Age categories, for cattle and goats <= 1 years, and camels <= 2 years, <sup>b</sup>for cattle and goats 1- <= 3 years, and for camels 2- <= 4 years, <sup>c</sup>for cattle and goats>3 years, and for camels>4 years.

**Table 2:** Kappa test for agreement between RBPT i-ELISA tests.

Animal species	RBT	iELISA		Kappa value	Interpretation of kappa value
		+	-		
Cattle	+	8	4	0.71	Substantial agreement
	-	2	197		
Camel	+	3	1	0.27	Fair agreement
	-	12	86		
Goats	+	40	8	0.81	Perfect agreement
	-	5	94		
Overall	+	51	12	0.73	Substantial agreement
	-	19	378		

**Table 3:** Associations with risk factors for *C. burnetii* seropositivity.

Risk factors		Number test negative	Number test positive (%)	Univariable OR (95% CI)
Species	Cattle	123	57 (31.6)	1
	Camel	9	81 (90.0)	19.0(8.9-41.2)
	Goat	44	54 (54.2)	2.7(1.7-4.2)***
Age class	1 <sup>a</sup>	43	17 (30.8)	1
	2 <sup>b</sup>	77	77 (49.6)	2.2 (1.3-3.9)**
	3 <sup>c</sup>	56	98 (62.7)	3.6(2.0-6.6)***
Sex	Female	140	170 (55.1)	1
	Male	36	22 (39.5)	0.6(0.4-1.1)

\*\*p<0.01; \*\*\*p<0.001

<sup>a</sup>Age categories, for cattle and goats <= 1 years, and camels <= 2 years, <sup>b</sup>for cattle and goats 1- <= 3 years, and for camels 2- <= 4 years, <sup>c</sup>for cattle and goats>3 years, and for camels>4 years.

### *C. burnetii* seroprevalence

A total of 368 sera were tested for antibodies against *C. burnetii*, (180 cattle, 90 camels and 98 goats), where by the median of samples tested per herd was 3 for all species, with range of 1-5 per herd. The seroprevalences were 31.6% (95% CI, 24.7-39.5%), 90.0% (95% CI, 81.8-94.7%) and 54.2% (95% CI, 46.1-62.1%), in cattle, camels and goats, respectively (Table 3). We found positive animals in all tested PAs for all animal species. Being camel and older animal were identified as risk factors for infection (OR=19.0, 95% CI, 8.9-41.2) and (OR=3.6, 95% CI, 2.0-6.6), respectively.

## Discussion

### Seroprevalence of brucella

The RBPT seroprevalence result of the present study is lower than many of the earlier reports. Seroprevalence, as high as 38.7% and 22%, have been reported from dairy farms in western and north-eastern parts of Ethiopia by Rashid [24] and Sintaro [25], respectively. Slightly higher individual serological prevalence of 5.6% was reported in cattle under different production systems in Eritrea [26], 5.9% cattle in Tanzania, 6.5% cattle in Sudan, 6.6% in cattle under pastoral production system in Chad, 9.9% cattle in Kenya, and 15.8% in dairy cattle in Uganda have also been recorded [18,27-29]. Differences in seroprevalence observed in this study, as opposed to those recorded by previous researchers, may be due to differences in herd size, different management systems, and the presence or absence of infectious foci, such as *Brucella*-infected herds, which could spread the disease among contact herds.

The seroprevalence of 9.6% of goats *Brucella* in this study is inline with the report of Ashenafi et al. [30]; however, higher than the results of Teklye and Kasali [31] from central Ethiopia, Teshale et al. [32]



from Somali region, and Megersa et al. [12] from Borena pastoralist. Management could be a factor for lower prevalence reported by Teklye and Kasali [31], since they studied goats under small holder mixed crop-livestock systems of central Ethiopia. Zero seroprevalence was reported in goats from Chad [18], and Zambia [33]. Difference in the management system used in different countries, or absence of infected goat herds may attribute to such variations. In addition, *Brucella* transmission is favored by a more humid climate, which prolongs the survival of the bacteria in the environment. However, our study sites were in arid and semi-arid regions.

The seroprevalence of <1% in camel is lower than previous reports from Borena pastoralists [12,34], and Jordan [35]. A comparable report was from Chad [18]. Inter-study variation may be due to differences in camel husbandry practices in different communities.

In our study, more camel reactors to ELISA than RBPT test were observed. Kappa statistic also shows fair agreement between the two serological tests in camels at kappa value of 0.27. This observation needs further evaluation of both tests, to validate their diagnostic use in camels.

The seroprevalence of *Brucella* was higher in goats than in the other two species of animals studied. It could be due to the highly contagious nature of the disease in goats. The higher pathogenicity of *B. melitensis*, and the close contact caused by the high density of the herds of goats, the intermixing of herds of different owners, and heavy exposure to housing during the night, can also contribute to this higher prevalence. The seroprevalence was also higher in females and older animals than in males and younger counterparts. This is in consent with the previous works [36]. It has already been shown that susceptibility to brucellosis is greater in sexually mature animals. Young animals are often resistant, although it should be noted that latent infections can occur, and such animals may present a hazard when mature [37].

### Seroprevalence of *C. burnetii*

The seroprevalence of *C. burnetii* found in this study is high in all the three animals species studied. The higher prevalence in camels is in agreement with previous reports from Chad [18]. But the seroprevalence in sera from cattle and goats are higher than previous reports from Central African Republic [38] and Chad [18]. The highest seroprevalence observed in camels may be due to genetic susceptibility of camels to *C. burnetii*, or host preference of tick vectors to camel. However, this observation needs further investigation.

### Limitations

Since we use the cluster sampling technique with fixed number of animals per herd and per village, in cases of low prevalent site, positive animals might be missed, and village or herd might be considered as negative. This study gives baseline information on Q-fever in pastoral livestock production in study area. However, before generalizing this result it needs further study, which includes large study population and area coverage.

The high seroprevalence of *C. burnetii* in all animal species studied, and the higher prevalence of *Brucella* in goats is a particularly important finding that pinpoints the hazard to the health of the pastoralists. The importance of these zoonotic diseases in impairing the health of the community needs to be further studied in the future.

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