Epidemiological survey of brucellosis in sheep and goats in selected pastoral and agro-pastoral lowlands of Ethiopia

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Summary

An epidemiological survey was conducted in pastoral regions of Ethiopia to investigate the distribution of brucellosis in sheep and goats. Between November 2004 and December 2007 a total of 6,201 serum samples were collected from 67 randomly selected peasant associations, 25 districts and eight pastoral zones of Ethiopia. The Rose Bengal plate test (RBPT) and complement fixation test were used in series. Samples for bacteriology were collected from three export abattoirs, where 285 goats were randomly selected and tested

by RBPT three days before slaughter. Tissue samples were collected from 14 strongly positive goats and cultured in dextrose agar and *Brucella* agar base. To confirm and subtype the isolates, staining, biochemical tests and polymerase chain reaction were used.

The overall standardised seroprevalence of brucellosis was 1.9%, ranging from 0.07% in Somali regions to 3.3% in Borena regions. There was statistically significant variation among the studied regions, zones, districts and peasant associations ($p < 0.05$).

Male goats and sheep were twice as likely to test positive as females (relative risk [RR]: 2.04; 95% confidence interval [CI]:1.7–3.4; $\chi^2 = 21.05$, $p < 0.05$). Adults (older than 1.5 years) were three times more likely to test positive than younger animals (RR: 2.76; 95% CI: 1.14–6.73; $\chi^2 = 5.18$, $p < 0.05$). Goats were around four times more likely to be infected than sheep (RR: 3.8; 95% CI: 2.4–6.1; $\chi^2 = 36.99$, $p < 0.05$).

Brucella melitensis was isolated from 2 of the 14 samples analysed. The widespread distribution of brucellosis in goats and sheep in these areas justifies the use of control measures to minimise the economic losses and public health hazards.

Keywords

Brucellosis – Complement fixation test – Identification – Isolation – Prevalence – Rose Bengal test.

Introduction

Brucellae are Gram-negative, facultative intracellular bacteria that can infect many species of animals, including humans. Ten species are recognised within the genus *Brucella*. There are six 'classical' species: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis* and *B. neotomae* (1, 2), and another four species have been recognised more recently (3). The principal manifestation of brucellosis is reproductive failure, including abortion, birth of unthrifty neonates and infertility (4, 5). *Brucella melitensis* (biovars 1, 2 or 3) is the main causative agent of caprine and ovine brucellosis (5).

Brucellosis is a worldwide re-emerging zoonosis that causes severe disease in humans, with non-specific clinical signs affecting numerous organs (6). Contact with infected animals, ingestion of contaminated animal products and handling of *Brucella* isolates in laboratories are risk factors.

Brucellosis in livestock and humans is still common in the Middle East, Asia, Africa, South and Central America, the Mediterranean Basin and the Caribbean. *Brucella melitensis* is particularly common in the Mediterranean basin, and it has also been reported from Africa, India and Mexico (7).

In sub-Saharan Africa, little is known about the prevalence of brucellosis, and most data are derived from small sero-epidemiological studies (8), although human cases have been reported in most of the countries (9). The prevalence in small ruminants ranges from 3.6% in Uganda (10) to 17% in the Sudanese region of Kartum (11).

In Ethiopia, most research work on brucellosis has been focused on intensive dairy cattle herds in urban and peri-urban areas. In 1987, the World Organisation for Animal Health (OIE) reported a prevalence of 20%; the prevalence was higher around large towns than in rural areas (12). In zebu cattle of the central highlands, Tekleye *et al.* (13) reported a prevalence of 4.2%. Eshetu *et al.* (14) found a prevalence of 10% in Addis Ababa, and in a study conducted on smallholder farms of central Ethiopia (the Wuchale-Jida district), Kebede *et al.* (15) reported a prevalence of 11%.

In cattle under extensive management systems, studies conducted in different regions of Ethiopia between 2003 and 2005 reported individual-level prevalence values of 0.8% and 3.2% and herd-level prevalence of 2.9% and 42.3% (16, 17). In other areas of the country, studies conducted between 2003 and 2004 reported an individual-level prevalence of 1.6% and a herd prevalence of 13.7% (18).

In small ruminants, a prevalence of 1.5% in sheep and 1.3% in goats was found in the central highlands by Tekleye and Kasali (19).

However, prevalence values of 5.6% in sheep and 13.2% in goats were reported by Teshale *et al.* (20) in the Afar and Somali regions respectively, and 3.2% of sheep and 5.8% of goats in the Afar region were reported to be positive by Ashenafi *et al.* (21).

When brucellosis is detected in a herd, flock, region, or country, international veterinary regulations impose restrictions on animal movements and trade, which result in huge economic losses. The economic losses as well as its zoonotic importance are the reasons why programmes to control or eradicate brucellosis in cattle, small ruminants and pigs have been implemented worldwide (22).

Limited financial resources, political instability and lack of commitment by local and regional governments, as well as the presence of other serious livestock diseases (i.e. peste des petits ruminants, Rift Valley fever, foot and mouth disease, contagious caprine pleuropneumonia, lumpy skin disease and African swine fever), in many African countries have diverted attention from important zoonotic diseases such as tuberculosis and brucellosis (8, 11, 23). There is no strategic control and eradication plan against brucellosis in Ethiopia under implementation (17, 18). Therefore, this study was designed to investigate the spread of this neglected zoonotic disease in the country. Its objectives were to determine the prevalence and to identify the species of *Brucella* circulating in the small ruminant population in the pastoral areas of Ethiopia.

Materials and methods

Description of study areas

Ethiopia's economy is mainly based on agriculture, which accounts for 46% of the national gross domestic product (GDP) and 85% of total employment (24). In Ethiopia, the pastoralist and agro-pastoralist areas such as Borena, Afar and Somali are considered the traditional source of livestock, supplying 95% of livestock destined for the export market (25).

Since 2003, exportation of both live animals and meat has been increasing rapidly. The annual export potential of Ethiopian export abattoirs is estimated at 72,000 tonnes of meat, valued at US$136 million (26). The small ruminant population of the country is estimated to be 29.64 million sheep and 25.85 million goats (27); 25% of the sheep and 73% of the national goat population inhabit the lowlands, mostly in pastoral areas.

The pastoral human population of Ethiopia makes up approximately 13.7% of the country's total population of 93.8 million (24, 28). The pastoral population is heterogeneous in its ethnic composition and social structure. Livestock production, trading and 'take-a-chance' crop farming (subsistence rain-fed farming) are the pastoral livelihood systems (28).

The administrative regions and study area are shown in Figure 1. These areas were selected for this study because:

– pastoral areas have a large population of small ruminants and supply more than 90% of the export animals

– seasonal mixing of flocks/herds of different origins may spread infectious diseases throughout these regions.

The traditional husbandry systems involve close contact between domestic animals and humans, and the dependence on livestock and livestock products for nutrition predisposes pastoral communities to brucellosis.

The sheep and goats are of the local indigenous Ethiopian breeds: Afar, Somali, Borana and South Omo. No *Brucella* vaccine has been used in the study area. The livestock population of the study area, according to the Central Statistics Agency of Ethiopia (CSA) (29), is shown in Table I.

Study design

A cross-sectional survey of small ruminants was carried out between November 2004 and December 2007 in selected pastoral zones (Afar

1–5, Jijiga, Borena and South Omo) of Ethiopia. For sample size calculations Win Episcope 2.0 software was used (30). The study was initially designed to estimate 5% prevalence with an accepted error of 0.75% and a confidence interval (CI) of 95% (3,245 animals). In the end the sample size was increased to 6,201 (3,694 sheep and 2,507 goats). Two-stage cluster sampling at different hierarchical levels was used. Peasant associations (PAs) and villages/flocks were designated as primary and secondary sampling units, respectively. The sampling frame consisted of 51 districts and 513 PAs, from which 25 districts and 67 PAs were randomly selected by lottery. In the Somali region, because of security problems, we were restricted to sampling in the districts and PAs of the Jijiga zone. Villages and/or flocks within PAs were considered as cluster units and animals were selected on the basis of their availability at the time of sampling.

Given the absence of a livestock identification system in the pastoral regions it was difficult to trace seropositive animals for further bacteriological sampling. Therefore it was decided to collect tissue samples from three export slaughterhouses where livestock from pastoral areas are slaughtered for the export market. Initially, 285 goats were randomly selected three days before slaughter and screened for brucellosis with the Rose Bengal plate test (RBPT). Tissue samples (inguinal lymph nodes, testes, spleen and lungs) from 14 positive goats were used for attempted bacterial isolation.

Serum sample collection and submission

Approximately 10 ml of blood was collected from each animal using a Vacutainer and needle. The samples were stored, tilted horizontally, overnight at room temperature to allow clotting. The serum from each animal was decanted into a single sterile cryogenic vial, labeled and transported to the National Animal Health Diagnostic and Investigation Center (NAHRC), maintaining the cold chain. The sera were stored at −20°C until tested.

Serological tests

The antigen used in the RBPT was a suspension of *Brucella abortus* colored with Rose Bengal (Institut Pourquier, 326 Rue de la Galera, 34097 Montpellier Cedex 5, France). For the complement fixation test (CFT), standard *Brucella abortus* antigen and control sera were obtained from the BgVV (Berlin, Germany), complement from Biomerieux (France), and haemolysin or amboceptor from Institut Pourquier (France).

The RBPT was used as a screening test. To increase the sensitivity of the test, the modified procedure recommended for sheep and goats by Blasco *et al.* (31) and the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Brucellosis (23) was followed. Briefly, 75 µl of test serum was dispensed on each of the circles of the plate and 25 µl of antigen (Institut Pourquier) was placed alongside the serum. Antigen and serum were mixed thoroughly and the plate was rocked by hand for about 4 min at room temperature. A magnifying glass was used to read the result and interpret it; the results were recorded as 0, +, ++, +++ according to the degree of agglutination.

All RBPT positive and approximately 50% of the negative samples were retested with the CFT for confirmatory diagnosis at the National Veterinary Institute (NVI) laboratory, Debre Zeit, Ethiopia, according to the protocols recommended by the OIE (22).

Bacteriology

Tissue samples (inguinal lymph nodes, testes, spleen and lungs) from 14 goats positive on the RBPT were used for attempted bacterial isolation. Samples were cultured on Sabouraud dextrose agar and *Brucella* agar base (BAB) containing antibiotics, and incubated at 37°C supplemented with 5–10% CO_2 for 5–8 days. Identification of *Brucella* organisms was carried out by a combination of assessment of colonial characteristics, morphology and biochemical tests. Positive cultures were stained with Gram's and modified Ziehl–Neelsen stains

to determine the morphology, and basic biochemical tests (catalase, oxidase and urease tests) were conducted (32, 33).

Polymerase chain reaction

The isolates were further tested with polymerase chain reaction (PCR) using forward primers specific for *B. abortus* and *B. melitensis* and a genus-specific reverse primer to confirm and subtype the *Brucella* isolates.

Extraction of genomic DNA from bacterial culture

Bacterial cultures (one to three colonies) were suspended in 50 µl of water (Molecular Biology Reagent, W4502, Sigma, Mannheim, Germany), heated to 99°C for 20 min, and centrifuged at 14,000 rpm for 1 min. The supernatant was used as the DNA template for PCR reactions.

DNA amplification by polymerase chain reaction

The primer sequences used were: genus-specific reverse primer/universal primer IS711R: 5′-TGC CGA TCA CTT AAG GGC CTT CAT-3′; species-specific forward primers *Brucella abortus* F: 5′-GAC GAA CGG AAT TTT TCC AAT CCC-3′ and *Brucella melitensis* F: 5′-AAA TCG CGT CCT TGC TGG TCT GA-3′ (Amersham Pharmacia Biotech, Barcelona, Spain). Both sets of primers were designed to amplify a PCR product that included a region of chromosome I from nucleotide (nt) position 963,334 to nt 964,065 (relative to the 5′ extreme). The PCR was performed in a 50 µl mixture containing: 5 µl template DNA; PCR buffer (10 mM Tris HCl [pH 8.4], 50 mM KCl, 1.5 mM $MgCl_2$); 200 nM of each of the PCR primers; 200 µM (each) of dATP, dCTP, and dGTP; 190 µM dTTP; 10 µM digoxigenin-11′-dUTP (Roche Diagnostics, Barcelona, Spain); and 1.25 U of *Taq* polymerase (Roche Diagnostics). The PCR cycling conditions consisted of preheating at 93°C for 5 min; 35 cycles of 90°C for 1 min, 60°C for 30 s, and 72°C for 1 min; and final extension at 72°C for 7 min.

Data treatment and analysis

The data collected from the field were stored in an Excel (Microsoft Corporation) spreadsheet. Prevalence values were standardised for the sheep and goat populations. The chi-square test was performed and relative risks (RRs) were calculated with Epi-Info (wwwn.cdc.gov/epiinfo); *p*-values less than 0.05 were taken to be significant. Confidence intervals (CI) for prevalence were calculated using EpiCalc 2000 (www.brixtonhealth.com/epicalc.html) and the standardised prevalence with Episheet (http://krothman.org/*episheet*.xls). Maps were created with Quantum GIS version 1.6. (www.qgis.org/).

Results

A total of 6,201 serum samples (from 3,694 sheep and 2,507 goats) were collected and tested; 4,882 (78.7%) of the animals sampled were female and 1,319 (21.3%) were male. Of the 6,201 sera tested, 320 (5.2%; 95% CI: 4.6–5.8) and 134 (2.2%; 95% CI: 1.8–2.6) were positive for brucellosis by RBPT and CFT respectively. All administrative zones, 84% of the districts and 58% of the PAs surveyed were found to be affected with brucellosis, but the distribution was not uniform. The region of Borena and the Afar zone had higher standardised prevalence values (3.3%; 95% CI: 1.7–4.9 and 2.8%; 95% CI: 2.2–3.4, respectively) than Jijiga and South Omo, where the prevalence values were 0.07% (95% CI: 0.0–0.17) and 0.26% (95% CI: 0.0–0.56). There was statistically significant variation in disease prevalence among the studied zones, districts and peasant associations ($p < 0.05$). The seroprevalence of brucellosis in each zone and district is summarised in Table II and Table III. Figure 2 and Figure 3 show the spatial distribution of brucellosis in districts and zones.

Table IV shows the variation of prevalence with species, sex and age group. Male goats and sheep were twice as likely to test positive as females (RR: 2.04; 95% CI: 1.7–3.4; χ^2: 21.05, $p < 0.05$). Adults (animals older than 1.5 years) were three times more likely to test positive than younger animals (RR: 2.76; 95% CI: 1.14–6.73; χ^2: 5.18,

$p < 0.05$), and goats were approximately four times more likely to be infected than sheep (RR: 3.8; 95% CI: 2.4–6.1; χ^2: 36.99, $p < 0.05$).

Bacterial growth was seen on only 2 of the 14 cultured plates. The PCR results for these two isolates were positive for *B. melitensis*, showing a band of amplified DNA of 731 bp, similar to the positive control containing *B. melitensis* DNA (Fig. 4).

Discussion

The prevalence of brucellosis observed in small ruminants in the Ethiopian pastoral areas was lower than most values reported in other African countries. This may be attributed to the low level of intensification, breed differences, flock size and composition, or the tests used to make the diagnosis. In the Somali region we were unable to include as many zones in the study as selected in the Afar region, because of security problems. However, we sampled five districts and 12 PAs, from which we collected 1,854 sera for the study. Therefore we believe that our sampling was representative and did not affect the overall result of our study.

A higher seroprevalence in goats than in sheep has also been described by other authors. Prevalence values between two- and four-fold higher in goats have been described in Eritrea (34), East Morocco, Tunisia and Egypt (11) and Nigeria (35), and between one- and two-fold higher in Sudan and the United Arab Emirates (11) and in Kenya (36). In other countries a higher prevalence has been detected in sheep, for example Somalia (37), Jordan (11) and Oman (38). Generally, goats are more susceptible to *Brucella* infection than sheep, and this could be partly due to the fact that sheep excrete the organism for shorter periods than goats. This may reduce the potential for spread of the disease within and between sheep flocks (4).

The higher prevalence observed in male than in female animals in this study was not in accordance with the findings of other authors. Hirsh and Zee (39) and Alton (40) reported lower susceptibility to *Brucella* infection in males, and Teshale *et al*. (20) and Ashenafi *et al*. (21) found no observable sex variation in the prevalence of brucellosis. A

possible reason for the findings of the current study is the sharing of male animals between villages, which could have led to increased likelihood of infection and may explain the higher prevalence.

Differences among regions may be related to husbandry practices. The higher prevalence in Afar and Borena flocks can be explained by the greater contact between flocks that use communal grazing and watering points, in contrast to Jijiga and South Omo, where the level of contact between flocks is lower.

In this ecosystem, people and animals move seasonally in search of pasture and water, and detailed herd information (i.e. animal identification, local herd census by sex, age and species) is not available for analyses. Therefore it is not possible to determine whether bias was introduced by the sampling procedure. In this study the available animals over six months of age were sampled in each PA. However, more females than males, more adults than young animals and more goats than sheep were sampled. This is partly because more females are kept in the herds/flocks than males. Male animals are sent for live animal export or slaughtered for the local and export markets. Furthermore, there is a regional variation in the preference for the species of small ruminant kept.

All administrative zones, 84% of the districts and 58% of the PAs surveyed were found to be affected with brucellosis. This implies that the livestock population in most of the pastoral areas is at risk. This provides clear evidence that control measures are required to minimise economic losses and the public health hazard.

Conclusion

The overall standardised prevalence of brucellosis found in this survey was 1.9%, which appears low, but 58% of the PAs, 85% of the districts and 100% of the zones surveyed contained animals seropositive for brucellosis. The bacterial isolation was successful, which indicates that it is possible to isolate circulating *Brucella* species from livestock in arid and semi-arid pastoral areas and other regions of Ethiopia. The results of the study show that most regions

and herds/flocks in the pastoral areas of Ethiopia are affected by brucellosis. There is clear evidence that control measures are required in order to minimise further economic losses and public health hazards.

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Table I

Livestock population of the study area

Source: Central Statistical Agency (29)

Region	No. of sheep (× 1,000)	No. of goats (× 1,000)
Afar	403.3	801.5
Somalí	1,162.7	1,374.5
Borena	1,274.1	669.1
South Omo	323.8	505.1

Table II

Seroprevalence of small ruminant brucellosis in the study zones, obtained using complement fixation test

Region	Zone	Goats			Sheep			Standardised prevalence (95% CI)
		Population (× 1,000)	Pos/*N*	Prevalence (95% CI)	Population (× 1,000)	Pos/*N*	Prevalence (95% CI)	
Afar	1		42/1,245	3.4% (2.5–4.6)		3/215	1.4% (0.4–4.4)	2.4% (1.4–3.3)
	2		2/79	2.5% (0.4–9.7)		0/8	0.0% (0.0–40.2)	1.2% (0–2.9)
	3		25/331	7.6% (5.0–11.1)		5/144	3.5% (1.3–8.3)	5.5% (3.4–7.5)
	4		6/156	3.8% (1.5–8.6)		1/53	1.9% (0.1–11.4)	2.8% (0.4–5.2)
	5		15/483	3.1% (1.8–5.2)		6/405	1.5% (0.6–3.4)	2.3% (1.3–3.2)
Sub-total: Afar		403.3	90/2,294	3.9% (3.2–4.8)	801.5	15/825	1.8% (1.1–3.1)	2.8% (2.2–3.4)
Oromia	Borena	1,274.1	21/474	4.4% (2.8–6.8)	669.1	3/135	2.2% (0.6–6.9)	3.3% (1.7–4.9)
Somali	Jijiga	1,162.7	0/371	0.0% (0.0–1.3)	1,374.5	2/1,483	0.1% (0.02–0.5)	0.07% (0–0.17)
SNNP	S. Omo	323.8	3/555	0.5% (0.1–1.7)	505.1	0/64	0.0% (0.0–7.1)	0.26% (0–0.56)
Total		3,163.9	114/3,694	3.1% (2.6–3.7)	3,350.2	20/2,507	0.8% (0.5–1.3)	1.9% (1.6–2.2)

95% CI: 95% confidence interval

N: number sampled

Pos: positive

Table III
Seroprevalence of small ruminant brucellosis at regional, zonal and district levels, by complement fixation test

Region	Zone	District	Tested	Positive	Prevalence (%)	95%CI
Afar	Zone 1	Chefira	280	16	5.7	3.41–9.29
		Dubti	134	9	6.7	3.32–12.74
		Mille	1,043	20	1.9	1.21–3.00
	Sub-total		1,457	45	3.1	2.29–4.15
	Zone 2	Afdera	58	1	1.7	0.09–10.46
		Koneba	29	1	3.5	0.18–19.63
	Sub-total		87	2	2.3	0.40–8.84
	Zone 3	Amibarah	60	7	11.7	5.21–23.18
		Awash Fentale	58	6	10.3	4.27–21.83
		Bure Mudaitu	87	1	1.2	0.06–7.13
		Gewane	270	16	5.9	3.54–9.63
	Sub-total		475	30	6.3	4.38–9.00
	Zone 4	Ewa	129	3	2.3	0.33–4.03
		Golina	58	2	3.5	0.60–12.95
		Yalo	28	2	7.1	1.25–24.96
	Sub-total		215	7	3.3	1.44–6.87
	Zone 5	Artuma	29	0	0	0.31–14.56
		Daliphage	566	10	1.8	0.86–3.17
		Dewey	58	1	1.7	0.09–10.46
		Fursi	57	1	1.8	0.09–10.63
		Semurobi	88	7	7.9	3.53–16.22
		Telalak	87	2	2.3	0.40–8.84
	Sub-total		885	21	2.4	1.51–3.66
Sub-total (region)			3,119	105	3.3	2.78–4.08
Oromia	Borena	Yabello	609	24	3.9	2.60–5.89
SNNP	South Omo	Benatsemay	619	3	0.5	0.12–1.53
Somali	Jijiga	Awabere	236	1	0.4	0.02–2.70
		Harshin	79	0	0	0.12–5.78
		Jijiga	1,249	1	0.1	0.00–0.52
		Medele	60	0	0	0.15–7.50
		Obre	230	0	0	
Sub-total (region)			1,854	2	0.1	0.02–0.44
Total			6,201	134	2.2	1.86–260

Table IV
Prevalence of small ruminant brucellosis by species, sex and age group

Category	No. tested	Positive	Prevalence (%)	95% CI	χ^2	*P*-value	Relative risk
Species							
Goat	3,694	114	3.1	2.69–3.57	36.99	<0.05	Goats in relation to sheep RR=3.8; 95% CI=2.4–6.1
Sheep	2,507	20	0.8	0.60–1.06			
Sex							
Male	1,319	50	3.8	3.34–4.31	21.05	<0.05	Male in relation to female RR=2.04; 95% CI=1.7–3.4
Female	4,882	84	1.7	1.40–2.06			
Age							
Adult*	5,592	129	2.3	1.95–2.71	5.18	<0.05	Adult in relation to young RR=2.76; 95% CI=1.14–6.73
Young**	608	5	0.8	0.60–1.06			
Total	6,201	134	2.2	1.86–2.60			

*Adult: >1.5 years

**Young: ≤1.5 years

95% CI: 95% confidence interval

Fig. 1
Study area

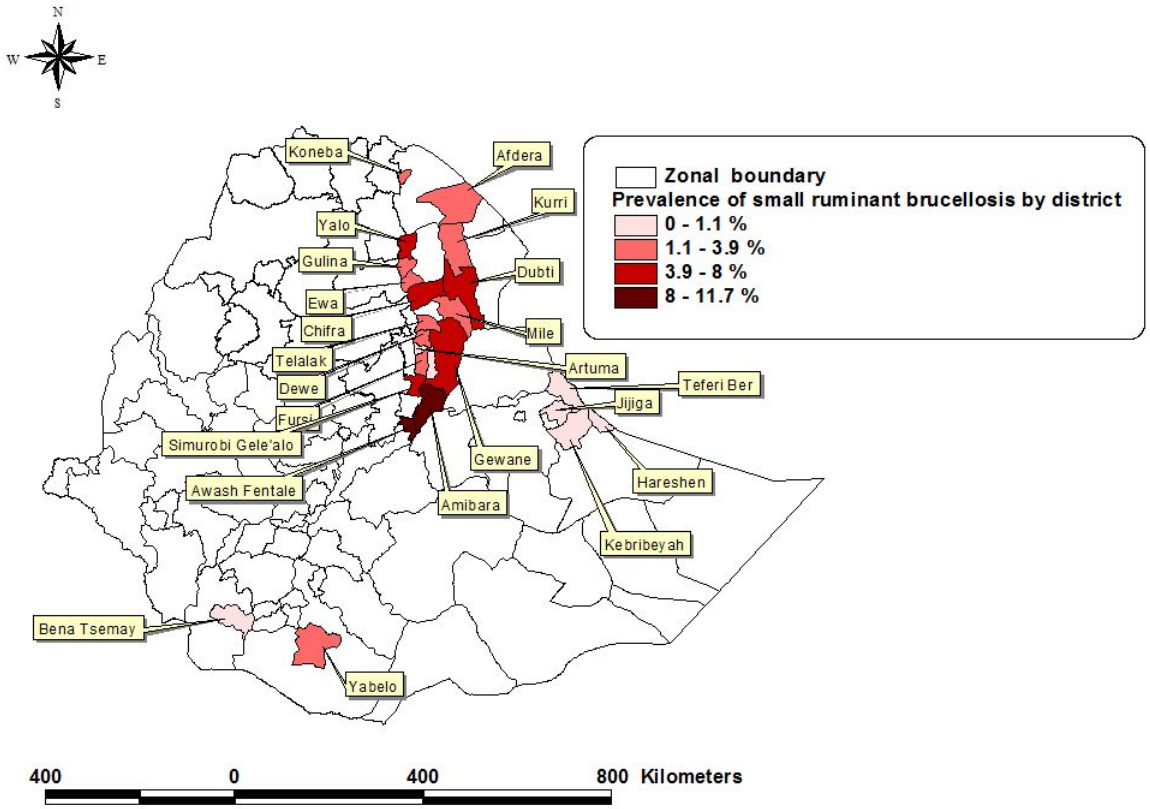

Fig. 2
Prevalence of brucellosis in small ruminants in the study districts ('Woredas')

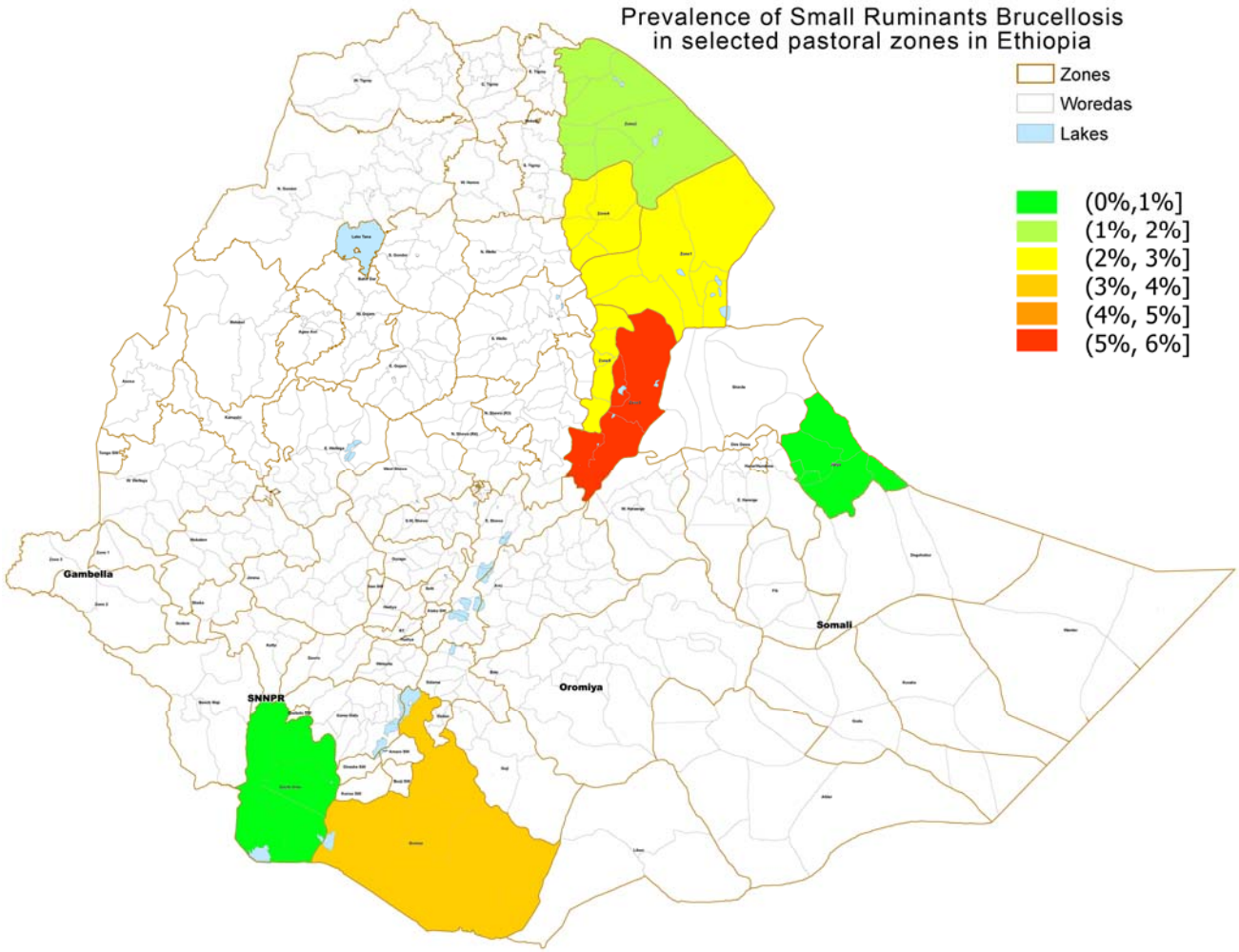

Fig. 3
Prevalence of brucellosis in small ruminants in selected pastoral zones

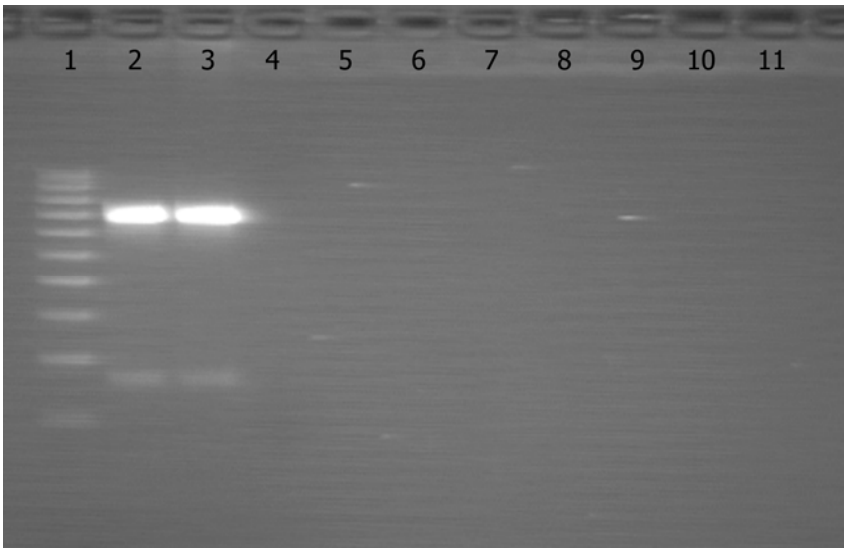

Fig. 4
Result of polymerase chain reaction on the isolate tested using *B. melitensis*-specific primers
A positive band was formed at 731 base pairs (bp), the same size as the band from a known isolate used as a positive control (National Animal Health Diagnostic and Investigation Center, Ethiopia, October, 2006)

Lane 1: Molecular ladder
Lane 2: *B. melitensis* positive control with band at 731 bp
Lane 3: The isolate coincides with the positive control, with a band at *731* bp
Lane 4: Negative control
Lane 5–11: Empty