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Brucellosis and bovine tuberculosis prevalence in livestock from pastoralist communities adjacent to Awash National Park, Ethiopia

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ABSTRACT

This cross-sectional study investigated the prevalence of brucellosis and bovine tuberculosis (BTB) in local cattle and goat breeds of Oromo and Afar pastoralist communities living in two distinct parts around the Awash National Park. A questionnaire survey was carried out to assess information on husbandry, milk consumption habits, and on knowledge–attitude–practice regarding both diseases.

Among a total of 771 animals from all sites tested by comparative intradermal tuberculin test (CIDT) none were BTB reactors with the >4 mm cut-off. Using the >2 mm cut-off, individual apparent prevalence was 0.9% (95%CI: 0.23–3.56%) in cattle and 0.7% (95%CI: 0.12–3.45%) in goats. Herd prevalence in Oromia and Afar sites was 0% and 66.7% respectively in goats and 16.7% and 50% in cattle. Among the 327 animals tested by enzyme linked immunoassay for brucellosis, 4.8% (95%CI: 1.2–17.1%) of cattle and 22.8% (95%CI: 5.98–29.5%) of goats were reactors. Highest individual prevalence of both diseases was found in Afar settlements with brucellosis being as high as 50%. Respondent ethnicity was the only risk factor for brucellosis positivity in goats in the univariable risk factor analysis. Knowledge about the diseases was poor. Raw goat milk was regularly consumed by women and children, putting them at risk for brucellosis.

This study highlighted an increased prevalence gradient of BTB and brucellosis from West to East along the study sites with high brucellosis individual prevalence and abortion rates among Afar settlements in particular.

1. Introduction

Brucellosis and bovine tuberculosis (BTB) are two important chronic milk-borne bacterial zoonoses, impacting on human and animal health, but also on animal productivity, thus having substantial financial consequences at national, regional and individual level (Meisinger, 1969; Grange, 2001; Zinsstag et al., 2006; Seleem et al., 2010).

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Bovine tuberculosis is caused by *Mycobacterium bovis*, a genetically close relative to *M. tuberculosis*, the main human tuberculosis pathogen and causing similar pathology, namely granulomas in lymph nodes and organs (Brosch et al., 2002; Mostowy et al., 2005). Brucellosis in animals is caused by various Brucella spp biovars, with *B. abortus* biovar found mainly in cattle and *B. melitensis* in small ruminants. The latter is considered to be the biovar with highest zoonotic potential (Acha and Szyfres, 2003). The pathogen is secreted via milk and uterine discharges, whereas *M. bovis* is mainly transmitted through aerosol exposure and/or through consumption of infected animal products (Phillips et al., 2003; Neill et al., 2005).

In humans, brucellosis is an acute febrile illness that can progress to chronic forms with complications involving musculoskeletal, central nervous and cardio-vascular systems (Regassa et al., 2009). The symptomatic profile of BTB is largely dependent on the location of the granulomas.

In the Ethiopian Highlands, both diseases were shown to be prevalent at low endemicity in rural areas where sedentary farmers keep local breeds under traditional husbandry (Berhe et al., 2007; Tschopp et al., 2009; Berg et al., 2009; Jergefa et al., 2009). The epidemiological setting in the pastoralist system, however, differs from the situation in the Highlands in terms of environment and husbandry practices. Pastoralists own large livestock herds, live in daily close contact with their animals on which their livelihood is directly dependent and often seasonally migrate with their animals. Only few studies on BTB and brucellosis were carried out amongst livestock of pastoralists in the Lowlands and they focused mainly on communities from Southern Ethiopia or Somali region (East Ethiopia) (Tschopp et al., 2010a; Gumi et al., 2012; Teshome et al., 2003; Dinka and Chala, 2009). Investigations in the Afar region (Lowland pastoralist communities in Eastern Ethiopia) were rare and were either focusing on only one of the two diseases, were outdated, had small sample size and/or were in a very different geographic setting to the current study (Ashenafi et al., 2007; Kassa et al., 2012).

The aims of this study were to assess the prevalence of BTB and brucellosis in cattle and goats in pastoral communities around Awash National Park, describe the potential risk factors of diseases, and knowledge–attitude and practice (KAP) of these communities towards both diseases.

2. Materials and methods

2.1. Study site

The study was carried out between January and August 2012 in two Woredas (districts) adjacent to Awash National Park (750 km^2), namely Awash Fentalle (Afar region, Zone 3), and Fentalle (Oromiya region). Altitude ranged between 600 and 1000 m above sea level and the climate was semi-arid with bi-annual rainfalls. Vegetation was characterized by semi-arid scrubland and open grass land. Main water supplies in the study area came from the Awash River, Lake Beseka and the Kesem River (Fig. 1).

The study area included Muslim pastoralists from different ethnic backgrounds, namely the Afar in the North and the Kereyu- and Ittu Oromo in the South (Fig. 1). Pastoralism was the main livelihood activity with some few villages in the South (Kereyu) engaging in recent years in agro-pastoralism. Pastoralists kept camels, cattle, goats and sheep. They usually lived in a permanent village from where some family members migrated annually and seasonally with their camels and cattle to secondary temporary settlements and/or grazing areas further away, in particular during difficult drought year and/or yearly flooding of the Awash River.

2.2. Study design and study animals

A list of pastoralist villages was obtained from the respective Woreda Agriculture Bureau. We performed a multi-stage sampling with following inclusion criteria for villages: logistic feasibility (accessibility by car; security; distance), proximity between villages and wildlife area (Awash National Park), and willingness of pastoralists to cooperate.

A cluster sampling proportional to the size of the livestock population was performed in which villages were considered as clusters. Sample size calculation performed according to Bennett et al. (1991) resulted in 380 goats and cattle to be tested for BTB assuming an intraclass correlation coefficient of *rho* 0.2, an expected prevalence of 5% and a standard error of 2.8%. The same number of cattle and goats was initially planned to be tested. However, most cattle had left the primary settlements at the start of the study and only few milking cows remained; more goats were therefore tested than initially planned. Within the villages, owners were selected randomly based on a list provided by the village leader; however their final inclusion depended on consent to have their animals tested. Between 1 and 20 animals were chosen per owner depending on the herd size. If an owner had a very small herd (<5 animals) all his animals were included. In bigger herds, animals were randomly chosen (e.g. every third one coming out from the Mboma). We chose more owners and less animals per owner rather than the opposite. A number was marked on the animal using a temporary animal marker so as to recognize it on the reading day. Animals younger than 6 months, females close to parturition, and animals that were visibly sick were excluded from the testing since these factors could influence the skin test results. When permission was granted by the owners, these animals were then further tested for brucellosis.

Livestock in Ethiopia are not vaccinated against brucellosis or BTB.

2.3. Disease diagnostics

2.3.1. Bovine tuberculosis

Cattle and goats were tested using the comparative intradermal tuberculin test (CIDT), using tuberculins with the following concentration: 30,000 IU/ml for bovine and 25,000 IU/ml for avium PPD (Prionics Lelystad BV, The Netherlands). Two sites on the neck (horizontal ipsi-lateral for cattle and contra-lateral for goats) were shaven, skin thickness measured with a caliper and 0.1 ml of bovine or avium PPDs were injected intradermally using an insulin syringe. Skin thickness was measured again after 72 h by

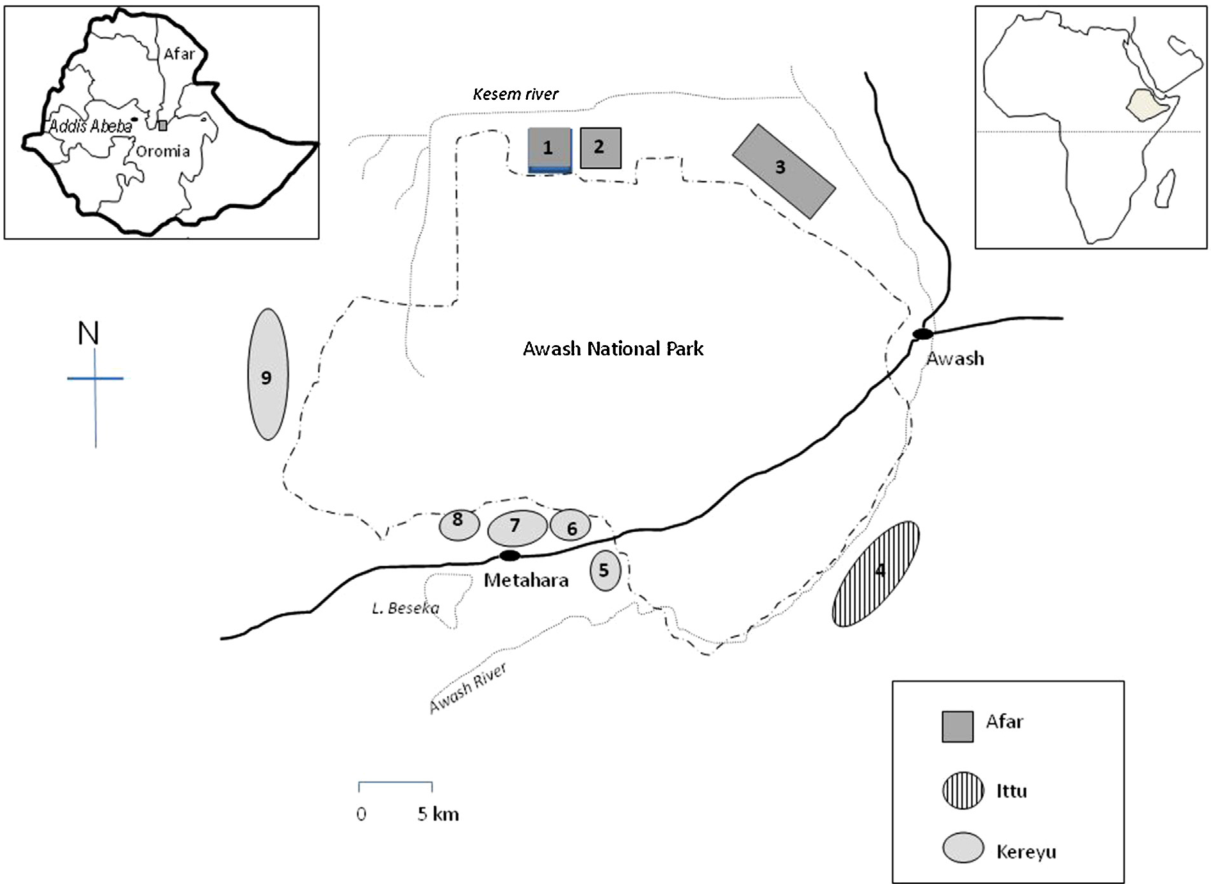

Fig. 1. Map of the study area showing the pastoral sites chosen. Fill color of sites depending on ethnic background. Numbers correspond to the settlement name (1: Wasero; 2: Alibeti; 3: Doho; 4: Fateledi; 5: Galcha; 6: Kobo; 7: Benti; 8: Ekue; 9: Ilala).

caliper and recorded along with other parameters such as body condition, sex and age. The same person performed all the testing. Test sensitivity and specificity for both cut-offs are described in Ameni et al. (2008).

2.3.2. Brucellosis

Whenever owners authorized the procedure, 5 ml and 10 ml blood was drawn into plain vacutainers (Plymouth, UK) from the jugular vein of goats and cattle respectively. Vacutainers were kept vertically at ambient temperature for 6–8 h. Serum was then collected into cryotubes using Pasteur pipettes and kept frozen until further processing at the Armauer Hansen Research Institute (AHRI) laboratory (Addis Ababa). Sera were analyzed in duplicates for the presence of *Brucella abortus* and/or *Brucella melitensis* antibodies using an enzyme linked immunoassay (PrioCHECK® Brucella Ab test, Prionics, Netherlands). According to the company, specificity ranged from 98% to 100% for cattle and goats respectively. Sensitivity ranged from 89% for cattle to 100% for goats. Positive samples were re-tested for confirmation with the same assay, but using a new kit.

2.4. Questionnaire survey

A questionnaire survey with open and closed questions in the local languages (amharic, oromifa, afarina) was pre-tested ($N = 8$) among pastoralists in the study area and back translated into English to assure questions were translated accurately. The survey was then performed among the willing owners in their native language (Afar and Oromiffa). The questionnaire captured general information on the interviewees (e.g. literacy, ethnicity, religion), on potential risk factors for both diseases as well as attitude of people (e.g. livestock presence and numbers, milk consumption habits, animal housing and herding, people attending animals, abortion data and afterbirth handling), on experienced disease symptoms and finally on disease knowledge (e.g. name, transmission) and treatment.

2.5. Statistical analysis

Data were entered into Microsoft Access tables and analyzed using Stata software (version 10.1, StataCorp, Texas, USA). Univariable risk factor analysis was done using logistic regression modelling with random effect on villages. Results were shown as OR, 95% for the OR and *p*-values.

Results were interpreted according to the World Organization for Animal Health Office (OIE) regulations (2009), namely, an animal was considered positive for bovine tuberculosis if the increase in skin thickness at the bovine site of injection was more than 4 mm greater than the

Table 1
Prevalence of bovine tuberculosis, PPD-A reactivity and Brucellosis in cattle and goats by villages.

		PPD 2 pos		PPD inconclusive*		PPD-A reactivity		Brucellosis	
Region	Village	Cattle	Goat	Cattle	Goat	Cattle	Goat	Cattle	Goat
Oromia	Ilala	0/8	0/57	2/8 (25)	0/57	0/8	3/57 (5.3)	ND	0/29
	Galcha	0/25	0/30	1/25 (4)	1/30 (3.3)	1/25 (4)	0/30	1/4 (25)	1/20 (5)
	Benti	0/30	0/48	1/30 (3.3)	0/48	0/30	3/48 (6.2)	1/7 (14.3)	3/19 (15.8)
	Kobo	1/31 (3.2)	0/36	1/31 (3.2)	0/36	2/31 (6.5)	0/36	0/8	5/25 (20)
	Fateledi	0/28	0/41	1/28 (3.6)	1/28 (3.6)	0/28	0/28	0/7	4/24 (16.7)
	Eka Idu	0/34	0/45	3/34 (8.8)	1/45 (2.2)	1/34 (2.9)	2/45 (4.4)	0/13	0/20
Afar	Doho	1/44 (2.3)	3/164 (1.8)	5/44 (11.4)	9/164 (5.5)	0/44	1/164 (0.6)	0/3	29/87 (33.3)
	Wasero	0/20	1/72 (1.4)	1/20 (5)	2/72 (2.8)	1/20 (5)	0/72	ND	14/28 (50)
	Alibeti	ND	0/58	ND	1/58 (1.7)	ND	0/58	ND	9/33 (27.3)
Total	Overall	2/220 (0.9)	4/551 (0.7)	15/220 (6.8)	15/551 (2.7)	5/220 (2.3)	9/551 (1.6)	2/42 (4.8)	65/285 (22.8)
	Oromia	1/156 (0.6)	0/257	9/156 (5.8)	3/257 (1.2)	4/156 (2.5)	8/257 (3.1)	2/39 (5.1)	13/137 (9.5)
	Afar	1/64 (1.6)	4/294 (1.4)	6/64 (9.3)	12/294 (4.1)	1/64 (1.6)	1/294 (0.3)	0/3	52/148 (35.1)

PPD 2 pos: number of positive PPD reactors using the >2 mm cut-off. PPD inconclusive calculated as (B2-B1)–(A2-A1) >1 mm & ≤4 mm; where B = skin reaction for bovine PPD, A = skin reaction for avium PPD, 2 = day 3 (72 h), 1 = day 1. ND: not done.

increase shown at the avian injection site. Reactions were considered inconclusive if the increase at the bovine site ranged from 1 to 4 mm greater than the increase at the avium site. In addition, results were also interpreted using a >2 mm cut-off for positive reaction as suggested by Ameni et al. (2008) to be more specific for the Ethiopian setting.

Animals were considered *Mycobacterium avium* complex (MAC) reactor if the reaction at the avium site showed a >4 mm skin-fold difference between day 1 and day 3, regardless of the bovine reaction, as previously described (Tschopp et al., 2010b).

Animals were considered brucellosis positive if seropositive in the ELISA assay in two separate assays. Villages were considered as herd. A herd was considered positive for any disease if it had at least one positive reactor for the specific disease. Animals were categorized by age whereby adult animals were defined as having reached breeding age.

2.6. Ethical clearance

The study has received institutional ethical clearance from the AHRI/ALERT Ethical Review Committee (AAERC), number PO04/12.

3. Results

3.1. CIDT results

In total, 771 animals were tested by CIDT, 220 cattle of local Zebu breeds (*B. indicus*) and 551 goats of local breed. The majority were female (N = 199/220; 90.5% in cattle; N = 539/551; 97.8% in goats) and adult animals (N = 197/220; 89.6% in cattle; N = 550/551; 99.8% in goats). Results of the PPD testing by species and settlements are shown in Table 1. None of the 771 animals were reactors with the >4 mm cut-off. All analysis was done using the >2 mm cut-off. Individual apparent BTB overall prevalence was 0.9% (95%CI: 0.23–3.56%; N = 2/220) in cattle and 0.7% (0.12–3.45%; N = 4/551) in goats. Overall prevalence was higher in the Afar villages than in those in Oromia (OR = 5.8; p = 0.108). Herd prevalence in Oromia was 0% for goats and 16.7% for cattle. Among the Afar villages, it was 66.7% in goats and 50% in cattle.

Overall, 6.8% (15/220) of cattle and 2.7% (15/551) of goats were inconclusive reactors, with a higher prevalence found in Afar. PPD-A prevalence was 2.3% (5/220) in cattle and 1.6% (9/551) in goats with higher prevalence in animals from the Oromia settlements. There was no statistical difference of PPD-A positivity and species (OR: 0.75; p = 0.619) and between PPD-A and PPD-B reactors (p = 0.999).

3.2. Brucellosis results

In total, 327 animals were tested for brucellosis. The majority were goats (N = 285; 87.1%). Three villages refused to have blood drawn from their cattle. The overall brucellosis prevalence was 4.8% (95%CI: 1.2–17.1%; N = 2/42) in cattle and 22.8% (95%CI: 5.98–29.5%; N = 65/285) in goats. Results by species and villages are shown in Table 1. Individual prevalence was highest in the Afar animals, particularly in goats (35.1%; N = 52/148). Fig. 2 shows the geographical clustering of brucellosis reactors and highlights a West-East prevalence gradient that overlapped with the ethnic background of the villages (Fig. 2). No animal was concurrently BTB and brucellosis positive.

3.3. Survey results

Overall, 67 pastoralists were interviewed on questions pertaining to information on owners, husbandry and milk consumption practices, as well as information on animal abortions and KAP. All respondents consumed daily raw milk products. At night, all animals were kept in species specific M'bomas for predator protection. During the day, livestock were shepherded by species, except in Afar where 27 (40.3%) kept their animals together. Women and young children looked after goats and sheep, while older boys shepherded the camel and cattle herds. Milking was performed by women and young children. Attending sick animals and animals giving birth was done by adults and children alike but with women and children in charge of small ruminants and men in charge of cattle and camels.

3.3.1. Abortion

Considering data given by respondents on number of livestock owned and number of abortions recalled during

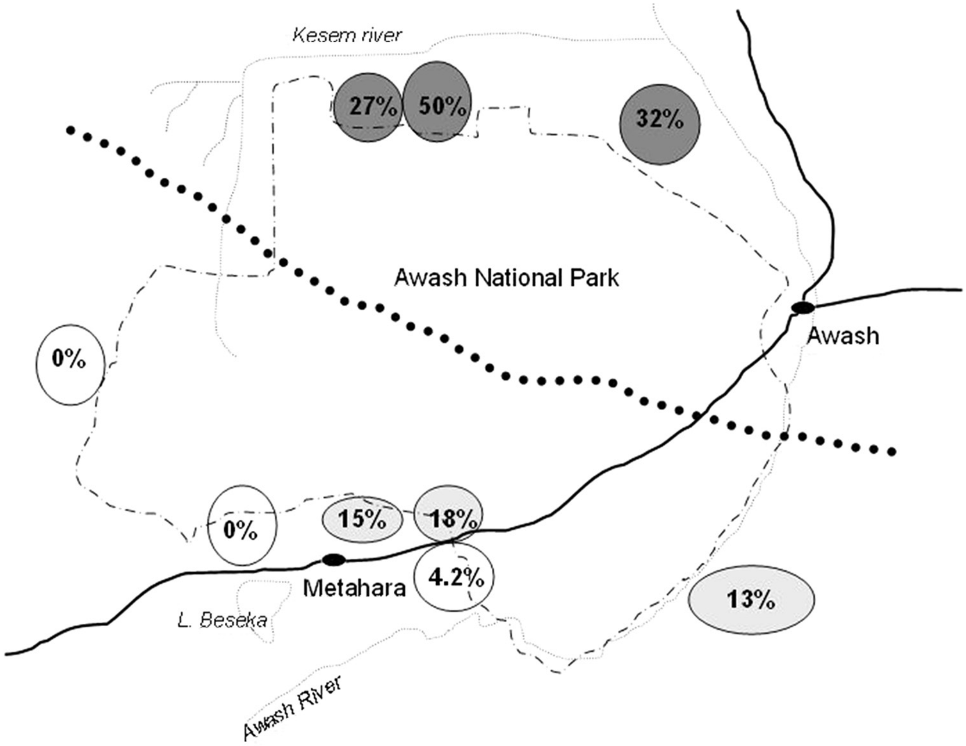

Fig. 2. Map of the study area showing in colored circles overall brucellosis individual prevalence in the different settlements (the darker the color the higher the prevalence). The thick dotted line represents roughly the border between the Afar and Oromia region.

the last 12 months, approximately 9.8% ($N = 142$) and 13.6% ($N = 82$) abortion were reported in small ruminants, 1.9% ($N = 10$) and 7.3% ($N = 25$) in camels, and 1.4% ($N = 5$) and 14% ($N = 58$) in cattle in Oromia and Afar respectively. There was a statistically significant higher risk of abortion in the Afar woreda as compared to the Oromia woreda (goats: $p = 0.040$; cattle and camel: $p < 0.001$).

3.3.2. Disease symptoms and knowledge–attitude–practice (KAP)

A list of clinical signs experienced chronically by the interviewees in the last 12 months is shown in Table 2. The majority (84.4%; $N = 38$) of the Oromia respondents had never heard of tuberculosis and hence did not know about transmission routes nor risk factors particular to the diseases. Among the Afar respondents, all knew TB and said it was transmitted by air (90.9%) or by sharing drinks and cups (9%), whereas 18.2% stated saliva as transmission vehicle. Twenty respondents (90.9%) knew that livestock can transmit TB to people. None of the respondents knew about brucellosis.

3.4. Risk factor analysis

Due to the very small number of BTB reactors, and small numbers of cattle tested for Brucellosis, risk factor analysis was performed for positive brucellosis goat reactors only (Table 3). The significant variable resulting from the analysis of 10 possible variables was ethnicity (p: 0.003). Multivariable analysis was therefore not performed.

Table 2
Perceived symptoms by the 67 pastoralist respondents that have chronically occurred in their household over the last 12 months.

Perceived symptom	Oromia	Afar
	Number of respondent (%)	Number of respondent (%)
Persistent cough lasting over 2 weeks	3 (6.67)	11 (50)
Haemoptysis	0	10 (45.4)
Chronic or recurrent fever	2 (4.5)	13 (59)
Weight loss	0	14 (63.6)
Night sweat	2 (4.5)	13 (59)
Lack of appetite	4 (8.9)	14 (63.6)
General fatigue	26 (57.8)	14 (63.6)
Joint pain	24 (53.3)	12 (54.5)
Headache	23 (51.1)	14 (63.6)
General body aching	23 (51.1)	10 (45.4)
Abortion	0	0

4. Discussion

Pastoralists keep large numbers of livestock and rely on them as nutritional milk and meat protein sources, thus putting them at risk for milk-borne zoonoses, in particular BTB and brucellosis. Using the official 4 mm as well as the 2 mm cut-off, our study showed an overall low BTB prevalence in cattle (0.5–2%), and its rarity in goats (0–0.5%). These results are in line with previously published reports that used the same cut-offs (Tschopp et al., 2010a; Gumi et al., 2012; Teshale et al., 2006; Megersa et al., 2011a; Megersa et al., 2011b). In support, *M. bovis* not having been

Table 3
Univariable analysis of risk factors for goat brucellosis using logistic regression with village as random effect.

Risk factor		Proportion% (nb/total)	*p*-Value	OR	95% CI for the OR
Respondent religion	Muslim	100 (63/63)			
Respondent literacy	No school	85.3 (52/61)			
	Primary school	19.7 (12/61)	0.822	0.77	0.08–7.14
Ethnicity	Kereyu	27 (17/63)	0.003		
	Ittu	38 (24/63)			
	Afar	35 (22/63)			
Goat herd size	Small (1–10)	27 (17/63)			
	Middle (11–30)	54 (34/63)	0.165	15.46	0.32–737.63
	Large (>30)	19 (12/63)	0.716	2.14	0.03–131.34
Common grazing of livestock during the day	Species kept separate	60.3 (38/63)			
	Species kept together	39.7 (25/63)	0.204	0.21	0.02–2.28
Livestock night housing	Species specific Mbomas	100 (63/63)			
Livestock grazing inside the National Park	Yes	60.3 (38/63)	0.389	3.62	0.19–67.96
	Never	39.7 (25/63)			
Households with goat abortions in the last year	Yes	73 (46/63)	0.534	0.53	0.07–3.89
	Never	27 (17/63)			
Touching afterbirth with bare hands	Yes	63.5 (40/63)	0.714	0.67	0.08–5.51
	Never	36.5 (23/63)			
Fate of afterbirth	Throw away	70.5 (43/61)			
	Left on the spot	6.6 (4/61)	0.611	0.45	0.02–9.30
	Fed to dog	6.6 (4/61)	0.611	0.45	0.03–9.30
	Hung in trees	16.3 (10/61)	0.68	0.5	0.03–10.26

isolated from any PPD goat reactors in a previous study in Afar (Kassa et al., 2012), suggest that the burden of *M. bovis* is probably low to negligible in pastoral communities in Ethiopia. More molecular studies are warranted to assess the true burden of *M. bovis* amongst PPD reactors in pastoralist livestock as well as the involvement of Non Tuberculous Mycobacteria (NTM), *M. tuberculosis and M. caprae*.

Our study reported the highest ever documented animal brucellosis prevalence in the country, in particular in Afar settlements and goat populations, where apparent individual prevalence reached 50% depending on villages. There were also a significant higher number of recalled abortions in the Afar settlements. Unfortunately, this study did not perform any brucellosis genotyping and it cannot be concluded with certainty as whether the epidemic was caused by *M. melitensis* or *B. abortus* or both serovars. In animals, brucellosis prevalence in pastoral areas varied among published reports and livestock species, between 1.2% and 15.2% (Teshome et al., 2003; Dinka and Chala, 2009; Ashagrie et al., 2011). These studies were done in pastoral communities from Southern Ethiopia and Somali region using the Rose Bengal Plate Test (RBPT) screening. The significant difference of results could be explained by the existence of geographical hotspots for the disease and/or the different diagnostic test used. East–West disease prevalence gradient (Fig. 2) which overlapped with the ethnic background of the villages (Fig. 1) suggested a geographical association of both diseases and supports the idea of “hotspots”. This variation is however, at this stage difficult to explain since husbandry practices, breeds involved and environment were similar in all study sites. More in depth investigation on possible risk factors are warranted in the area such as animal interactions at watering points, trading system, seasonal grazing areas and migration routes/patterns of the different pastoralists groups that could shed light on this interesting observation of stark difference in disease prevalence in an area of close proximity. However, goat herds do not migrate and remain all year in and around the villages. They also tend to be shepherded separately and not communally with herds from other owners, hence favoring a lower risk of inter-herd disease transmission. During the night, goats are kept in overcrowded enclosures with continuous close physical contact, thus perpetuating infection in a pen, and intra-herd clustering of infected animals and leading to localized high prevalence.

Geographical variation of brucellosis prevalence between the Afar and Oromo communities could further be explained by the fact that pastoralists tend to trade animals with pastoralists from their own ethnicity/clan rather than with neighboring pastoralists of other ethnic groups.

Main clinical signs (Table 2) as perceived by the respondents, although not pathognomonic for Brucellosis infections alone, were highly suggestive for the existence of the disease in these communities. Accurate brucellosis diagnosis relies entirely on laboratory testing (serology, culture), and the treatment requires a minimum of a 6 week course of an antibiotic cocktail (Skalsky et al., 2008). This proves challenging for the pastoralist communities of this study site due to remoteness of settlements, lack of access to a laboratory service, lack of trust in the medical system with often unwillingness to have blood taken, as well as poor medical attention given to these communities (personal communication Health Bureau of Metahara; personal observation). Interviewees, particularly in the Oromia study sites, stated that their families very rarely visited health facilities in neighboring towns when sick and were complaining that there was no medical outreach system in place. There is to our knowledge no published information on brucellosis prevalence amongst pastoralists in Afar. The high animal brucellosis prevalence found in our study, particularly in goats as well as the handling of afterbirths and the consumption of raw goat milk, highlighted the high zoonotic risk, particularly for women and children since they generally are in charge of the goats. Lack of specific disease awareness and the existence of potential risk factors are likely to be an important contributing factor to disease transmission between animal and human.

5. Conclusions

Our study showed a very low BTB prevalence and a brucellosis individual animal prevalence varying between nil and 50% in pastoral villages across a distance of roughly 30 km with a clear West–East gradient coinciding with ethnic background. Reasons for such hotspots in a small geographical area could in this study not be explained with "classical" risk factors such as difference in husbandry practices or types of breeds involved. More research is warranted to assess risk factors in this type of setting.

Taking in account the high illiteracy grade of the respondents, the lack of knowledge of the disease, the potential risk factors for brucellosis transmission to people through raw milk consumption and animal handling, control of brucellosis in the study site will strongly benefit from a One-Health collaborative approach between the public and animal health sector, the education sector as well as direct involvement of the communities in the choice of adequate strategies in order to be accepted by them.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

RT conceived and designed the study, performed the PPD testing and blood collection in the field, analyzed the data and drafted the manuscript. SB processed samples in the field, performed laboratory analysis, helped in the revision of the manuscript. TM was involved in laboratory sample analysis and revision of the manuscript. AA and DY were involved in the conception of the study, data interpretation and the revision of the manuscript. All authors were involved in the revision of the draft.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.prevetmed.2015.03.004.

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