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Cross-sectional survey of brucellosis and associated risk factors in the livestock–wildlife interface area of *Nechisar* National Park, Ethiopia

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Abstract

A cross-sectional survey was carried out to investigate the seroprevalence of ovine and bovine brucellosis in the livestock-wildlife interface area of Nechisar National Park, Ethiopia. Furthermore, producer's knowledge about brucellosis and its zoonotic potential was assessed using a structured questionnaire. A total of 268 cattle and 246 goat sera were collected from 50 herds and 46 flocks and subjected to Rose Bengal test (RBT) and enzyme-linked immunosorbent assay (ELISA) in parallel to detect anti-Brucella species antibodies. Positive reactions were further confirmed with compliment fixation test (CFT). Flock and herd level seroprevalence rate was 12.8% (95% CI 4.8-25.7) and 32.0% (95% CI 19.5-46.7) in goats and cattle, respectively. An overall animal-level seroprevalence of 4.5% (95% CI 2.25-7.86) and 9.7% (95% CI 6.44-13.89) was recorded for goats and cattle, respectively. Seroprevalence showed an increasing trend with age, where adult cattle > 2 years. Goats (>1 year) recorded relatively higher seroprevalence, but the differences were not statistically significant. Similarly, female cattle and goats recorded a relatively higher seroprevalence, 11 and 5.6%, respectively, compared to males but the difference was not significant. However, a significant (P < 0.01) variation of seroprevalence was noted for parity (bovine), higher in animals in second parity, and abortion history, in both species, higher in animals that experienced abortion. Interviews revealed lack of awareness about brucellosis and food safety related to the zoonotic potential from consuming raw animal products (milk and meat). Ninety-eight percent of respondents did not consider handling abortion material is risky, and only a very low proportion (8%, n = 50) was able to mention limited zoonotic diseases (anthrax and Taenia cysticercosis) could be transmissible to people. The study indicated that brucellosis is endemic in domestic animals in the interface area and calls for further broad epidemiological investigation of the disease in livestock, human and wildlife following 'one health' unified research approaches beside enhancing public awareness.

Keywords Brucellosis \cdot Cattle \cdot Goats \cdot Seroprevalence \cdot Livestock-wildlife interface

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Introduction

Brucellosis is a zoonotic disease caused by *Brucella abortus* or *B. melitensis*, characterised by abortion, metritis, orchitis and epididymitis; stillbirths are leading to impaired fertility in cattle or small ruminants with significant economic losses and even pose a potential barrier for international trade. The disease is among the most common bacterial zoonoses worldwide (Pappas et al. 2006; WHO 2006) as a milk-borne zoonosis or contamination from abortion materials especially in production system where livestock and people live more

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closely and raw milk consumption is a favourite (McDermott and Arimi 2002; Refai 2002). This was mainly attributed to lifestyles, feeding habits, close contact with animals, low awareness of the transmission and poor hygienic conditions which favour infections (Schelling et al. 2003; Regassa et al. 2009). Human brucellosis has been reported to be increasing in populations with high prevalence of HIV in developing world (Faye et al. 2005).

Reliable estimates of the frequency of brucellosis among ruminants in Ethiopia are also lacking, more specifically in wildlife-livestock interface areas. The surveys conducted so far in different foci of the country established various seroprevalence levels, 1.4-10.6%, in the pastoral production system in cattle (Megersa et al. 2011a; Megersa et al. 2011b; Gumi et al. 2013). Similarly, low bovine brucellosis seroprevalence (< 5%) was reported from central Oromia Region and Sidama Zone of SNNPR (Jergefa et al. 2009; Asmare et al. 2010). The disease in smallholder dairy farms in central Ethiopia was reported to be around 11% (Kebede et al. 2008). Depending on the production system, 0.6 to 9.6% seroprevalence was reported in small ruminants (Ashenafi et al. 2007; Megersa et al. 2011a; Bekele et al. 2011; Asmare et al. 2013; Gumi et al. 2013). Study in adjacent areas to Awash National Park has documented brucellosis seroprevalence of 4.8 and 22.7% in bovine and caprine species, respectively (Tschopp et al. 2015). Nevertheless, the situation of the disease in livestock-wildlife interface areas of other national parks in Ethiopia was not duly investigated and documented. In Zambia, Muma et al. (2007a, b) has documented that herds coming into contact with wildlife had higher likelihood of acquiring infection than those without contact. Hence, the objective of the study was to establish the serological status of brucellosis in cattle and goats in livestock-wildlife interface area around the Nechisar National Park, Ethiopia. Furthermore, producer's knowledge about brucellosis, its zoonotic potential, management of herds and their observation of interaction of livestock with wildlife in the interface areas was assessed.

Materials and methods

Description of the study area and selection of villages

The study was conducted around *Nechisar* National Park, from May to June 2013, more specifically in the areas with a presumed wildlife–livestock interface. *Nechisar* is located in the Southern Nations, Nationalities, and Peoples Region (SNNPR) in the southern part of Ethiopia as part of the great East Africa Rift Valley. Its surroundings are inhabited by pastoralist and agro-pastoralist people who keep different livestock species and practice cropping. Wildlife in the park includes zebra (*Equus burchelli*), Grant's gazelle, Dik-dik, the greater kudu and the endangered Swayne's Hartebeest endemic to Ethiopia. The park has also populations of bushbuck (*Tragelaphus scriptus*), bush pig, Anubis baboon, vervet monkeys, black-backed jackal and Burchell's zebra.

There are permanent waterbodies bordering the park in the west (Abaya and Chamo lakes) and seasonal rivers in the eastern part of the park. In the study area, villages were purposely selected, in consultation with part experts and scouts, to include locations where livestock is believed to share grazing and watering sources with wild animals, particularly, during the dry season when there is limited pasture and water sources for livestock. Accordingly, all the accessible villages in the interface area were visited and animals sampled (Fig. 1).

Study design, sample size and epidemiological data collection

A cross-sectional study with purposive selection of study area and villages was chosen. Households were randomly selected while individual animals were selected systematically. From each village, 6-7 households were selected and on average, 5 animals for each species/household were sampled. Information on individual animal variables (species, age, sex and history of abortion and parity for females) were recorded separately on the sample data sheet. Additionally, herd level data including animal species kept by an individual's herd size, stock replacement history of purchases or sell of animals and management practices (related to grazing, watering, bull/sire sharing) were collected during a face-to-face interview using a pre-tested structured questionnaire. Age, gender, educational status and the knowledge or awareness of the respondents about disease with abortion syndrome, zoonotic disease and consumption habits of animal products were gathered. Potential mix of herds/flocks with wildlife at grazing and watering points, based on herder's observation, was also assessed.

Sample collection and sample testing

Blood samples were collected from selected animals to harvest serum. In total, 50 herds and 45 flocks in 7 villages were visited and sampled. To this end, 268 bovine and 246 caprine sera were collected and tested for brucellosis using two tests: Rose Bengal tests (RBT) and ELISA (SERELISA[®] Brucella OCB Ab Mono Indirect, Synbiotics corporation, Lyon, France) in parallel. The RBT was performed by mixing 30 μ L of serum with 30 μ L of antigen on a white glass plate which was agitated gently for 4 min at ambient temperature on a three-directional agitator (OIE 2004a). For caprine sera, modified RBT was performed by mixing 75 μ L of serum with 25 μ L of antigen (Blasco et al. 1994; OIE 2004b). Sample showing any degree of agglutination was considered to be

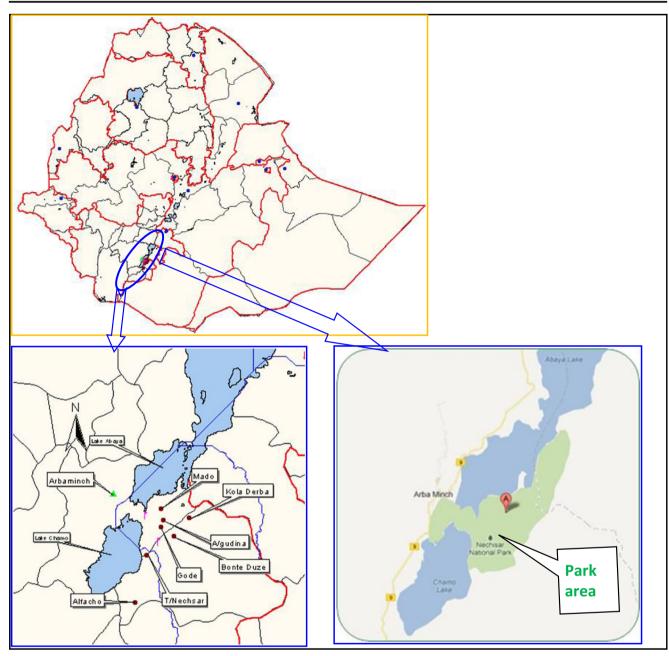


Fig. 1 Map of Ethiopia showing the study area and visited villages

positive. The tests were performed according to the manufactures instruction or standard operating procedures of the National Animal Health Diagnostic and Investigation Centre (NAHDIC). The positive sera in both RBT and ELISA tests were subjected to complement fixation test (CFT) at NAHDIC for *Brucella* spp. antibodies. Additionally, 10% of negative serum both in RBT and ELISA were tested in CFT. The "SERELISA[®] Brucella OCB Ab Mono Indirect" kit uses an indirect immunoenzymatic technique allowing the detection of *Brucella* lipopolysaccharide antibodies in individual bovine, ovine and caprine serum samples. A seropositive herd or flock was defined as one in which at least one animal tested positive in CFT.

Data analysis

Questionnaire and individual animal data were entered into an excel spreadsheet and analysed for the association of the potential risk factors with the outcome-seropositivity using STATA 11 (StataCorp, 4905 Lakeway Drive, College Station, TX 77845, USA). Data on the proportion of seropositive individual animal or herds/flocks and respondent knowledge on the brucellosis and its zoonotic potential were analysed and presented descriptively with binomial exact 95% confidence interval (CI). We used the two-sided Fisher exact test to assess the association between individual seropositivity and the categorical risk factors, and the level of significance was set at P < 0.05.

Results

Animal level

Table 1 shows animal level seroprevalence in the different study villages. The animal level seroprevalence was 4.5% (95% CI 2.3–7.9) and 9.7% (95% CI 6.4–13.9) in goats and cattle, respectively. There was no goat among the sampled animals detected to carry anti-*Brucella* antibodies, in three villages (Derba, Bonte Duze and Alfacho). Similarly, all the cattle tested in Alfacho village were found to be negative for anti-*Brucella* antibodies.

Seropositivity was observed to be relatively higher in female than that in male animals (odds ratio; OR = 2) both for cattle and goats (Table 2), but not statistically significant. Significant association (P < 0.05) between anti-*Brucella* antibodies and abortion history was observed in individual animal sampled and tested. Seropositivity was found to be higher in dry cow (16.4%, n = 268) and goats (8.0%, n = 246) than lactating or animals in other functional status (heifers or males).

Flock and herd level

Table 3 shows flock and herd level seroprevalence in the seven villages. Flock and herd level seroprevalence was 12.8% (95% CI 4.8–25.7) and 32.0% (95% CI 19.5–46.7) in goats and cattle, respectively. Interestingly, the disease showed to be more clustered in a certain flocks or herds within a village where by flocks or herds in *Tinshu Nechisar* village were more affected than others (Table 3).

Interviewee details and assessment of their knowledge

Ninety-four percent (n = 50) of the interviewed persons were males (Table 4). Education wise 60% were uneducated, i.e. never attended formal education. None of them went to a college. Only 26% (n = 50) of them were able to mention a disease, trypanosomiasis, that they believed could result in abortion (Table 4).

High proportion of respondents assumed that handling abortion materials has no risk or did not consider animal disease is transmissible to human (Table 4). But limited proportion (8%) mentioned anthrax and *taeniasis* as a transmissible disease from animals to human. Moreover, drinking raw milk is common practice in most pastoralist households (98%).

Considerable proportion of owners reported or having introduced animal as sharing bulls or sirs from outside for breeding (Table 4). Similarly, a considerable proportion of respondents (62%) reported that livestock shares common grazing and/or watering points with wildlife in the park.

Discussion

According to the livestock producers information, none of the cattle and goats from the studied areas had been vaccinated against brucellosis, implying that the antibodies detected were more likely to be due to natural infection with *Brucella* spp. Hence, the study has established that *Brucella* infection is endemic in the cattle and goats reared in the interface areas of *Nechisar* National Park.

The animal-level bovine seroprevalence recorded in this study is higher than that reported by Haileselassie et al. (2010) in Tigray Region (1.2%) and Asmare et al. (2010) in Sidama Zone (1.7%) both in extensive production system, Gumi et al. (2013) in Southeastern pastoralist zones of Somali and Oromia regions (1.4%) and Tschopp et al. (2015) from areas adjacent to Awash National Park (4.8%)

Table 1Animal levelseroprevalence for anti-Brucellaantibodies in different villagesaround Nechisar National Park,2013

Villages		Caprii	ne			Bovine			
		n	Pos	% pos	95% CI	n	Pos	% pos	95% CI
1	Gode	37	1	2.7	0.07-14.2	36	2	5.6	0.7–18.5
2	A/gudina	37	2	5.4	0.7 - 18.2	38	2	5.3	0.6-17.7
3	Derba	33	0	0		47	1	2.1	0.05-11.3
4	Bonte Duze	30	0	0		44	8	18.2	8.2-32.7
5	Alfacho	60	0	0		42	0	0	
6	T/Nechisar	19	6	31.6	12.6-56.6	31	10	32.3	16.7–51.4
7	Mado	30	2	6.7	0.8-22.1	30	3	10.0	2.1-26.5
Total		246	11	4.5		268	26	9.7	

n number of animals tested, Pos positive, CI confidence interval

 Table 2
 Brucellosis

 seroprevalence and association
 with different risk factors,

 Nechisar National Park, 2013
 Park, 2013

Variables	Category	Bovine			Caprine		
		n	% pos	Р	n	% pos	Р
Sex	Female	199	11.1	0.245	195	5.6	0.127
	Male	69	5.8		51	0.0	
Age*	1	23	4.4	0.544	40	2.5	0.490
	2	54	13.0		79	2.5	
	3	191	9.4		127	6.3	
Parity	1	36	5.6	0.007	35	0.0	0.211
	2	50	24.0		57	8.8	
	3	90	7.8		75	6.7	
Abortion	No	125	5.6	0.000	111	1.8	0.001
	Yes	51	27.5		56	14.3	
Functional status	Dry	61	16.4	0.206	75	8.0	0.182
	Lactating	115	9.6		92	4.4	
	Young female	23	4.4		28	3.6	
	Male	69	5.8		51	0.0	

n number of animals

		1	2	3
*Age categories:	cattle	6mths -2y	2-4y	>4y
	Goats	6mths -1y	1y-2y	>2y

but less than the report of Megersa et al. (2011a) from Borana pastoralist area of Yabello (10.6%). Seroprevalence in caprine is in agreement to the report of Ashenafi et al. (2007) who has documented seroprevalence of 4.5% in sheep and goats of Afar. However, it is higher than the one reported from Jijiga area (1.5%), Somali Region of Ethiopia (Bekele et al. 2011), and in Borana pastoralist area (1.5 to 1.9%) by Debassa et al. (2013) and Megersa et al. (2011a), and less than reported (9.6%) by Gumi et al. (2013) from goats in Southeastern pastoralist zones of Somali and Oromia regions and Tschopp et al. (2015) from areas adjacent to Awash National Park (22.8%). Asmare et al. (2013) also recorded an overall prevalence of

 Table 3
 Flock and herd level seroprevalence for brucellosis in different villages around Nechisar National Park, 2013

Village/name		Caprine			Bovine		
		n	Pos	%	n	Pos	%
1	Gode	7	1	14.3	7	2	28.6
2	A/gudina	7	1	14.3	7	2	28.6
3	Derba	6	0	0.0	9	1	11.1
4	Bonte Duze	6	0	0.0	7	4	57.1
5	Alfacho	11	0	0.0	8	0	0.0
6	T/Nechisar	4	3	75.0	6	5	83.3
7	Mado	6	1	16.7	6	2	33.3
Total		47	6	12.8	50	16	32.0

n number of herds

1.9% across different production systems including a prevalence of 7.6% in the pastoral production system, substantiating further that the odd of small ruminant *Brucella* seropositivity is also the highest in the pastoralist system compared to agro-pastoralist and sedentary production systems.

Overall, the seroprevalence was relatively higher for cattle sampled from the villages in the interface areas (or resident on the main land of the park) compared to those sampled from the buffer zone/areas (Kola Derba, Alfacho and Bonte Duze). The latter villages were observed to have lower number of animals per holding (on average 13 cattle/household) compared to 58 in the other villages and that they kept lesser number of goats (3 goats/household) compared to 13 in the other villages. Seropositivity was observed to increase with herd size in cattle of Tigray Region, Ethiopia (Berhe et al. 2007; Haileselassie et al. 2010). Moreover, the villages in the buffer zone are inhabited by herders that practice crop farming with limited mobility in terms of herd mix-up than those who were purely pastoralist. Earlier, the odds of brucellosis seropositivity were reported to be higher in Ethiopia in animals owned by pastoral than in animals from the mixed crop livestock system (OR = 2.8) (Tadesse 2016) and 3.5 times greater among pastoral herds than agro-pastoral herds in Nigeria (Mai et al. 2012) partly attributed to enhanced movement in the pastoralist production system for search of water and pasture. Similarly, Boukary et al. (2013), in Niger, indicated that risk of brucellosis transmission associated with transhumance.

Seropositivity was observed to be relatively higher in female than that in male animals both for cattle and goats. This Table 4Descriptive summary ofinformation on the livestockherders, herd/flock managementpractice, knowledge on zoonoticdiseases in the Nechisar NationalPark areas, 2013

Variables	Category	Number	%	Remark
Sex	Female	3	6.0	
	Male	47	94.0	
Education	Uneducated	30	60.0	
	Elementary	12	24.0	
	6-12 grade	8	16.0	
	>12 grade	0	0.0	
Know-abortion cause (disease)	No	37	74.0	
	Yes	13	26.0	Trypanosomiasis
Consider abortion material risky	No	49	98.0	
	Yes	1	2.0	
Animal diseases-transmissible	No	46	92.0	
	Yes	4	8.0	Anthrax, cysticercosis
Drink raw milk	No	1	2.0	
	Yes	49	98.0	
Eat raw meat	No	3	6.0	
	Yes	47	94.0	
Had introduced animals from outside	No	36	72.0	
	Yes	14	28.0	
Sold livestock	No	26	52.0	
	Yes	24	48.0	
Share bull	No	22	44.9	
	Yes	27	55.1	
Share sire	No	9	23.7	
	Yes	29	76.3	
Share grazing area with other herd/flock	No	10	20.0	
	Yes	40	80.0	
Share watering area with other herd/flock	No	5	10.0	
-	Yes	45	90.0	
Wildlife share water points with LS	No	16	32.0	
*	Yes	34	68.0	
Wild-life graze on same field	No	19	38.0	
-	Yes	31	62.0	

is in agreement with reports in Cameroon and Nigeria that showed significantly higher prevalence in females than in males (Bayemi et al. 2009; Junaidu et al. 2008). But Tadesse (2016) argued, in a meta-analysis of several studies of brucellosis in Ethiopia, that the tendency of females to be slightly more seropositive than males could have been due to sampling bias rather than the effect of sex per se. Indeed, females are kept longer than males, so that the population of females was generally higher than that of males and more females were sampled. In a large cattle seroprevalence survey in the states of Nigeria, Mai et al (2012) observed a significantly higher seroprevalence in males (38.2%) than in females (24.7%) and in non-pregnant females (27.8%) than in pregnant females (17.2%).

Additionally, the proportion of animals with anti-*Brucella* antibodies was found to have increased with age and parity,

where older animals and female in the second parity had high percentage of seroreactor for both cattle and goats. This could be related to the likelihood of exposure to *Brucella* infections as animal age advances as well as animals we going through repeated breeding cycle before culled from herds or flocks. On top of this observation, seropositivity was also found to be relatively higher in dry cow (16.4%, n = 268) and goats (8.0%, n = 246) than in lactating ones or in animals in other functional status (heifers or males). This is in agreement with the cattle study in Nigeria (Mai et al. 2012) and could be attributed to the absence of culling of infected animals that might lead to development of chronic infection in animals.

Interestingly, a significant association between anti-*Brucella* antibodies and abortion history was observed in individual animal sampled and tested, in agreement with observations in Ethiopia (Haileselassie et al. 2010; Alemu et al. 2014), among traditional animals reared in proximity in the Kafu flat of Zambia (Muma et al. 2007b) and in the Zambian side of greater Limpopo Transfrontier conservational area (Gomo et al. 2012). These suggest that *Brucella* is one of the principal factors responsible for abortion of the animals in the study area. Nevertheless, about 26% (n = 50) of the interviewed owners have indicated that trypanosomiasis is the disease claimed to have caused abortion in their livestock. As the area is one of the known endemic areas for tsetse-transmitted trypanosomiasis, their assertion is acceptable and hence, abortion might also be due to concurrent effect of several diseases.

The disease showed to be more clustered in a certain herds or flocks within a village where by herds or flocks in *Tinshu Nechisar* village were more affected than others (Table 3). Similarly, Tschopp et al. (2015) observed that the disease was more clustered in a certain villages than the others what was explained by the possible existence of geographical 'hot spots' for brucellosis. The small data set of 50 herds meant that any slight changes in any variable could have affected the results of the analysis. Further, the number of samples taken from each herd may have influenced the classification of herds as positive or negative.

The villages with higher proportion of seropositivity are actually located in the main lands of the park areas, and the likelihood of sharing grazing area and water with wildlife is higher than for other villages located in the peripheral areas. Over 58% of the owners mentioned that their livestock shared either watering points or grazing fields with wildlife. Grazing in the national park or adjacent areas seems to be a risk factor increasing brucellosis infection in cattle (Table 1) since animals from villages in/near the park were found to have relatively higher seropositivity rate. Although the scope of the present study did not considered wildlife for sampling, and hence significance of wildlife in the epidemiology of brucellosis has not been adequately described, a study in Zambia showed that cattle with a history of grazing in the park recorded a significantly higher seroprevalence compared to those with no history of grazing in the park (Muma et al. 2007a) what could be attributed to a closer contact with wildlife. Similarly, Gomo et al. (2012) demonstrated that cattle with a history of grazing in the Zimbabwean side of the Great Limpopo Transfrontier conservation area (in the park) recorded a significantly (P < 0.01) higher Brucella seroprevalence (13.5%) compared to those with no history of grazing in the park (4.9%). Increased interaction between wildlife and farm animals in communal grazing systems in Uganda has been observed to increase the prevalence of Brucella reactors in goats (Kabagambe et al. 2001).

During the interviews, we learned that the knowledge of livestock owners is very much limited with regard to diseases that could cause abortion in animals and they described only trypanosomiasis as possible cause. Regarding the necessary precautionary measures that should be taken in handling abortion material, 98% did not consider handling abortion material as a risky practice. This showed obviously knowledge limitation on the possible causes of abortion in animals and zoonotic potential of animal disease to people in the study area. Similarly, Regassa et al. (2009), in the assessment of human brucellosis and knowledge, found that none of the patients with clinical disease had knowledge about brucellosis underlining limited health education in the area.

Almost all the interviewed owners reported to consume unpasteurized milk and to eat raw meat. Their knowledge and awareness with regard to associated zoonotic potential of milk and meat is very much limited so that only a very low proportion (8%) of them were able to mention zoonotic diseases (anthrax and *cysticercosis*) transmissible to people. It has been described that close contact with animal and consumption of unpasteurized milk product were associated with human brucellosis (Jennings et al. 2007; Tumwine et al 2015). Similarly, Regassa et al. (2009) indicated that in the pastoralist areas of Ethiopia (Borana and Hamer), ingestion of raw milk was associated with infection of brucellosis with increased odds (3.33). *B. abortus* has been isolated from raw and sour milk of Fulani cattle in Nigeria (Bale and Kumi-Diaka 1981).

Practices like sharing of bulls or sire among livestock producers or introduction of animals from outside for breeding purposes might also serve as disease transmission way even if the most important spread of brucellosis takes place from cow to cow, with infected cows contaminating the pasture and uninfected animals becoming infected by ingestion when grazing (Madsen 1989). In the Nigerian pastoralist production system, bull sharing was indicated as a potential risk factor for brucellosis (Mai et al. 2012).

In general, the inherent pastoralist way of live characterised by livestock movement is believed to intensify the risk of disease transmission and spread. The permanent waterbodies bordering the park in the west (Abaya and Chamo lakes) and the seasonal rivers in the eastern part of the park ideally create an interface environment favouring interactivity between livestock and wildlife.

The public health situation of brucellosis was not assessed in the present study. Nevertheless, given lack of diagnostic facilities in most of the health centres in the country, it is likely that the disease is misdiagnosed for other febrile disease. The epidemiological link between brucellosis in wildlife and brucellosis in livestock and people is widely recognised (Godfroid et al. 2013). Hence, given the limited or very little knowledge of the respondents on brucellosis and other infectious zoonotic diseases, and its impact on human health, information and health extension is important to help them understand the need to take care for themselves.

Moreover, in order to demonstrate the benefit or to suggest any kind of rational intervention for the disease control, it is highly desirable to provide up-to-date convincing quantitative epidemiological data to decision-makers regarding the seroprevalence of brucellosis infection among animals, human and wild life. To this end, it is imperative to extend such a survey to understand the disease in people targeting health centres/hospitals following the "one health approach" in collaboration with Medics. Similarly, as the current study is only a preliminary survey and as prevalence, spread and transmission dynamics of brucellosis may differ between areas, which as well may change over time, it is necessary to design a larger study that integrates livestock, wildlife (including other national parks) and human beings.

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Compliance with ethical standards

Ethical standards The manuscript does not contain clinical studies or patient data

Conflict interest The authors declare that they have no conflict of interest.

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