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Seroepidemiological study of livestock brucellosis in a pastoral region

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SUMMARY

A seroepidemiological study of *Brucella* infections in multiple livestock species in the Borana pastoral system of Ethiopia was performed between December 2007 and October 2008. A cross-sectional multi-stage sampling technique was employed to select 575 cattle, 1073 camels and 1248 goats from the target populations. Sera were collected from the animals, and serially tested using Rose Bengal test and complement fixation test. Overall prevalence and prevalence with respect to explanatory variables were established, and potential risk factors for seropositivity were analysed using a multivariable logistic regression. The results showed that 8·0% (95% CI 6·0–10·6), 1·8% (95% CI 1·1–2·8) and 1·6% (95% CI 1·0–2·5) of the tested cattle, camels and goats, respectively, had antibodies to *Brucella* antigen. Positive reactors were found in 93·8% of the villages with more frequent detection of positive cattle (93·3%) than camels (56·3%) and goats (37·5%). Risk factors identified for cattle were: keeping more livestock species at household level (OR 4·1, 95% CI 1·9–8·9), increasing age of the animal (OR 2·8, 95% CI 1·3–6·0) and wet season (OR 3·3, 95% CI 1·6–6·9). Increase in household-level species composition (OR 4·1, 95% CI 1·2–14·2) and wet season (OR 3·7, 95% CI 1·5–9·1) were found to be risk factors for seropositivity in camels and goats, respectively. Existence of more than one seroreactor animal species in most villages and association of increased livestock species composition with seropositivity may add more credence to the possibility of cross-species transmission of *Brucella* infections. Although no attempt to isolate *Brucella* spp. was made, our results suggest that cattle are more likely maintenance hosts of *Brucella abortus* which has spread to goats and camels. This should be substantiated by further isolation and identification of *Brucella* organisms to trace the source of infection and transmission dynamics in various hosts kept under mixed conditions. In conclusion, the present study suggests the need for investigating a feasible control intervention and raising public awareness on prevention methods of human exposure to brucellosis.

Key words: Brucellosis, Ethiopia, livestock, pastoral system, prevalence, risk factors.

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INTRODUCTION

Brucellosis is one of the most widespread zoonoses, mainly caused by *Brucella abortus*, *B. melitensis* or *B. suis*, and is transmitted to people from various animal species. The economic and public health impacts of the disease remain of particular concern in developing countries. It poses a barrier to trade of animals and animal products, and can seriously impair socioeconomic development of livestock owners [1]. Many developing countries with limited resources, including Ethiopia, are concerned with other priority diseases that are more significant and have not yet fully introduced programmes featuring any aspects of brucellosis intervention. The epidemiology of the disease in livestock and humans, and cost-effective prevention measures are not well understood, particularly in sub-Saharan countries [2]. Brucellosis is known to cause abortion in livestock with the subsequent excretion of a large number of organisms which are easily acquired by other animals. Hence, it remains endemic and continues to be a major public and animal health problem in this region of the world [3].

Antibodies against *Brucella* spp. have been detected in various domestic animals in Ethiopia, with large disparities between various regions, production systems, livestock species and time periods as generally seen in sub-Saharan Africa [2]. Seroprevalence records predominantly document cattle [4–8], and there are few investigations on camels [9, 10] and small ruminants [11, 12]. Despite the widespread distribution of brucellosis in animals and the close contact between humans and animals, only sparse published information is available regarding the zoonotic transmission of brucellosis in Ethiopia [13, 14]. So far, isolation and characterization of *Brucella* spp. has never been attempted in Ethiopia and all available reports are based on serological evidence. One marked limitation of brucellosis serology is that the tests used worldwide detect antibodies directed against epitopes associated with the smooth lipopolysaccharide (s-LPS) which is shared to a great extent by the different smooth *Brucella* spp. Thus, it is not possible to ascribe which *Brucella* spp. (*B. abortus, B. melitensis, B. suis*) induces antibodies in a given animal species. There are also other cross-reacting organisms that have been extensively reviewed by Corbel [15]. Therefore, in the absence of isolation of *Brucella* spp., additional information is needed in order to describe brucellosis epidemiology in husbandry systems in which several animal species, susceptible to different *Brucella* spp. are maintained together.

Animal brucellosis constitutes significant public health importance for a pastoral community where close intimacy with animals, raw milk consumption and low awareness on zoonoses facilitate zoonotic transmission of the disease. Milk is a major staple food, and is an important source of protein and vitamins for households. Raw milk, which is the mode by which almost all the pastoral community consume it, is also a source of infection with milk-borne zoonoses such as brucellosis [16]. The overall infection risk is also influenced by the pattern of *Brucella* spp. present; as *B. melitensis* often represents a more serious public health hazard than *B. abortus* [1]. To date, the occurrence of brucellosis has not been investigated in different livestock species sharing common ecozone and management under a pastoral setting in Ethiopia. The present study therefore aimed at investigating the epidemiological situations of brucellosis in the major livestock species kept together in the Borana pastoral system of Ethiopia.

MATERIALS AND METHODS

Study area

The study was conducted in Borana pastoral area, Oromia Regional state of Ethiopia. Generally, the Borana plateau represents a lowland area where altitude gently slopes from North (1650 m a.s.1.) to South (1000 m a.s1.). The area has a bimodal rain pattern with annual average precipitation ranging from 300 mm to 700 mm. The main rainy season (65% of precipitation) extends from March to May, and a minor rainy season is between mid-September and mid-November. The main dry season extends from December to February [17]. As surface water is very scarce in the area, deep wells, shallow ponds, and large machine-excavated ponds are important sources of water for both livestock and humans. Traditional wells are owned by clans while large ponds are communal and often responsible for aggregation of large numbers of animals at the water points.

The livestock production system is predominantly extensive, where animals are allowed to forage freely during day time and kept in open enclosures during the night. The major livestock species kept by households in Borana include cattle, goats, camels and sheep [18]. These livestock species share common

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grazing areas and watering points, and probably intermingle at villages although separate enclosures are used for each species. Mobile herds are often maintained together with five or more village herds to reduce labour demand, a condition which facilitates transmission of the disease from infected to susceptible herds.

The pastoral village, *Olla* in Borana, is characterized by the clustering of households with close proximity of houses in a pastoral camp. Each village we visited, varying in size between 7 and 20 households, is traditionally administered by a village chief, *Abba Olla*, who is an important contact person in facilitating cooperation between livestock owners. Keeping multiple livestock species and seasonal herd mobility are part of the dynamic nature of the pastoral production system. Livestock constitute the principal source of livelihood for Borana households. Nearly 70% of household cash revenues come from pastoral sources, mainly from livestock sales with sales from dairy products constituting only a small proportion [17].

Study design and sample size determination

The study was performed between December 2007 and October 2008. Administratively, the Borana zone is subdivided into districts, pastoral associations (PA) and villages. Yabello district was selected for convenience, considering its livestock species diversity, spatial distribution patterns of ethnic groups, existence of laboratory facilities and its central geographical location in the zone. This study involved a cross-sectional multistage sampling technique. Selection of six out of 18 PAs in Yabello, and 16 villages from a total of 80 villages was based on random sampling. However, in some cases restrictions on selection were imposed, based on the accessibility of the villages by vehicle, the proximity to roads, and the presence of the three livestock species. Briefly, a total of 37 villages with the three livestock species were listed and used as a sampling frame. Taking the minimum number of each animal species to be sampled from each village into account, it was feasible to randomly sample about half of the eligible villages. Subsequently, sampling of households was by convenience, with the assumption that there were an average of 10 households per village and 50% of them may keep two or more livestock species. Households with two or more livestock species were identified and approached for permission to sample their animals. Two of the selected villages could not be sampled due to road damage and inaccessibility, and were replaced by accessible villages. Furthermore, two camel herders and one cattle herder were uncooperative, complaining that their animals were in poor condition due to dry period feed shortage and should not be bled for serum sampling. The design was thus a mix of random selection and, by necessity, some convenience decisions which may constrain the study.

Cattle, camels and goats are the three major livestock species kept in most villages and are regarded as study animals. The average number of animal species per household was estimated to be 20 cattle, 15 goats and 10 camels with possible variation between ethnic groups [17, 18]. Factors such as presence of three animal species per village, species of animals per household, willingness of herders to cooperate and availability of herds during the visit were taken into consideration to estimate the number of each animal species to be sampled per village. Within these constraints, we aimed to sample at least 30 cattle in each village (from a finite population of 200 cattle on average); corresponding to a confidence level of 95% and expected prevalence of 10%, using the formula to detect disease. Similarly, sampling of 60 animals each in cases of camels and goats was targeted from an estimated village population of 150 goats and 100 camels with expected prevalence of 5% and a confidence level of 95% [19]. It worth noting that sampling to detect the presence of disease is fundamentally different from sampling to estimate the prevalence of disease. We assumed that if a contagious disease such as brucellosis was present in a population, it would be most unlikely that $<1\%$ of the population would be infected [19]. Based on this assumption, the required sample size for one village (a finite population) can be reasonably calculated this way. With availability of field logistic facilities, a total of 575 cattle, 1073 camels and 1248 goats were serum sampled from targeted villages. Villages were visited and sampled during the dry (December 2007 to January 2008) and wet (April to May, 2008) seasons to investigate possible seasonal effects. Cattle from one village (Bildim) had moved location and were unavailable for sampling. Some goats were also additionally sampled from villages other than those selected.

Serum sample collection and testing

For ease of access to animals and convenience to livestock owners, blood sample collection was

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performed early in the morning before the animals were taken out for grazing. Blood samples of about 10 ml were aseptically collected from cattle and camels, and about 5 ml from goats using plain tubes through jugular venepuncture. Serum was separated within 12 h of collection, transported to the laboratory using an ice box and stored at −20 °C until tested. Information on potential risk factors related to environment, animal factors, and husbandry practices was recorded separately for each animal species during blood sampling. Serum samples were tested by Rose Bengal test (RBT) using RBT antigen (Institut Pourquier, France) as a presumptive test. Briefly, RBT antigen (30 μl) was added onto a glass slide next to an equal amount of cattle or camel serum sample, but a threefold amount of goat serum sample was used. For goats, in order to improve the sensitivity of RBT it is recommended to use alternatively one volume of antigen and three volumes of serum (e.g. 25 μl with 75 μl) instead of an equal volume of each [20]. The antigen and test serum were mixed thoroughly in a plastic applicator, shaken for 4 min, and agglutination was read immediately.

RBT-positive samples were subjected to complement fixation test (CFT) as a confirmatory test at the National Veterinary Institute (NVI), Debre Zeit, Ethiopia. CFT was performed using *Brucella* antigen and control sera (positive and negative) produced by Veterinary Laboratories Agency (UK). The antigen was standardized at 1:20 working dilution (strength). Serial dilutions of test sera (1:5, 1:10, 1:20, 1:40) were prepared in microtitre plates prior to adding *Brucella* antigen, complement and 3% sensitized sheep red blood cells. The warm fixation method was used in this study by incubating serum, antigen and complement at 37 °C for 30 min. CFT was regarded positive when the reading was as partial fixation (50% haemolysis) or complete fixation (no haemolysis) at 1:10 dilution. This cut-off point, which is used by National Veterinary Institute, was taken to optimize the specificity of the test and to ensure that seropositive cases resulted from *Brucella* infection. The test was validated if the negative and positive control sera showed complete haemolysis and inhibition of haemolysis, respectively. It is also worth reiterating that serological tests developed for the detection of brucellosis in cattle, sheep and goats have not been validated in camels, and there is as yet no standard set of serological tests for the diagnosis of brucellosis in camels. In our study, the test procedure outlined for cattle was used to detect brucellosis in camels [1]. An animal was considered positive if it tested seropositive on both RBT and CFT in serial interpretation. Similarly, a herd or a village was considered seropositive when at least one animal in a herd or one of the animal species in a village tested positive.

Data collection and analysis

Putative biological and environmental factors believed to be associated with epidemiology of brucellosis were recorded. These included individual animal identification, sex, age, species, herd size and stock composition. Information on ethnic group, village size (number of households per village), study season and type of permanent water sources were also recorded. Data entry, dataset establishment and storage were performed in Microsoft Excel. All statistical analyses were performed using Stata SE 10 for Windows (StataCorp, USA). The overall seroprevalence for each livestock species was established based upon the Stata survey command with seropositivity as outcome variable of interest. A univariable analysis of association between explanatory variables and seropositivity to *Brucella* antigen was assessed using logistic regression analysis. Subsequently, a multivariable logistic regression model was established to identify risk factors associated with seroprevalence with adjustment for clustering by village. Variables with a $P<0{\cdot}25$ from univariable analysis were included in the multivariable logistic model. The final model was built using a backward-selection procedure with a likelihood-ratio test at $P=0{\cdot}05$ as variable selection criteria. Prior to building a final model, variables were tested for interaction effects using cross-product terms and any collinearity using a multicollinearity matrix index. The validity of the model to the observed data was assessed by computing the Hosmer–Lemeshow goodness-of-fit test using a default approach of grouping the dataset into 10 categories. The ability of the model to predict brucellosis seropositivity was assessed by establishing the receiver operating characteristic (ROC) procedure.

RESULTS

Out of 575 cattle, 1073 camel and 1248 goat serum samples screened by RBT, 54 (9·4%), 23 (2·1%), and 25 (2·0%), respectively, were found to be seropositive. With subsequent serial testing, the overall animal-level seroprevalences were 8·0% (95% CI 6·0–10·6), 1·8% (95% CI 1·1–2·8) and 1·6% (95% CI 1·0–2·5),

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Table 1. *Village-level seropositivity to* Brucella *infection by animal species in Borana, Ethiopia*

	Cattle		Camels		Goats	
Sampled villages	Animal no. (%)*	Herd no. (%)	Animal no. (%)	Herd no. (%)	Animal no. (%)	Flock no. (%)
Aradaya'a	24 (0·0)	3 (0·0)	59 (0·0)	3 (0·0)	49 (2·0)	5 (20·0)
Bake	25 (12·0)	3 (66·7)	47 (4·3)	7 (28·6)	38 (0·0)	2 (0·0)
Bernyole	41 (2·4)	4 (25·0)	86 (5·8)	6 (50·0)	53 (0·0)	2 (0·0)
Bildim	—	—	105 (0·0)	9 (0·0)	19 (0·0)	1 (0·0)
Boya	22 (18·2)	3 (100)	128 (0·8)	16 (6·3)	33 (9·1)	3 (33·3)
Cholkasa	39 (2·6)	4 (25·0)	74 (1·4)	9 (11·1)	22 (0·0)	1 (0·0)
Dartu	45 (4·4)	5 (20·0)	119 (1·7)	14 (14·3)	19 (0·0)	2 (0·0)
Didahara	66 (3·0)	6 (33·3)	22 (0·0)	2 (0·0)	42 (0·0)	3 (0·0)
Didayabello	60 (6·7)	4 (75·0)	25 (0·0)	2 (0·0)	77 (5·2)	4 (25·0)
Gotu	33 (9·0)	2 (50·0)	33 (6·1)	6 (33·3)	29 (3·4)	3 (33·3)
Jijido	45 (2·2)	3 (33·3)	47 (2·1)	4 (25·0)	54 (0·0)	4 (25·0)
Kadale	49 (14·3)	5 (80·0)	68 (0·0)	6 (0·0)	20 (0·0)	1 (0·0)
Kella	45 (8·9)	6 (33·3)	27 (3·7)	2 (50·0)	69 (5·8)	5 (25·0)
Kellasora	24 (12·5)	2 (100)	55 (0·0)	5 (0·0)	30 (0·0)	4 (0·0)
Korke	35 (20·0)	4 (100)	95 (4·4)	6 (50·0)	75 (0·0)	6 (0·0)
Surupa	45 (8·9)	2 (100)	83 (0·0)	10 (0·0)	19 (0·0)	1 (0·0)

* No. is number of animals or herds sampled per village; (%) is percent of positive samples.

respectively, for cattle, camels and goats. The herd-level prevalence was 51·7% (30/58) for cattle, 15·0% (16/107) for camels and 13·3% (13/98) for goats. The mean within-herd prevalence was 15·5% (range 4·8–50·0%) for cattle, 8·9% (4·4–33·3%) for camels and 10·5% (5·0–25%) for goats.

Table 1 illustrates village-level seropositivity to *Brucella* infection by animal species. Seropositive animals were found in 93·8% (15/16), 43·8% (7/16) and 18·8% (3/16) of the villages with at least one, two and all three positive animal species, respectively. Village-level seropositive reactors were more frequently detected in cattle (93·3%) than in camels (56·3%) and goats (37·5%). The average number of positive animals per positive herd was generally low and comparable in the three species, cattle (1·5), goats (1·5) and camels (1·2), suggesting a slow within-herd spread of the disease.

Table 2 shows association of individual explanatory variables with respect to seropositivity in each species. More variables were found to be associated with seropositivity to *Brucella* antigens in cattle compared to camels and goats.

The results of multivariable logistic regression analysis are presented by Table 3. The results show that age (OR 2·8, 95% CI 1·3–6·0), livestock species composition (OR 4·1, 95% CI 1·9–8·9) and wet season (OR 3·3, 95% CI 1·6–6·9) were the major risk factors for cattle seropositivity to *Brucella* antigen. Seropositivity was found to significantly increase with age, with higher prevalences recorded in mature than young cattle. Cattle kept with multiple livestock species were fourfold more likely to be seropositive than those kept together with less (one) animal species. As the wet season occurs concurrently with parturition time of the animals, this variable is linked to increased parturition or abortion with likely excretion of *Brucella* organisms that could facilitate transmission and exposure to the pathogen. Unlike in cattle, only one factor for each was found to show association with seropositivity in camels and goats. Increase in household-level species composition (OR 4·1, 95% CI 1·2–14·2) was the risk factor in camels while wet season (OR 3·7, 95% CI 1·5–9·1) was found to be associated with seropositivity in goats.

The Hosmer–Lemeshow goodness-of-fit test showed that the model fitted the data (cattle: $\chi^2 = 14{\cdot}8$, $P = 0{\cdot}071$; camels: $\chi^2 = 2{\cdot}45$, $P = 0{\cdot}118$; goats: $\chi^2 = 1{\cdot}01$, $P = 0{\cdot}315$). The ability of the model to rationally predict occurrence of brucellosis cases, if applied to the reference population in the study area, exhibited an acceptable level of reliability (area under ROC curve $\geqslant 0{\cdot}74$).

DISCUSSION

Diagnosis of *Brucella* infection is almost exclusively based on serological methods since bacteriological

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Table 2. *Univariable analysis of explanatory variables associated with seropositivity to* Brucella *infections in cattle, camels and goats in Borana, Ethiopia*

		Cattle		Camels		Goats	
Variables	Levels	No. (%)	*P* value	No. (%)	*P* value	No. (%)	*P* value
Overall		**575 (8·0)**		**1073 (1·8)**		**1248 (1·6)**	
Herd size*	Small	277 (8·3)		510 (2·1)		582 (1·4)	
	Large	298 (7·7)	0·796	563 (1·3)	0·654	666 (1·8)	0·555
Sex	Male	135 (7·4)		188 (0·7)		277 (1·8)	
	Female	440 (8·2)	0·772	885 (1·9)	0·425	971 (1·5)	0·761
Age†	Young	241 (3·7)		309 (0·6)		385 (0·8)	
	Adult	334 (11·1)	0·002	764 (2·1)	0·096	863 (2·0)	0·135
Ethnicity	Gabra	130 (3·4)		725 (1·5)		358 (1·4)	
	Borana	445 (9·4)	0·026	348 (2·3)	0·367	890 (1·7)	0·714
Species composition‡	2	212 (4·7)		453 (0·7)		683 (1·5)	
	>2	363 (9·9)	0·030	620 (2·6)	0·029	565 (1·8)	0·669
Village size	⩽10 HH	193 (6·2)		271 (2·4)		549 (0·7)	
	>10 HH	382 (8·9)	0·265	802 (1·4)	0·915	699 (2·3)	0·039
Water point§	Small	102 (6·9)		224 (1·6)		472 (1·5)	
	Large	473 (8·2)	0·641	849 (1·7)	0·584	776 (1·7)	0·793
Season	Dry	271 (4·8)		789 (1·2)		1035 (1·1)	
	Wet	304 (10·9)	0·009	284 (2·4)	0·306	213 (4·2)	0·002

HH, Households.
Potential risk factors ($P \leqslant 0{\cdot}25$) were selected for inclusion in the multivariable model.
* Herd or flock size below median value is regarded as small and above median value as large.
† Age: young <3 years (cattle), <4 years (camel) and <1 year (goat) while above is adult.
‡ Species composition: number of livestock species kept by households.
§ Permanent water point for home-based herd: large for large ponds, and small for traditional wells or small ponds.

examination is not practicable for routine application [1, 20]. A remarkable limitation of brucellosis serology is that the tests used worldwide detect antibodies directed against epitopes associated to s-LPS, which is shared by the different *Brucella* spp. and other cross-reacting organisms such as *Yersinia enterocolitica* O:9 [15]. Thus, as no single serological test is appropriate in all epidemiological situations, the application of two tests in series is usually recommended for maximal specificity [15, 20, 21]. When test specificities are conditionally independent of each other, the resulting expected specificity of serial testing is said be higher than the corresponding individual specificities of each test [19]. Application of series testing in diseased populations maximizes specificity and positive predictive values, but may have the risk of missing true positive cases. Given the serial nature of the testing, it is not possible to exclude that some RBT-negative animals may be positive by CFT and/or c-ELISA. Conversely, serial testing using pairs of specificity-correlated serological tests (RBT, CFT, c-ELISA) has been argued to have lower specificity than expected when applied to disease-free populations [22]. When such a test is applied to a low disease prevalence (<1%) or disease-free population, the positive predictive value of the test falls closer to zero and the increased proportion of non-infected animals are classified as seropositive [19, 22]. Test cut-offs have different diagnostic goals depending on their context, e.g. a screening situation *vs.* a confirmatory diagnostic situation; where a diagnostic cut-off is selected is always a trade-off between false negatives and false positives, due to the overlap between normal and diseased populations [19]. In this study, the cut-off point used may increase the specificity of the test thereby ensuring that seropositive cases are resulting from *Brucella* infection, but may have the shortcoming of missing positive cases.

The present study documents serological evidence of *Brucella* infections in the animals kept for milk production under a pastoral system in Borana. The recorded higher prevalence in cattle (8·0%) compared to camels (1·8%) and goats (1·6%), is consistent with the serosurvey findings of brucellosis in different livestock species sharing the same ecosystem. Similar patterns of brucellosis seroprevalence were reported from pastoral camps in Chad, as being higher in cattle (7·0%), lower in camels (0·4%) with

Table 3. *Multivariable logistic regression analysis of explanatory variables associated with seropositivity to* Brucella *infections in cattle, camels and goats* (*adjusted for clustering by village*)

Variables	Levels	OR	95% CI	*P* value
Cattle				
Age	Young	1·0	—	
	Adult	2·8	1·3–6·0	0·009
Species composition	2	1·0	—	
	>2	4·1	1·9–8·9	0·000
Season	Dry	1·0	—	
	Wet	3·3	1·6–6·9	0·001
Camels				
Age	Young	1·0	—	
	Adult	3·7	0·8–16·0	0·084
Species composition	2	1·0	—	
	>2	4·1	1·2–14·2	0·037
Goats				
Season	Dry	1·0	—	
	Wet	3·7	1·5–9·1	0·005
Age	Young	1·0	—	
	Adult	2·0	0·6–6·9	0·289

OR, Odds ratio; CI, confidence interval.
Hosmer–Lemeshow goodness-of-fit test data: cattle ($\chi^2=14{\cdot}8$, $P=0{\cdot}071$), camels ($\chi^2=2{\cdot}45$, $P=0{\cdot}118$), goats ($\chi^2=1{\cdot}01$, $P=0{\cdot}315$).

the absence of seroreactors in small ruminants [16]. Cadmus *et al*. [23] reported a relatively higher seroprevalence in Nigerian cattle (5·8%) than goats (0·9%). In a study performed in Sudan [24], seroprevalence was higher in cattle and camels than sheep and goats. Comparable seroprevalence reports were also obtained from different ruminant species in Eritrea [25] and Egypt [26]. Conversely, records of higher seroprevalence were documented in camels and goats from Middle East areas [27–29]. These results suggest that prevalence in different species of animals sharing a common ecosystem could vary from region to region depending on the presence of *B. abortus* and *B. melitensis*, and their respective preferential hosts, i.e. cattle and small ruminants, respectively.

In one village (Aradaya'a) only a seropositive goat was found, while no positive case was detected in 24 cattle and 59 camel samples (Table 1). This could be explained by either a false-positive result linked to the imperfect specificity of the test or absence of seropositive cases in cattle and camels due to the small sample size of tested animals. Since a serial testing method was applied to enhance specificity, the latter justification seems to be more plausible. Furthermore, the mobile nature of pastoral herds or animals may also lead to the assumption that an infected animal or flock might have been introduced to the village recently, so that only one seroreactor animal was detected.

In pastoral and agropastoral systems, seroprevalence of bovine brucellosis is often greater than 5% [2, 4, 30], while prevalence is generally low in pastoral camels [31]. *B. abortus* has been isolated from cattle in different African countries [32]. On the contrary, only sparse information exists on the isolation of *B. melitensis* from small ruminants in sub-Saharan Africa for the last decades [32]. Indeed, *B. melitensis* biovar 3 was isolated from a testicular hygroma in a ram from a nomadic flock of sheep, and in goats serologically positive for brucellosis and with a history of occasional abortions in Western Sudan [33], whereas three outbreaks (in 1965, 1989, 1994–1996) of *B. melitensis* have been recorded in goats and sheep in South Africa [34]. In camels, the occurrence of *B. melitensis* or *B. abortus* was found to be linked to their contacts with the preferential hosts of the pathogens, i.e. small ruminants and cattle, respectively [7, 28, 29, 31, 35].

Although, this study made no attempt to isolate *Brucella* spp., it is of note, based on serological records, that higher prevalence in cattle than in goats or camels is most likely due to the fact that *B. abortus* is present in cattle and might have spilled over to goats and camels. In classical brucellosis (i.e. *B. abortus* in cattle, *B. melitensis* in sheep and goats, *B. suis* in pigs) where control measures are not in place, a state of endemicity is reached at the herd or flock level which is characterized by a high seroprevalence [3]. However, in cases of spillover from the preferential host to the accidental host such a state of endemicity is not likely to be reached in the accidental host and, thus, a low seroprevalence record is anticipated. This presumption can be augmented by the findings of *B. abortus* infection in sheep in Nigeria [36], *B. melitensis* infection in cattle in France [37] and *B. suis* infection in cattle in Denmark [38], which have been linked to the presence of infections in their preferential host reservoirs: cattle, small ruminants, and hares (*Lepus europeanus*), respectively.

Our seroprevalence finding of 8·0% in cattle (Table 2) closely agrees with the findings of 11·0% in Ethiopia [5], 5·8% in Nigeria [23], 5·0% in Egypt [26] and 7·0% in Chad [16]. However, it is higher than most of the previous reports from mixed

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farming systems in Ethiopia [4, 6–8]. The higher seroprevalence of the present study could be attributed to the nature of pastoral herds; large herd size, high herd mobility and diverse species composition.

Our finding of low seroprevalence (1·8%) of camel brucellosis is also in line with the findings of different authors from pastoral camels in Ethiopia [9, 10], Eritrea [25], Chad [16] and Somalia [39]. In contrast, higher seroprevalences than the present finding were reported in slaughter camels from Egypt [40], Nigeria [41] and Sudan [24] which could be due to increased age of slaughter animals. Relatively higher seroprevalences (12·1% and 15·8%) were also recorded from camels in Jordan [28, 29]. Prevalence was also found to be high in camels kept with cattle, sheep and goat in Sudan [35] and in camels in contact with small ruminants in Jordan [28]. This was further substantiated by isolation of *B. melitensis* biotype 3 [29, 35] and *B. abortus* biotype 6 [35] from camel samples. The prevalence status of brucellosis in camels, therefore, appears to depend much on husbandry practices and the transmission of infection from maintenance hosts for *B. melitensis* and *B. abortus* sharing the same habitat [31, 42].

In the present study, seroprevalence of caprine brucellosis was generally low (1·6%) and comparable with the findings of other authors from Ethiopia [11], Nigeria [23] and Eritrea [25]. However, some authors, reported a relatively higher prevalence of 5·8% from Ethiopia [12] and even much higher prevalence of 27·7% from Jordan [27] compared to our finding. Such contrasting results are mainly related to differences in husbandry practices as well as the *Brucella* spp. involved. Indeed, seroprevalence is higher in areas like the Middle East where goats infected with *B. melitensis* are kept in large flocks, a condition that favours the spread of infection [27]. Infection due to *B. abortus* occurs less frequently in goats and may result in low prevalence [1] although abortion due to *B. abortus* has been documented under an experimental condition [43].

The observed significant association between increased livestock species composition at household level and seropositivity in cattle and camels substantiates the existence of cross-species transmission of *Brucella* infection (Table 3). Keeping small ruminants with cattle or camels was reported by different authors to be a risk factor for brucellosis transmission between different animal species [28, 35, 44, 45]. Thus, animal-to-animal contact, owing to increase livestock composition at pastoral villages or households, play a considerable role in the spread of *Brucella* infections within and between animal species sharing a common environment.

A significant association of season with seropositivity to *Brucella* antigen results from concurrent occurrence of parturition along with increased excretion of *Brucella* organisms into the environment. The breeding cycle (parturition or abortions) in pastoral areas is often naturally synchronized with wet season feed availability, a condition which facilitates contamination and maintenance of the organisms in the environment. Gul & Khan [46] described peak occurrence of a brucellosis epidemic from February to July and related the event to the months that coincide with parturition and abortion in animals.

Association of higher seropositivity with increasing age in cattle is in agreement with earlier findings [4, 5, 44, 47], and is linked to increasing susceptibility to *Brucella* infection with sexual maturity [48]. Seroprevalence may also increase with age as a result of prolonged duration of antibody responses in infected animals and prolonged exposure to infection. In traditional husbandry practice, female animals are maintained in herds over a long period of time and have ample opportunity to acquire infections. Hence, the practice of culling breeding animals with reduced reproductive performance and old age could reduce the risk of within-herd spread of brucellosis and its zoonotic hazard to humans.

Although brucellosis has been controlled or eradicated in most developed countries, many developing countries such as Ethiopia have not been able to initiate intervention measures, and the disease continues to be a major public and animal health problem. Adherence to traditional farming practices and a preference for fresh dairy products [14, 49, 50], and occupational risks [13] have been reported to be risk factors for human exposure. In our study area, close intimacy with livestock; nursing infected livestock closely, assisting during parturition without protective equipment and the tradition of raw milk consumption may facilitate zoonotic transmission of the disease.

In conclusion, the study shows that antibodies to *Brucella* organisms are prevalent in cattle, camels and goats, and different explanatory variables were found to be associated with seropositivity. The presence of brucellosis in animals kept for milk production,

certainly poses a threat to the public health of pastoral communities. Hence, the need for investigating feasible control measures in animals and raising public awareness of prevention methods of human exposure to *Brucella* infection is becoming more evident.

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DECLARATION OF INTEREST

None.

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