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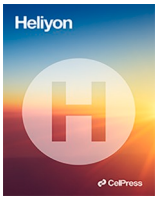

Research article

Sero-epidemiological study of brucellosis in cattle under pastoral/agro-pastoral and mixed crop-livestock systems in South Omo, southern Ethiopia

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ABSTRACT

Background: In the pastoral/agro-pastoral communities in Ethiopia, like in South Omo, brucellosis constitutes a serious health threat for livestock and the public. The public health risk is especially high in these communities, as their way of life is highly linked with their herds.
Objective: The study was conducted to estimate the seroprevalence and identify potential risk factors of cattle brucellosis in South Omo zone in southern Ethiopia.
Methods: A total of 614 traditionally managed local zebu female cattle, above six months old, were bled and data on hypothesized risk factors were collected using a semi-structured questionnaire. The preliminary screening of the sera for *Brucella* antibodies was done using Rose Bengal Plate Test (RBPT) and positive sera were further subjected to complement fixation test (CFT).
Results: The overall animal level seroprevalence of brucellosis was 2.8 % (95 % CI: 1.72–4.41) while herd level prevalence was 11.3 % (95 % CI: 6.5–19.0). Among the risk factors considered, seroprevalence was associated with herd size, new animal introduction, district, history of occurrence of abortion, and retained fetal membranes (RFM), at both individual- and herd-level ($p < 0.05$). Higher seroprevalence of brucellosis was observed in cows than heifers and in animals older than 4 years ($p < 0.05$). *Brucella* seroprevalence was higher in herds in lowland areas than those in mid-altitude and highlands ($p < 0.05$).
Conclusion: The individual and herd level prevalence observed in our study indicates endemicity of brucellosis and the potential public health threat it poses in pastoral and agro-pastoral areas of southern Ethiopia. The results of the study also suggest that the disease might be responsible for significant losses in cattle productivity due to impaired reproductive performance.

1. Introduction

Brucellosis is an important disease of animals and people with worldwide distribution. It is one of the most important zoonoses in many areas of the world, though controlled and eradicated in many countries [1–4]. In cattle brucellosis is usually caused by *Brucella*

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abortus, less frequently by *B. melitensis,* and rarely by *B. suis* [4,5]. The most important clinical signs of brucellosis in cattle are abortion at the first gestation and infertility [6,7]. Brucellosis causes loss through spontaneous abortion or birth of weak offspring, reduced milk production and infertility [8].

During abortion, billions of *Brucella* spp. Are excreted and this is a major source of infection for congeners and for people in contact with aborted materials [7]. Grazing on infected pasture, or consuming other feedstuffs and water supplies contaminated by discharges and fetal membranes from infected cows, and contact with aborted fetuses and infected newborn calves are the most common methods of spread [5]. In addition, chronically infected cattle can shed lower numbers of organisms via milk and reproductive tract discharges, and can even vertically transmit infection to subsequently born calves [3,9]. In humans, brucellosis is mainly transmitted through direct contact with infected animals, particularly when they are aborting, and consumption of infected raw milk and dairy products made of unpasteurized milk [3,10]. Airborne transmission through inhalation of infected aerosolized particles can also occur [10,11].

Serological studies suggest that brucellosis is prevalent and widespread in livestock and humans in Sub-Saharan Africa [3]. Similarly, several serological studies conducted in livestock [12–19] and humans [20–22] in different parts of Ethiopia have demonstrated the endemicity of brucellosis in the country. In a study involving human patients with febrile illness in Hammer district of South Omo zone, one of the districts bordering the study area, 29.4 % of the patients were found to be sero-positive to *Brucella* [21].

A recent meta-analysis of 20 years published data in Ethiopia has estimated the animal and herd level pooled prevalence of cattle brucellosis at 2.6 % and 16.3 %, respectively with variability along production systems and regional administrations. The prevalence being higher in the pastoral/agro-pastoral system compared to mixed crop-livestock and intensive systems [23]. In the pastoral communities brucellosis constitutes a serious public health threat because their way of life is closely interlinked with their large livestock herds [24].

Understanding the local epidemiology of a disease is vital for designing relevant control strategies. However, there is no sufficient up-to-date data regarding the status of brucellosis in South Omo zone. Therefore, the study was conducted to assess the seroprevalence of brucellosis in cattle in South Omo zone and to identify potential risk factors for exposure to the infection.

2. Materials and methods

2.1. Description of the study area

The study was conducted from December 2020 to June 2021 in Malle, Bena Tsemay and South Ari districts of South Omo zone of SNNP Region in southern Ethiopia (Fig. 1). Some of the major constraints of livestock production in the area include shortage of feed, animal diseases and poor genetic makeup of the local animals [25].

South Ari district is an intensively cultivated highland area of South Omo zone with average altitude of 1600 m. a.s.l. The district receives an average annual rainfall of 900 mm with a bimodal distribution. The mean annual temperature is 20 °C. Mixed crop-livestock farming is the livelihood of the farmers [26,27]. In mixed crop-livestock production system both crop and livestock productions are practiced, and livestock are raised on limited communal and/or private grazing areas and crop residues and stubble [28].

Malle and Bena Tsemay districts are characterized by semi-arid and arid climatic conditions, with mean annual rainfall increasing from the extreme south lower part, with some 350 mm, to the upper part where it ranges to 838 mm. The rainfall is bimodal, which occurs between September and November, and between March and May. In general, the area has an erratic, variable rainfall and high ambient temperature ranging from 26 to 35°C. The communities' livelihoods in the districts are based on pastoralism and agro-pastoralism [29]. The pastoral production system is based on extensive communal grazing while agro-pastoralists are characterized

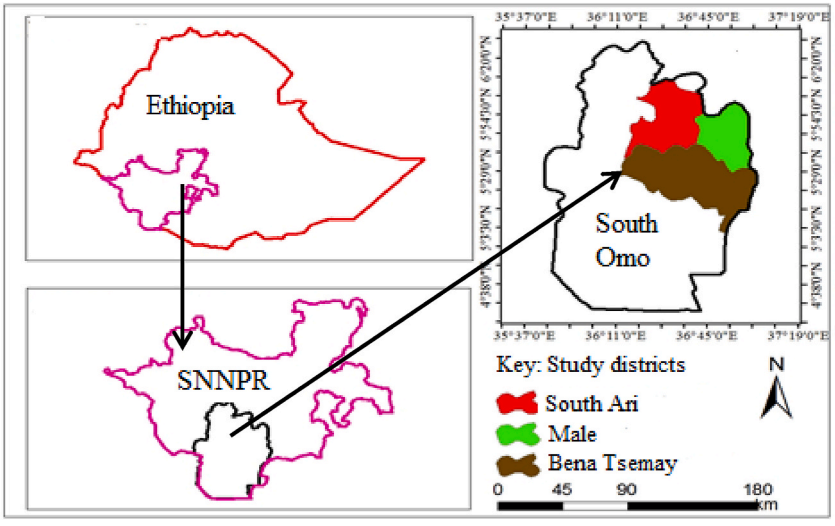

Fig. 1. Map showing the study districts.

by a combination of both pastoral and mixed crop-livestock production [28]. Pastoralists own large, mixed livestock species herds, upon which their daily livelihood depends on from a social, economic and dietary point of view [24]. Pastoralism and agro-pastoralism are generally practiced in the arid and semi-arid lowlands of Ethiopia and involve seasonal migration of pastoralists with their animals in search of grazing and water [24,30].

2.2. Study animals

The study involved 614 indigenous zebu cattle reared under traditional extensive production system where cattle are usually kept with other species of domestic animals (i.e. goats, sheep and camels). Female cattle above six month of age were included in the study. The study animals had no history of vaccination against brucellosis, as vaccination against brucellosis is not practiced in Ethiopia.

2.3. Study design, sampling and sample size

The study was a cross-sectional study conducted from December 2020 to June 2021 in three districts of South Omo zone, southern Ethiopia. Three districts (Bena Tsemay, South Ari and Malle) and 4 villages from each district (12 villages in total) were selected purposively for the study based on their relative high cattle population, Study herds (n = 106) were selected using simple random sampling method from a list of 214 herds identified in the study districts with the help of local development agents. Individual animals were selected using systematic random sampling technique as they were released from night enclosures for grazing. The 12 villages selected for the study were Gudo, Makana, Kamba-Bobo, Tike-Boko, Gurimamero, Keyafer, Dizi-Ama, Ansonda, Singal, Gazer, Baytsemali and Tolta.

The number of animals required for the study from each district was calculated using expected seroprevalence of 3.3 % [31], 95 % confidence level and 5 % desired absolute precision [32]. Accordingly, the calculated minimum sample size was 147 animals per district (441 in total). However, 614 animals were included in the study with 206 from Malle and Bena Tsemay each, and 202 from South Ari district.

Individual animal data (age, parity, reproductive status, history of abortion and retained fetal membranes) and herd level information (herd size, new animal introduction, history of abortion and retained fetal membranes) were collected using semi-structured questionnaire from herd owners.

According to the local community perception herd size was classified into three: small (1–15 heads of cattle), medium (16–30) and large ($\geq$31).

2.4. Blood sample collection

Blood samples were collected early in the morning as animals were moved out for grazing. About 10 ml of blood was drawn from the jugular vein into plain (with no anticoagulant) vacuum tubes after disinfecting the venipuncture area with 70 % ethanol. Each collected blood sample was labeled with the cow's identification information. The tubes containing the blood samples were kept in slant position overnight at room temperature for the blood to clot and contract. Serum was then collected into appropriately labeled cryo vials of 2 ml capacity using Pasteur pipettes. The sera samples were then transported to Jinka Regional Veterinary Laboratory where they were stored at −20°C.

2.5. Laboratory tests

Sera were analyzed serially for the presence of *Brucella* antibodies using Rose Bengal plate test (RBPT) for screening (Screening test) and then complement fixation test (CFT) for confirmation (Confirmatory test) following the standard procedures described by the OIE [1]. RBPT is a simple brucellosis diagnostic method, which is affordable, quick and efficient screening test [33,34]. But it generates false positive results in vaccinated animals due to its high sensitivity. CFT is widely used as confirmatory diagnosis. It is very specific, but less sensitive than RBPT. It cannot differentiate between infected and vaccinated animals, and also early stage of infection is associated with negative results due to low IgG titers [33–35].

The screening with RBPT was performed at Jinka Regional Veterinary Laboratory, and positive sera were transported to National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta, Ethiopia for CFT. Sera were always kept cold in an icebox during transportation.

To perform the RBPT, 30 μL of serum was placed onto a microscope glass slide, and an equal volume of RBPT antigen was dispensed near the serum spot. The serum and antigen were then thoroughly mixed using wooden toothpick and the mixture was manually agitated for about 4 min. Any visible colored agglutination formed was considered to be a positive reaction.

For CFT, test sera were inactivated for 30min in a water bath at 60°C. The diluted inactivated test sera were placed to the wells of standard round bottom microtitre plates. Then diluted antigens and complements were added to the wells. The plates were incubated at 37°C for 30min and sensitized sheep RBCs were added to the wells. The plates were re-incubated at 37°C for 30min and the results were read after the plates had been centrifuged at 1000 g for 10 min [1].

2.6. 2.5data management and analysis

All collected data were entered into Microsoft Excel spread sheet and checked for completeness and validity. Descriptive statistics

were used to summarize and present the data. Animal level prevalence was calculated by dividing the number of CFT positive animals to the total number of animals tested, while herd level prevalence was calculated by dividing the number of herds with at least one sero-reactor with CFT to the number of herds tested. True prevalence (TP) of *Brucella* was calculated by adjusting the AP for specificity (Sp) and sensitivity (Se) of the tests using the formula, $TP = AP - (1 - CSps)/1 - [(1 - CSes) + (1 - CSps)]$ [36], where TP represents true prevalence, AP represents apparent prevalence, CSes represents the combined sensitivity of the test series (SeRBPT × SeCFT), and CSps represents the combined specificity of the test series (1-(1-SpRBPT) × (1-SpCFT)). The sensitivity and specificity of RBPT and CFT have been reported as 0.981 and 0.960, and 0.998 and 0.998, respectively [37]. Association between sero-positivity to *Brucella* and factors considered in the study were analyzed using Pearson's chi-square or Fisher's exact test, based on the number of observations. All analyses were done using STATA version 13 statistical software (Stata Corp, College station, Texas 77,845, USA). The study considered 95 % confidence level and 5 % desired level of precision. A p value < 0.05 was considered statistically significant.

3. Results

3.1. Sero-prevalence

Out of the total of 614 animals screened with RBPT 21 (3.4 %; 95 % CI: 2.24–5.19) were found to be positive for *Brucella* antibodies. However, the subsequent confirmatory test using CFT revealed only 17 (2.8 %; 95 % CI: 1.7–4.4) were positive for brucellosis. The true animal-level prevalence estimate was 3.0 % (95 % CI: 1.8–4.7). Of the 106 herds evaluated, 12 (11.3 %; 95%CI: 6.5–19.0) had at least one seropositive animal. At district level seroprevalence ranges from 0 % for South Ari to 4.9 % (95 % CI: 2.6, 8.8) for Malle (Tables 1 and 2).

3.2. Association with risk factors

All factors considered at individual animal level, except agro-ecology, were found significantly associated ($P < 0.05$) with serostatus (Table 1). There was a tendency for increasing seroprevalence with increasing herd size, increasing age and increasing parity. Proportion of seropositive animals was higher in Bena Tsemay and Malle districts compared to South Ari. Higher seroreactor rate was observed in animals from herds with history of introduction of new animals. Significantly higher proportions of cows with history of abortion and retained fetal membranes were found seropositive to *Brucella*.

All factors considered in the analysis (herd size, new animal introduction, district, agro-ecology, history of abortion, history of retained fetal membranes) were found significantly associated ($p < 0.05$) with herd sero-status. Herds with larger size, new animal introduction, in Malle and Bena Tsemay districts, located in lowland areas, with history of abortion, and RFM were more likely to have seroreactors than their counterparts (Table 2).

4. Discussion

The overall seroprevalence of bovine brucellosis observed in this study was low (2.8 %) at individual animal level but moderately

Table 1
Association of *Brucella* serostatus with risk factors, and abortion and retained fetal membranes at individual animal level.

Variable	Category	No tested	No positive (%)	95 % CI	χ^2 -value	*p*-value
Age	<2 years	151	0 (0)	–	16.26	0.000
	2–4 years	210	2 (1.0)	0.2, 3.7		–
	>4 years	253	15 (5.9)	3.6–9.6		
Parity	Nulliparous	361	2 (0.6)	0.1, 2.2	16.22	0.000
	1–3	107	7 (6.5)	3.1, 13.1		
	>3	146	8 (5.5)	2.8, 10.6		
Herd size	Small	162	1 (0.6)	0.0, 4.3	9.14	0.015
	Medium	227	4 (1.8)	0.7, 4.6		
	Large	225	12 (5.3)	3.0, 9.2		
New animal introduction	No	551	7 (1.3)	0.6, 2.6	44.78	0.000
	Yes	63	10 (15.9)	8.7, 27.2		
District	South Ari	202	0 (0)	–	9.38	0.002
	Bena Tsemay	206	7 (3.4)	1.6, 7.0		
	Malle	206	10 (4.9)	2.6, 8.81		
Agro-ecology	Mid altitude	96	0 (0)	–	6.00	0.061
	Highland	107	1 (0.9)	0.1, 6.4		
	Lowland	411	16 (4.0)	2.4.6.3		–
Abortion	No	238	6 (2.5)	1.1, 5.5	138.99	0.000
	Yes	20	9 (45.0)	24.9, 66.9		
RFM	No	242	11 (4.5)	2.5, 8.0	55.53	0.000
	Yes	11	4 (36.4)	13.6, 67.5		
Overall		614	17 (2.8)	1.7, 4.4		

RFM: Retained fetal membranes.

Table 2
Association of *Brucella* serostatus with risk factors, and abortion and retained fetal membranes at herd level.

Variable	Category	No tested	No (%) positive	95 % CI	χ^2 -value	*p*- value
Herd size	Small	35	1 (2.9)	0.4–18.5	6.48	0.042
	Medium	40	4 (10.0)	3.7–24.3		
	Large	31	7 (22.6)	10.9–40.9		
New animal introduction	No	95	5 (5.3)	2.2–12.2	33.46	0.000
	Yes	11	7 (63.6)	32.2–86.6		
District	South Ari	39	0 (0)		7.88	0.008
	Bena Tsemay	34	6 (17.6)	8.0–34.6		
	Malle	33	6 (18.2)	8.2–35.5		
Agro-ecology	Mid altitude	17	0 (0)		7.88	0.015
	Highland	22	0 (0)			
	Lowland	67	12 (17.9)	10.3–29.2		
Abortion	No	88	5 (5.7)	2.3–13.1	16.41	0.000
	Yes	18	7 (38.9)	19.2–63.1		
RFM	No	92	7 (7.6)	3.6, 15.3	9.56	0.002
	Yes	14	5 (35.7)	15.0, 63.3		
Overall		106	12 (11.3)	6.5, 19.0		

RFM: Retained fetal membranes.

high (11.3 %) at herd level. This pattern of low individual but high herd seroprevalence is consistent with what has been described for endemic areas [6]. Studies in many areas in Ethiopia documented similar observations [12,13,19,38,39]. The individual animal level prevalence was in agreement with previous reports from different parts of Ethiopia with prevalence ranging from 2.0 to 3.2 % [12,13,38–41]. The current finding was also consistent with a 2.6 % pooled prevalence estimate obtained by a recent comprehensive review of 20 years published data in Ethiopia [23]. Similarly, comparable seroprevalence (2.9 %) was reported from Ghana (2.9 %) in West Africa [42]. On the other hand, much higher [16,19,43,44] and lower [17,45–48] seroprevalence of brucellosis were reported from different parts of the country.

The herd level seroprevalence of 11.3 % observed in the present study is moderately high and corroborates with earlier reports in the country [13,14,19,31,39,48]. However, there are some reports of much higher herd level prevalence from Ethiopia [12,38,49] and elsewhere in Africa [40,48,50]. Differences in prevalence among studies may be explained by differences in geographic locations, diagnostic tests and production systems [23]. The reported herd level prevalence could be seen as evidence to the potential of the disease to flare up when conditions promoting transmission occur [6].

In this study brucellosis was highest (5.9 %) in animals older than four years of age. This observation is in line with previous reports [12,15,19,40,45], which recorded association between older age and higher brucellosis seroprevalence. Higher prevalence of brucellosis in older cattle may be attributed to higher chance of acquiring the infection due to longer exposure time during the animals' life. As once infected, animals remain infected for life even if they can lose their antibody titer [7]. It should also be noted that steady increase in prevalence with age might indicate relatively constant infection pressure in the area [3].

The significantly higher seroprevalence observed in cows (parous) than heifers (nulliparous) in the current study is consistent with some earlier reports [51,52]. This might associate with the higher seroprevalence observed in older animals in the current study.

New animal introduction into herds was significantly associated with higher seroprevalence at the herd and individual animal level. It is highly likely that diseases are introduced to herds through incoming animals, as there is virtually no practice of screening replacement animals against any disease in the country. Purchase of infected replacement animals is one of the ways of introduction of the disease into unaffected herds [5].

Seroprevalence was higher in animals from large herds and in large herds at animal and herd level, respectively. This observation is consistent with earlier reports from various parts of the country [13,15,18,19,38,45]. The possible reason could be that larger herds are associated with a higher density of animals that facilitate close contact and environmental contaminations that lead to higher chances of transmission, especially, during cases of abortion and parturition [5].

Seroprevalence of brucellosis was higher in Malle and Bena Tsemay than South Ari District. Malle and Bena Tsemay districts are areas where pastoralism/agro-pastoralism is practiced, while South Ari is a district where intensively cultivated highland areas predominate with mixed crop-livestock production system. Our observation is consistent with reports of higher prevalence in pastoral/agro-pastoral production systems where there is extensive movement and commingling of cattle at common grazing and watering points. On the other hand, prevalence is usually low in crop-livestock system reflecting very low level of cattle to cattle contacts [3]. In agreement with earlier reports from different parts of Ethiopia [12,13], the highest proportion of seropositive herds was observed in lowland areas where pastoral management systems prevail over sedentary ones.

Higher sero-positivity was observed in herds with abortion history and animals having history of abortion. In a general agreement with the current observation various authors [12,16–18] reported significant association between abortion and sero-positivity to brucellosis. It is well established in the literature that brucellosis is characterized by reproductive disorders such as abortion, stillbirth and birth of weak offspring [5].

The individual and herd prevalence observed in the present study indicates that brucellosis continues to be endemic in cattle population in pastoral and agro-pastoral areas in southern Ethiopia. This in turn entails the significant public health risk the disease poses especially to pastoralists and agro-pastoralists that usually practice risky activities such as consumption of raw milk [24]. Our

results also suggest that brucellosis might be causing significant loss in productivity of cattle in the affected areas through impaired reproductive performance. However, to fully understand the epidemiology of *Brucella* there is a need to look for its status in other livestock species, as *Brucella* is a multi-host pathogen. Moreover, identifying the circulating biotype using microbiological or molecular techniques is indispensable to identify the primary host.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Wondimagegn Demissie: Data curation, Conceptualization. **Kassahun Asmare:** Methodology, Formal analysis. **Melaku Legesse:** Data curation, Conceptualization. **Kassaye Aragaw:** Writing – review & editing, Writing – original draft. **Desie Sheferaw:** Writing – original draft, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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