Sero-prevalence of camel brucellosis and the associated public health risks in Kereyu pastoral area of Oromia Region, Ethiopia

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Abstract

Camel brucellosis has been reported in almost all camel-rearing countries in Africa and Asia. A cross sectional study was carried out with the objective of estimating sero-prevalence of brucellosis among camels and identify risk factors associated with sero-positivity to *Brucella* infection in Kereyu pastoral area of Oromia Region of Ethiopia. A total of 324 sera were collected randomly from apparently healthy camels in Fentale district, East Shewa zone of Oromia Region, Ethiopia. Data related to risk factors such as sex, age, herd size, and herd composition were collected and camel herders were interviewed to assess their awareness about brucellosis on the sampling day. The Rose Bengal plate test (RBPT) was used as a screening test and those RBPT-positive serum samples were further confirmed by complement fixation test (CFT) and competitive enzyme-linked immunosorbent assay (c-ELISA). Out of the collected sera, 21.0% (68/324, 95% CI: 16.4 - 26.2) were positive by the RBPT and of the RBPT-positive samples, 29 (9.0%, 95% CI: 5.9-12.3) and 31 (9.6%, 95% CI: 6.5-13.0) sera samples were positive by CFT and c-ELISA, respectively. Based on c-ELISA, the animal-and herd-level prevalence in the study area were 9.6% (95% CI: 6.5-13.0) and 35.4% (95% CI: 23.1-43.5), respectively. While sex, age and livestock composition had no significant effect on the prevalence of camel brucellosis ($p > 0.05$), herd size was the major risk factor for the presence of

the disease. Accordingly, camels kept in large herds were 4 times higher at risk for *Brucella* infection than camels kept under small herd size (OR = 4.024; 95% CI: 1.667-9.716; $p < 0.005$). Out of 65 camel herders interviewed, 64 had no awareness about the zoonotic importance of brucellosis. This study demonstrated that brucellosis has a moderate occurrence in the study area and may pose health risks to the public. Therefore, there is a need for implementation of better management practices such as regular testing of all animals, typing of infecting strains, culling of positive animals and increasing public awareness on brucellosis.

Keywords: Camel brucellosis; c-ELISA; CFT; RBPT; Risk factor; Sero-prevalence.

Introduction

Global camel population is estimated to be more than 40 million with countries of East Africa, such as Ethiopia, Sudan, Kenya and Somalia having more than 60% of the world dromedary camel population (Faye, 2020). In pastoral areas of Ethiopia, camels are vital livestock with a population of approximately 4.8 million (Kena, 2022). Camels are used for income, food security, and transportation. However, their production and productivity are affected by infectious diseases, such as brucellosis, which can cause loss of newborns due to abortion, birth of weak newborns that die soon after birth, impaired fertility, decrease milk yield and loss of man-hours in infected people (Matope *et al.,* 2010; Mai *et al*., 2012; OIE, 2016). The spread of brucellosis is increasing as infected animals do not show pathognomonic symptoms and milk distributions chains in developing countries encourage supply of unprocessed milk (Wareth *et al.,* 2014) and, hence spread of diseases (Racloz *et al*., 2013).

An evidence-based conservative estimate indicated an annual global incidence of 2.1 million human brucellosis (Laine *et al*., 2023). Infected camels and their products could be a source of human brucellosis, leading to severe arthritis, fever, infertility, and in some cases, chronic infections following misdiagnosis (Liu *et al*., 2021). Most of the time, human cases are linked to direct occupational exposure to livestock in rural areas and consumption of unpasteurized dairy products in urban environments (Obradovic´ and Velic´, 2010; Perrett *et al*., 2010).

Camels are not known to be primary hosts of *Brucella.* However, they are susceptible to *B. melitensis* and *B. abortus* (Gwida *et al*., 2012). Camels become infected with *Brucella* especially when they are in contact with infected large and small ruminants. Though brucellosis is rare in camels not in contact with ruminants, it remains a concern in pastoral areas due to lack of awareness and consumption of raw milk (Islam *et al*., 2023).

Brucellosis can be diagnosed by assessing specific cell-mediated or serological responses to *Brucella* antigens. A primary test, Rose Bengal plate test (RBPT) and a confirmatory test, the complement fixation test (CFT) are widely used serum-testing procedures for the diagnosis of *Brucella* infections. Though no validation for camel sera (Gwida *et al*., 2011), serological tests used for the diagnosis of *Brucella* infection in cattle may also be adequate for the diagnosis of brucellosis in camels (OIE, 2016). Competitive enzyme-linked immunosorbent assay (c-ELISA) is also one of the commonly used confirmatory tests for brucellosis in a variety of animal species (Abdel-Hamid *et al*., 2017).

Many countries, such as the United Kingdom, Australia, Japan, as well as some countries in North and West Europe have successfully eradicated brucellosis through intensive health control measures. However, camel brucellosis remains a widespread disease in camel producing countries such as Middle East (Franc *et al*., 2018; Alrawahi *et al*., 2019; Mohamed *et al*., 2019; Abedi *et al*., 2020; Al-Sherida *et al*., 2020; Manivannan *et al*., 2021; Alhussain *et al*., 2022), West Africa (Tanimoun *et al*., 2021; Akinyemi *et al*., 2022), North Africa (Ahmed *et al*., 2015; Benfodil *et al*., 2022; Selmi *et al*., 2024), and North-East Africa (Kadle *et al*., 2017; Lokamar *et al*., 2022; Hazem *et al*., 2023; Mohammed *et al*., 2023). In East Africa, the highest rate of camel brucellosis (40% herd level prevalence) was recorded (Omer *et al*., 2010).

Brucella infections in animals and humans have been reported in Ethiopia and the findings were quite varying. Brucellosis prevalence of 2.90% (Ahad *et al*., 2024), 2.86% (Giro *et al*., 2022) and 0.90% (Gumi *et al*., 2013) were recorded using c-ELISA, indirect ELISA and ELISA, respectively, in different regions. Other reports range from 2.00% - 7.60% in different parts of Ethiopia using CFT (Megersa *et al*., 2011; Zewold and Haileselassie, 2012; Tilahun *et al*., 2013; Tassew and Kassahun, 2014; Zeru *et al*., 2016; Wegi *et al*., 2021; Waktole *et al*., 2022). Similarly, in human 3.33% (Wegi *et al*., 2021), 2.0% (Ahad *et al*., 2024), 15% (Zewold and Haileselassie, 2012), 35% (Zerfu *et al*., 2018) prevalences were reported in pastoral areas of Ethiopia by CFT. Moreover, Mehari

et al. (2021) and Ibrahim *et al.* (2021) reported 15.8% and 2.8%, respectively, in different regions. Close physical contact with animals and the tradition of consumption of unpasteurized milk are risk factors for contracting brucellosis in pastoral communities (Abbas and Agab, 2002).

There is insufficient awareness and knowledge of brucellosis among livestock owners and herders in Africa and Asia (Zhang *et al*., 2019). In some pastoral areas of Ethiopia, livestock herders had no awareness on zoonotic importance of brucellosis, usually drink raw milk and discard abortion materials in the open surroundings with minimal protection (Wegi *et al*., 2021; Tschopp *et al*., 2022). Moreover, they lack clear understanding about brucellosis as one of the diseases that cause abortion in their animals (Legesse *et al*., 2018). Generally, the tradition of mixing different livestock species (Wegi *et al*., 2021), occurrence of brucellosis in different animal species (Gumi *et al*., 2013; Ibrahim *et al*., 2021; Ahad *et al*., 2024) and habits of consuming raw milk (Wegi *et al*., 2021; Tschopp *et al*., 2022) in the pastoral areas urge the need for routine investigation of brucellosis. Hence the present study was designed to investigate sero-prevalence of camel brucellosis, identify potential risk factors for camel brucellosis and assess the awareness of Kereyu pastroalists on zoonotic brucellosis in Fentale district of Oromia Region, Ethiopia.

Materials and methods

Study area and study animals

The study was conducted in Fentale district (Figure 1). Fentale is located in the Awash lowland area of the East Shewa zone of Oromia Region, Ethiopia. The administrative center of Fentale district, Metahara town, is located 193 km east of Addis Ababa on the main highway joining Ethiopia with Djibouti. The area falls within an altitude range of 800-1100 meter above sea level (masl). The district takes its name from Mount Fentale, which has high peaks reaching elevations of up to 2,007 masl (Gedda, 2003). The Kereyu are Oromo-speaking transhumant pastoralists and are the indigenous inhabitants of the Metehara Plain and Mount Fentale areas of this district. Fentale district is currently composed of 20 kebeles, 18 rural peasant associations and two urban kebeles.

The livestock population of the district comprises about 53,682 cattle, 106,931 sheep, 129,424 goats, 20,298 camel, 13,005 equine and 6,446 poultry (Lemma and Fufa, 2023). The main watering point is the Awash River and the main

common grazing areas of the district are also in the valleys of this river. The area is affected by recurrent droughts due to disrupted rainfall patterns. The annual rainfall ranges from 400 to 700 mm. Temperature ranges from 29 to 38°C. The district lies in one of the most geologically active areas of the world (Gedda, 2003). Apart from livestock herding, the Kereyu are agro-pastoralists practicing both rain-fed and irrigated agriculture mainly as a response to the subsequent weakening of their pastoral means of livelihood (Gebre, 2001).

The study animals were non-vaccinated camels (*Camelus dromedarius*) kept under extensive husbandry. Only camels older than 6 months of age were sampled.

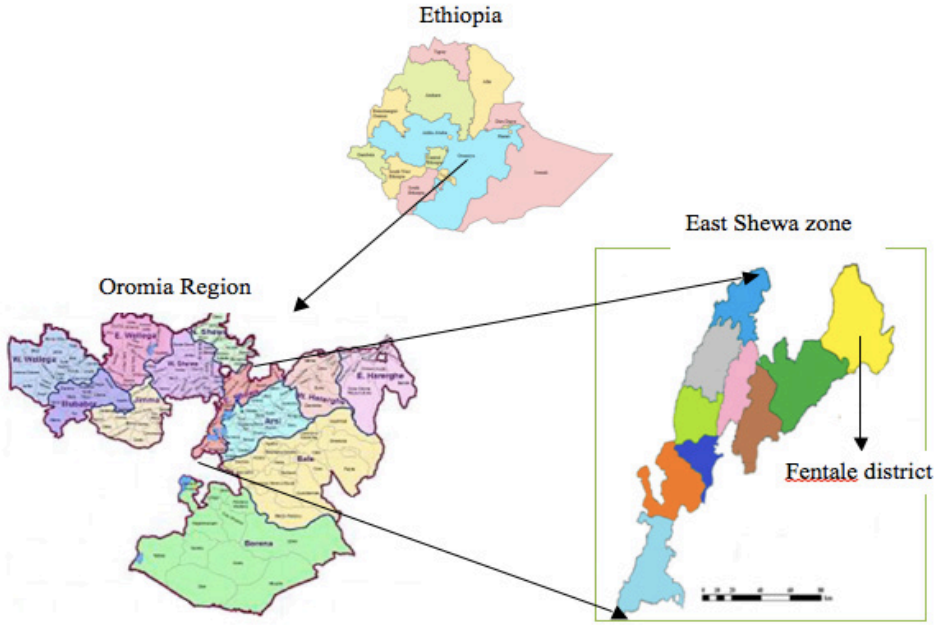

Figure 1. Map of the study area (modified and adopted from Bahru *et al*., 2021)**.**

Study design and sample size determination

A cross sectional study was conducted from December, 2018 to October, 2020 to estimate the sero-prevalence of *Brucella* infection in camels and to identify potential risk factors associated with sero-positivity in Fentale district. The sample size was determined by the formula $N = (1.96)^2 \times P \times Q/D^2$, where N =

sample size, P = expected prevalence, Q = 1-P and D = level of precision (5%) (Thrusfield, 2005). Taking the previous report of 7.6% sero-prevalence of camel brucellosis in Awash-Fentale district (Zewold and Haileselassie, 2012), the calculated sample size was, $N = (1.96)^2 \times (0.076) \times (1 - 0.076)/ (0.05)^2 = 108$. This sample size was inflated three times to account clustering of camels at household level. Hence, a total number of 324 camels were sampled.

Random sampling technique was used in selecting the households. From the 18 peasant associations (PAs) found in Fentale district, 5 PAs were selected based on accesses to transportation and security reasons. Then, a total of 65 households were randomly selected from these 5 PAs (13 households from each PA). In randomly selected households, all camels were sampled from a herd with less or equal to five animals. In herds with more than five animals, only five animals were randomly selected for sampling. All camels of both sexes above the age of six months were sampled from the randomly selected herds. Based on their age, camels were grouped into young (camels below 4 years of age) and adult (camels at 4 years of age and older) according to Ahad *et al* (2024). Moreover, camel herds were classified as small (< 20 camels), medium (21-50 camels) and large (> 50 camels) according to Gizaw *et al.* (2017) and Ahad *et al.* (2024) with some modifications.

Sample collection

About 10ml blood samples were collected from the jugular vein of each animal using plain vacutainer tubes. The blood containing tubes were given identification numbers. The blood samples were allowed to clot overnight at room temperature. The serum was separated from the clotted blood by decanting to cryovial tubes. The separated sera were stored at -20 °C until tested. Data including the owner's names, sex, age, herd size, livestock composition and other relevant information were recorded at the time of blood collection.

Serological examination

The Rose Bengal plate test (RBPT) and complement fixation test (CFT) were conducted at the National Veterinary Institute, Bishoftu, Ethiopia for the detection of camel brucellosis antibodies. All RBPT-positive sera samples were also shipped to University of Nairobi, Nairobi, Kenya and further tested using competitive enzyme-linked immunosorbent assay (c-ELISA).

Rose Bengal plate test (RBPT)

Following the procedure described by Staak *et al.* (2001), all camel sera samples were tested using RBPT at the National Veterinary Institute, Bishoftu, Ethiopia. Briefly, 30µl of RBPT antigen was mixed with equal amount of serum on a plate. The plate was rocked by hand for 4 minutes and the test was read by comparing with the positive and negative control sera by examining for agglutination in natural light. Magnifying glass was used to detect micro-agglutination. Results of RBPT were interpreted as 0, +, ++ and +++ as described by Staak *et al.* (2001). 0 = no agglutination; + = barely visible agglutination (seen by using magnifying glass); ++ = fine agglutination and +++ = coarse agglutination. Samples with no agglutination (0) were recorded as negative while those with +, ++ and +++ were recorded as positive. The results were recorded and stored in Microsoft Excel.

Complement fixation test (CFT)

All RBPT-positive samples were confirmed with a complement fixation test using the OIE protocol (OIE, 2016) at the National Veterinary Institute, Bishoftu, Ethiopia. Antigen standardization was done with dilution strength of 1:20. *Brucella* antigen, complement and 3% sensitized sheep red blood cells were added to serially diluted test sera on microtitre plates after serial dilution. The plates were incubated at 37°C for 30 minutes. At 1:10 dilution, positive results were considered for partial fixation (50% hemolysis) or complete fixation (no hemolysis). The negative control serum exhibited complete hemolysis, while the positive control serum exhibited inhibition of hemolysis.

Competitive enzyme-linked immunosorbent assay (c-ELISA)

All the RBPT-positive camel sera samples were further tested using a commercial COMPELISA 160 & 400, a competitive ELISA kit for the detection of antibodies against *Brucella* in serum samples (APHA Scientific, Weybridge, New Haw, Addlestone, Surrey, KT15 3NB, United Kingdom) at Department of Public Health, Pharmacology and Toxicology, University of Nairobi, Nairobi, Kenya. The test was conducted according to the manufacturer's protocol using 96-well polystyrene plates pre-coated with *Brucella* lipopolysaccharide antigen.

Case definition

Animals were considered positive to brucellosis when they tested positive on either RBPT/CFT or RBPT/c-ELISA tests. Similarly, a herd was considered sero-positive when at least one animal in a herd tested positive. Since there is no history of vaccination against brucellosis in Ethiopia, sero-positivity observed in this study was considered to be due to natural infection.

Questionnaire survey

During the interview, the objectives of the survey were explained to camel owners and verbal consent was obtained. A structured questionnaire format was developed and administered to camel herders in the local language (Afan Oromo). The questionnaire focused on knowledge about brucellosis and other zoonotic diseases, the habits of animal product consumption and handling, and dead animal/aborted fetus disposal practices. All 65 camel herders whose animals were bled for serological examination were interviewed.

Data management and analyses

Data obtained during sample collection and serological tests were stored in a Microsoft Excel spreadsheet (Microsoft Corp.). Sero-prevalence was calculated as a percentage by dividing the number of positive samples for *Brucella* to the total number of samples. Animal- and herd-level sero-prevalence were calculated by dividing the number of positive test results by the total number of animals and herds sampled, respectively.

The data were analyzed using SPSS 20 statistical package. The presence of associations between explanatory variables (risk factors) such as sex, age, herd size, livestock composition and positive sero-prevalence were analyzed using logistic regression. Non-collinear variable that presented p-value of < 0.25 in univariable logistic regression analysis was offered to the multivariable regression model. The degree of associations between the disease and risk factors was assessed using odds ratio (OR). A p-value of less than 0.05 was considered for statistically significant difference.

Results

Of the total 324 sera samples examined, using RBPT screening test, 68 (21.0%, 95% CI: 16.4-26.2) camels were found positive for brucellosis. However, of the

68 sero-positive reactors further tested, using CFT and c-ELISA, 29 (9.0%, 95% CI: 5.9-12.3) and 31 (9.6%, 95% CI: 6.5-13.0) camels, respectively, were confirmed to be sero-positive for brucellosis, showing that the apparent prevalence of camel brucellosis in the study area was 9.6%. The herd level sero-prevalence of brucellosis based on c-ELISA was 35.4% (95% CI: 23.1-47.7). The herd-level prevalence of antibodies against *Brucella* spp. was 18.2% (95% CI: 4.5-36.4), 16.7% (95% CI: 0.0-33.3) and 54.8% (95% CI: 38.7-71.0) in small, medium and large camel herds, respectively, using c-ELISA.

Higher prevalence in females than males (9.1% vs. 5.9% by CFT and 9.8% vs. 5.9% by c-ELISA, respectively) were recorded in this study. However, sero-positivity among the sex groups was not statistically significant ($p = 0.600$). Both CFT and c-ELISA showed higher prevalence of brucellosis in adults than young camels (10.0% vs. 7.5% by CFT and 11.6% vs. 6.7% by c-ELISA, respectively) with no statistical significance ($p = 0.147$) (Table 1).

Herd size was found statistically associated with camel brucellosis ($p < 0.05$) with higher prevalence rate in large herds (22.9%, 95% CI: 13.0-31.4), followed by medium (7.6%, 95% CI: 3.5-11.8) and small (3.6%, 95% CI: 0.9-7.3) herds using c-ELISA. Despite the fact that the highest (20.0%, 95% CI: 0.0-50.0) brucellosis sero-prevalence was found in herds composed of camel only, followed by camel-cattle (14.0%, 95% CI: 4.3-24.0), camel-small ruminant (12.0%, 95% CI: 5.1-19.0) and camel-cattle-small ruminant (6.1%, 95% CI: 2.4-9.8), livestock composition was found not statistically significant in determining sero-positivity ($p > 0.05$) (Table 1).

Table 1. Univariable logistic regression analysis of camel brucellosis by risk factors.

Risk factors	No. tested	c-ELISA positive (%, 95% CI)	Univariate OR (95% CI)	*p*-value
Sex				
Male	17	1 (5.9, 0.0-17.6)	1.733 (0.222-13.530)	0.600
Female	307	30 (9.8, 6.5-13.4)		
Age				
Young (<4 year)	134	9 (6.7, 3.0-10.4)	0.550 (0.245-1.235)	0.147
Adult (≥4 year)	190	22 (11.6, 7.4-17.3)		
Herd size				
Small (<20 camels)	110	4 (3.6, 0.9-7.3)	7.852 (2.502-24.640)	0.000
Medium (21-50 camels)	144	11 (7.6, 3.5-11.8)	3.582 (1.562-8.218)	0.003
Large (>50 camels)	70	16 (22.9, 13.0-31.4)		
Livestock composition				
Camel only	10	2 (20.0, 0.0-50.0)	0.260 (0.049-1.389)	0.115
Camel-cattle	50	7 (14.0, 4.3-24.0)	0.399 (0.143-1.110)	0.078
Camel-small rum	100	12 (12.0, 5.1-19.0)	0.476 (0.198-1.147)	0.098
Camel-cattle-small rum	164	10 (6.1, 2.4-9.8)		
Total	324	31 (9.6, 6.5-13.0)		

No.: number; OR: odds ratio; CI: confidence interval

In general, sex, age, and livestock composition had no effect on sero-positivity to brucellosis ($p > 0.05$). Herd size, however, was found to have a significant effect on the prevalence of brucellosis in individual animals. To measure the degree of association between sero-positivity and risk factors, multivariable logistic regression analysis was carried out (Table 2). The association between sero-positivity and herd size was found statistically significant ($p < 0.005$). Higher sero-positivity to brucellosis was found in large herds (22.9%) than medium (7.6%) and small (3.6%) camel herds. Accordingly, camels kept in large herds were 4 times higher at risk for *Brucella* infection than camels kept under small herd size (OR = 4.024; 95% CI: 1.667-9.716) (Table 2).

Table 2. Multivariable logistic regression analysis for the association of camel brucellosis and risk factors.

Risk factors	No. tested	c-ELISA positive (%)	OR (95% CI)	*p*-value
Age				
Young	134	9 (6.7)	2.123 (0.906-4.978)	0.083
Adult	190	22 (11.6)		
Herd size				
Small	110	4 (3.6)	8.546 (2.609-27.997)	0.000
Medium	144	11 (7.6)	4.024 (1.667-9.716)	0.002
Large	70	16 (22.9)		
Livestock composition				
Camel only	10	2 (20.0)	0.741 (0.128-4.286)	0.738
Camel-cattle	50	7 (14.0)	0.421 (0.145-1.226)	0.113
Camel-small rum	100	12 (12.0	0.429 (0.171-1.077)	0.072
Camel-cattle-small rum	164	10 (6.1		
Total	324	31 (9.6)		

The questionnaire survey revealed that 98.5% (64/65) did not know about brucellosis, and that their practices could potentially expose them to the disease. All the 65 camel herders interviewed said they usually consume whole milk without boiling. All of them informed abortion materials are handled with bare hands, and they do not safely destroy these materials. Abortion materials are usually left for dogs and wild carnivores. Milk collected from camels kept around nearby towns and cities (Olenchiti, Adama and Modjo) was being marketed at big cities such as Adama and Addis Ababa.

Discussion

The present study indicated a moderate prevalence of camel brucellosis. About 324 camel sera samples were examined for the presence of brucellosis using RBPT. All RBPT-positive sera were further tested using CFT and c-ELISA. A total of 68 (21.0%) RBPT positive, 29 (9.0%) CFT positive and 31 (9.6%) c-ELISA positive camels were found in the study area. The disparity between results of CFT and c-ELISA is due to the variation in sensitivity of the tests. For the diagnosis of brucellosis, the c-ELISA test is more sensitive, it can be used on a variety of animal species and it is suitable to use on poor-quality samples such as those affected by hemolysis as compared to CFT (Perrett *et al.*, 2010). CFT, on the other hand, is prone to prozone effect which could lead to false negative results (Corbel, 2006). Moreover, CFT is associated with negative results in

early stage of infection due to low IgG titers (Taleski, 2010; Khan *et al*., 2017; Legesse *et al*., 2023).

In Ethiopia, previous serological surveys showed that camel brucellosis is endemic and widespread (Zewold and Haileselassie, 2012; Hadush and Pal, 2013; Tilahun *et al*., 2013; Tassew and Kassahun, 2014; Zeru *et al*., 2016; Wegi *et al*., 2021; Waktole *et al*., 2022; Waji and Neja, 2023; Ahad *et al*., 2024). The 9.6% sero-prevalence recorded in current study is comparable with the 9.23% overall prevalence of camel brucellosis reported by Dadar *et al.* (2022). Similarly, Osoro *et al.* (2015) reported 11.1% sero-prevalence in camels in Marsabit County, Kenya. Zewold and Haileselassie (2012) and El-Boshy *et al.* (2009) also recorded, comparably, moderate prevalence, 7.6% in Afar region and 7.3% in Egypt, respectively. The current finding is much lower than the 17 % (Mohammed *et al*., 2023) and 23.8% (Musa *et al*., 2008) reported in Sudan, 20% (Lokamar *et al*., 2022) reported in Baringo County, Kenya and 19.4% (Dawood, 2008) reported in Jordan. However, it is higher than that of Hadush *et al.* (2013) who reported 4.1% in Afar region. Kadle *et al.* (2017) also reported a lower sero-prevalence (3.9%) in Somalia. The variation in the prevalence rates between the current finding and similar studies undertaken in Ethiopia could be due to differences in herd size, absence or presence of *Brucella*-infected herds, sample size, and sensitivity and specificity of tests used. Whereas, the observed differences in the current and related studies from other countries might be due to differences in management and husbandry practices (Al-Majali *et al*., 2008) and coverage and quality of veterinary services (Mohammed *et al*., 2023).

Brucellosis is considered as a disease of herd importance. The 35.4% (23/65) herd-level sero-prevalence of camel brucellosis recorded in this study is in agreement with the 35.1% (Al-Majali *et al*., 2008) and 31.3% (Kadle *et al*., 2017) herd-level sero-prevalence reported in Jordan and Somalia, respectively. The high herd-level sero-prevalence recorded in this study might be related to the frequent migration of camels that facilitates the sharing of grazing sites and watering points. Direct interactions between herds also increase their exposure to brucellosis.

Brucellosis prevalence of 9.77% in female and 5.88% in male camels was recorded in the present study with no statistical significance ($p > 0.05$). This statistical insignificance between sexes regarding sero-positivity is against the established fact. The small number of male camels randomly sampled, as

the camel herders in the study area were keeping very small breeding males, might predictably bias the statistical analysis.

The current finding, however, was supported by similar trends observed by Ahad *et al.* (2024) who reported higher prevalence in females (4%) than males (0.7%) and Waktole *et al.* (2022) who reported higher prevalence rate in females (9.2%) than males (7%) with no statistical significances. Brucellosis infected male animals usually show clinical signs such as epididymitis and orchitis and could be culled more quickly leading to lower brucellosis prevalence in males. Moreover, male camels are kept for short period. They are usually fed and sold off, except for few individuals that are kept for breeding and transport purposes (Salisu *et al*., 2018). On the other hand, female animals are kept for longer period for breeding purposes and have more physiological stress than males which can be considered as risk factors for brucellosis. Conversely, Bekele *et al.* (2013) reported a higher prevalence in male camels than females and this could be attributed to the differences in the proportion of male and female animals included in the study.

In this study, adult camels had a higher prevalence (11.6%) of brucellosis compared with young camels (6.7%). Similar trends were observed by Alhussain *et al.* (2022), Dadar *et al.* (2022), and Hughes and Anderson (2020). This can be explained by the fact that brucellosis is a disease of adult animals since susceptibility increases after sexual maturity and pregnancy as erythritol sugar in these animals trigger expression of virulence traits in *Brucella* (Petersen *et al*., 2013). Young animals have short-time contact with infected animals or with the environment which also contributes to the lower prevalence of brucellosis in young (Megersa *et al*., 2011). Moreover, young animals tend to be more resistant to infection and frequently clear infections, although latent infections do occur (Walker, 1999).

Sero-positivity to brucellosis was higher in large herds (22.9%) than medium (7.6%) and small herds (3.6%). The difference in the sero-positivity in the three herd categories was found to be statistically significant ($p < 0.005$). Accordingly, camels kept in large herds were 4 times higher at risk for *Brucella* infection than smaller herds (OR = 4.024). Gizaw *et al.* (2017) and Zewold and Haileselassie (2012) have shown herd size to significantly affect prevalence of brucellosis. The current finding was also in agreement with that of Omer *et al.* (2010) who reported high prevalence in large herds than medium and small herds in Sudan. However, in a study conducted by Wegi *et al.* (2021) no sta-

tistically significant difference in *Brucella* sero-positivity was observed among different herd groups of camels which disagrees with the current finding. The higher prevalence of brucellosis in large herds could be the result of easy contact among the animals in the herds favoring higher chances of brucellosis transmission (Yawoz *et al*., 2012).

Highest sero-prevalence was recorded in camels kept alone (20.0%), and no significant difference observed in the prevalence of brucellosis between camels kept alone and camels co-herded with cattle (14.0%), small ruminants (12.0%) and cattle and small ruminants (6.1%) in this study. This looks contrary to the established fact that contact of camel herds with other livestock species is a contributing risk factor to brucellosis (Al-Majali *et al*., 2008). In the study area, although milk producing camels were kept separate around cities and towns for the whole lactation period, they could come in contact with ruminants from other households at communal pasture and watering points. Besides, other non-lactating camels which usually migrate alone very far in search of feed and water could also come in contact with ruminants from other households.

Milk producing camels in the study area are kept around nearby towns and cities for the whole lactation period. This is practiced to access milk markets as far as the capital, Addis Ababa. This tradition was observed during sample collection and could also be observed by anyone through the number of camels seen along the road.

Regarding questionnaire survey, almost all interviewed camel herders (98.5%) had no clear knowledge on zoonotic importance of brucellosis and this may predispose the community to the disease. Traditions such as bare-hand handling of aborted fetuses and fetal membranes, and the consumption of raw milk are risks for the transmission of the disease. Moreover, aborted materials are left for dogs and wild carnivores, and this is likely to play a role in the transmission of brucellosis in the study area. Waji and Neja (2023) reported that over 75% of animal owners are unaware of the risks of zoonotic camel brucellosis, and over three-quarters of pastoralists engage in at least one activity that increases the risk of transmission. In a study conducted by Hassan *et al*. (2022), 13 (39.4%) brucellosis diagnosed patients had history of contact with aborted materials and 28 (23.9%) consumed undercooked meat or unpasteurized milk. Similarly, drinking raw milk from aborted animals, touching aborted materials or fetuses, and occupation were among the risk factors for human brucellosis (Dagnaw *et al*., 2024).

Limitations of this study were, some camel herders refused to allow blood sample collection from their lactating camels contending that this practice could reduce milk production. In those cases, other households from neighbors were replaced. Besides, the kebeles surveyed were limited to areas with less security concerns.

Conclusions

In conclusion, the findings of this study showed that brucellosis is prevalent in camels in the study area. Among the potential risk factors assessed, herd size was found significantly associated with *Brucella* sero-positivity. The presence of brucellosis in milk-producing camels poses a significant risk to human health as milk collected from such camels is consumed raw and even distributed to big cities like Adama and Addis Ababa. Moreover, unsafe handling and disposal of aborted materials is a common practice. Thus, further extensive epidemiological studies involving all domestic and wild animals and humans need to be undertaken in the study area. Moreover, awareness on public health importance of camel brucellosis and its prevention is quite necessary.

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Conflict of interest

The authors declare that they have no competing interest.

Ethical considerations

Ethical clearance for the study was obtained from the college research ethics committee of College of Veterinary Medicine and Agriculture, Addis Ababa University. Before sampling and data collection, the objectives of the study were explained to animal owners. Blood samples were collected following good animal handling practices to minimize animal suffering.

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