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Brucella exposure in camels and health impact in Lega Hida, Ethiopia: seroprevalence, risk factors, and public perception

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Abstract

Brucellosis is a communicable zoonotic disease that is endemic in Eastern Africa and other countries, humans contracted this infection from diseased animals. This study aimed to evaluate the seroprevalence and risk factors associated with brucellosis in dromedary camels and the knowledge, attitude, and practice of camel owners regarding brucellosis at the human-animal interface that could endanger humans in the study area. A cross-sectional study determined the seroprevalence of camel brucellosis in 404 camels. A questionnaire survey involving 120 participants was also conducted to assess the knowledge, attitude, and practice of camel owners regarding the disease. The seroprevalence of camel brucellosis was estimated as 6% by the Rose Bengal plate test and 0.5% (95% CI: 0.1-1.8) by indirect enzyme-linked immunosorbent assay (iELISA). Pastoralists are in close contact with their animals, and the consumption of raw milk and handling of abortive material is common, which exposes them to a higher risk of infection. The questionnaire survey indicated that about 40% of respondents had previous knowledge about the disease. Almost, 17% and 40% knew that the disease can be transmitted from camel to humans and between camels, respectively. About 67.5% of the respondents disposed of aborted fetuses and placenta in the open field and 86.7% of the respondents consumed raw milk. This indicates that an awareness campaign is important to equip pastoral communities in the study areas with knowledge about the disease and change their malpractice to protect themselves from brucellosis. Qualitative and quantitative research methods were used in the study. The present study revealed a low seroprevalence (0.5%). Although the seropositive animals in this study appeared to be healthy, the results indicated that the disease may manifest as a carrier and adversely affect productivity and public health.

Introduction

Within pastoral and agro-pastoral systems, camelids made significant socio-economic contributions. The main sources of income in arid and semi-arid regions of northeast, east, south, and southeast Ethiopia are the production of milk, meat, and hides, as well as transportation, draught power, and pharmaceuticals (1, 2). Nevertheless, camels are more vulnerable to various infectious and non-infectious diseases, as well as ailments related to

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reproduction and the desert, due to increased stress caused by droughts and desert conditions. The primary cause impeding production and productivity is camel brucellosis (3). Russia published the first report of brucellosis in camels in 1931 [4]. *Brucella abortus* and *Brucella melitensi* are the primary cause of brucellosis in camels. Although each brucella species is typically linked to a specific host, other species can also be infected, especially if they are kept near each other. Different findings state that milk, aborted fetal samples, and vaginal swabs from suspected camels are the most common sources of isolation for both species of brucella (5, 6).

Brucellosis is a serious disease that affects cattle, sheep, goat, and camel populations in sub-Saharan Africa. It is especially harmful to pastoralists and agro-pastoral systems, where human infection is common (7). The first case of camel brucellosis in Ethiopia was reported from the Sidamo, Harar, and Tigray regions, with a seroprevalence of 4.4% (8). Furthermore, a study on camel brucellosis was conducted in the lowlands of Borena, where the seroprevalence was 1.8% (9). Moreover, reports of brucellosis in camels from various pastoral areas have been made; the incidence varies considerably, ranging from 0.73 to 11.9% for the Rose Bengal plate test (RBPT) and from 0.53 to 9.6% for the Complement Fixation Test (CFT) (10).

The differences in animal husbandry and management techniques used by pastoral and agricultural societies are thought to cause this discrepancy in the seroprevalence of camel brucellosis (11). The size of a herd, husbandry practices, the use of maternity pens, the lack of vaccination, and common grazing and watering with mixed ruminant animals are among the management factors that influence the transmission of *Brucella* species, which can enter hosts through ingestion, inhalation, sexual contact, and broken skin (12). The presence of Erythritol in fetal tissues and the uterus causes *Brucella* species to exhibit a preferential tissue tropism for female reproductive organs. The disease is characterized by placentitis, but can also cause epididymitis and orchitis in male camels. Symptoms of the disease include late-term abortions, weak calves, stillbirths, and infertility (13, 14).

According to the World Health Organization, the Food and Agriculture Organization, and World Organization for Animal Health, the third most significant zoonotic disease worldwide is brucellosis (15). The likelihood of contracting the disease has been increased for those who come in contact with the discharge of sick animals, such as farmers, butchers, and veterinary staff (16). Among the most common illnesses in the world, brucellosis has major negative effects on the economy (17). This disease is causing large reproductive losses in sexually mature animals (18), impeding the free movement of animals, restricting the trade in cattle, and delaying exporting (17, 18). In camel production pastoral areas in Ethiopia, brucellosis is one of the diseases that must be reported when there are reproductive problems (such as delayed puberty and calving age, increased calving interval, infertility, and decreasing milk yield) (17).

Brucellosis is a zoonotic disease that can affect humans causing disastrous health and economic effects (19). It is well known that pastoral community life is dependent on livestock and their products. Significantly, the role of camels in family growth is very high in pastoral areas. Camels can survive in harsh environments, but various unknown diseases have hindered their production. Pastoralists are in close contact with their animals, and consumption of raw milk and handling of aborted materials is common making them more exposed to the risk of infection in the study area. This study aimed to evaluate the seroprevalence and risk factors related to brucellosis in dromedary camels and the knowledge, attitudes, and actions of camel owners regarding brucellosis at the human-animal interface that could endanger humans in the study area.

Material and Methods

Description of the study area

The study was conducted in the Lega Hida district in East Bale Zone, Oromia Region, Southeastern Ethiopia (figure 1; figure 2). It is located between 6º0 ’0’’ and 8º3 0’ 0” N latitude, and 40º30’0” and 41º40’0” E longitude, about 108 km from the capital of East Bale Zone and 528 Km from the capital of Ethiopia, Addis Ababa. It is bordered by Somali Regional State to the east, East and West Hararge Zone to the north, Gololcha to the west, and Sawena district to the south. The area experiences a bimodal rainfall occurring from March to the end of June and a small rainy season Usually from September to late October. The mean annual rainfall ranges from 500 to 800 mm and temperatures from 25°C and 28°C are recorded (20). The production system of the district is pastoralism. The majority of the communities in this district get income from livestock and livestock products. This district has 27 villages. Among this, the study was conducted in five villages namely Adele, Goni, Wanjisa, Hida hunda, and korba. The livestock population in the area comprises about 394,368 goats, 39,159 sheep, 293,413 cattle, 21,116 donkeys, 11,520 mules, three horses, 50,058 camels, and 25,560 poultry. Although camels can survive in a harsh environment, various unknown diseases in the region are the main obstacle to getting enough production of camels (Agriculture Office, 2023 (unpublished)).

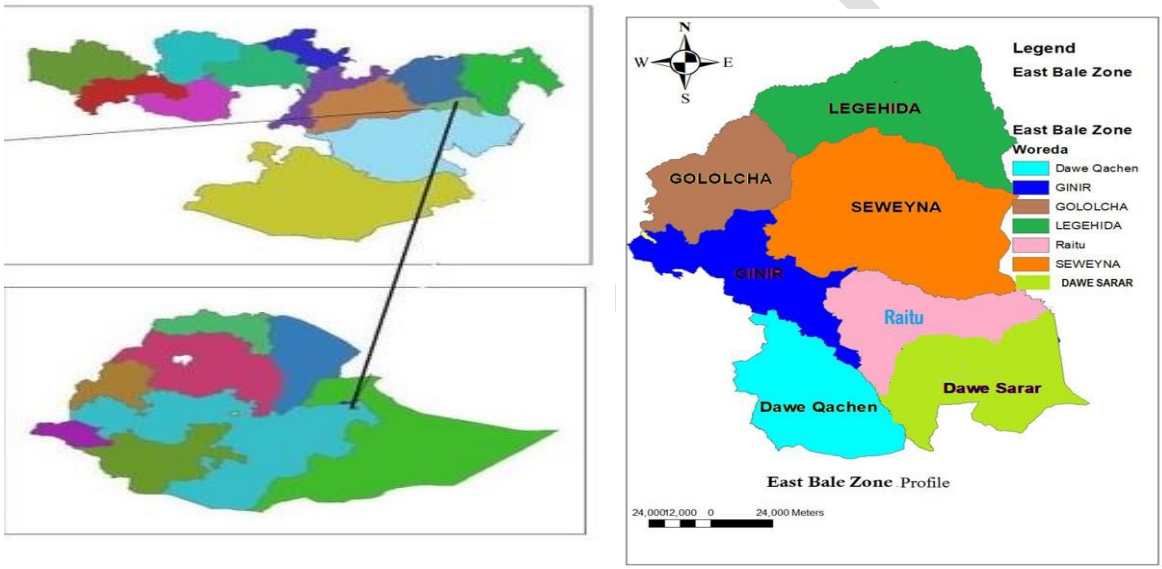

Fig. 1. Location map of east Bale zone, Oromia, Ethiopia.

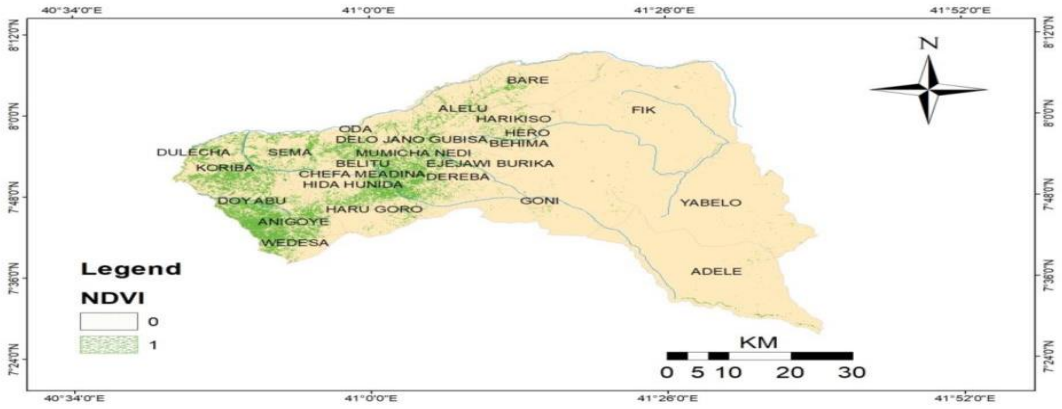

Fig. 2. Map shows Lega Hida district with its villages.

Study population and animals

Dromedary camels raised under extensive pastoral production, allowing for free browsing in Lega Hida, made up the research population. Camels above six months of age, were not vaccinated against *brucella* species and both sexes were considered. The total camel population of the district is estimated to be about 50,058. Camels' age was classified into ≤4 yrs, 4–10 yrs, and >10 yrs as young, adult, and old age groups, respectively, according to Fekadu et al. (21).

Study Design

A cross-sectional study was conducted using serological tests. The RBPT and the indirect enzyme-linked immunosorbent assay (ELISA) test were used to determine the seroprevalence of brucellosis in dromedary camels. Data were collected to determine the risk factors associated with the seropositive animals in the study area. Zone and district were selected based on the camel population, while the study villages were randomly selected.

Sample size determination and sampling technique

The sample size was determined based on the method described in Thrusfield (22). Since the seroprevalence of brucellosis in dromedary camels in the Lega Hida district had not previously been reported, the calculation took into account an absolute precision of 5% and a 95% confidence interval. The result was 50% expected seroprevalence (Pexp). The minimum number of camels required was computed using the following formula:

$$n = \frac{Z^2 Pexp\ (1-Pexp)]}{d^2}$$

$$n = \frac{(1.96)^2(0.5(1-0.5)}{0.05} = 384$$

Where;

n = required sample size

Pexp = Expected prevalence = 0.5

d=margin of error = 0.05

Z= 95% CI =1.96

The sample size increased to 404 (5%) to increase the chance of observation and estimate the wide distribution of brucellosis in the study area. Simple random sampling was used to choose the study animals. The minimum sample size needed to determine the prevalence was calculated to be 384.

Questionnaire survey

Before the interview began, the respondents verbally agreed and were informed of the survey's goals. Questionnaires were prepared first in English language and translated into the local language Afaan Oromoo. The questionnaire centered on knowledge of zoonotic diseases, attitudes toward the danger of contracting brucellosis and preventing exposure to animal birth products, and strategies for mitigating the risks of contracting diseases from animal products.

Laboratory examination

5-10 blood samples were collected from the jugular vein of camels. The collected blood samples were allowed to clot at room temperature. Then, the serum was separated and decanted into cryovial 1.7mL tubes. Collected sera were stored at -20 °C until the laboratory tests were performed using RBPT and iELISA. The serological examinations were done in the Asella Regional Veterinary Laboratory.

Rose Bengal Plate Test (RBPT)

Brucella antibodies were found in the serum samples by screening them with a standardized buffered Rose Bengal Test antigen. After being removed from the refrigerator, the sera and antigen were allowed to come to room temperature for a minimum of half an hour. Next, each plate's 12 circles received 75 µl of test sera. After giving the antigen bottle a gentle shake, a 25-µl drop of RBPT antigen was added to the serum. The Rose Bengal plate test has some limitations, including the possibility of false negative results in cases of early primary infection and late disease stages. The antigen and serum were thoroughly mixed using an applicator stick, and the plate was manually rocked for approximately 4 minutes before the agglutination reactions were read under a good light source (23).

Indirect Enzyme Linked Immuno-sorbent Assay

As directed by the manufacturer, the test was conducted using an indirect enzyme-linked immunosorbent assay (iELISA) kit to detect antibodies against brucella in serum samples. The micro-wells are diluted at a ratio of 1/20 before the specimens be tested and the controls are added. The wells were coated with purified *Brucella abortus*. Antibody-antigen complexes are formed by anti-brucella antibodies if they exist. The micro-wells are filled with a multispecies horseradish peroxidase (HRP) conjugate. It forms an antigen-antibody conjugate HRP complex by attaching itself to anti-brucella antibodies. After 30 minutes of 21°C incubation, empty the well and wash to remove any remaining excess conjugate. Next, add the substrate solution (TMB). The amount of antibody in the specimen analyzed determines the final color. The blue solution that forms in the presence of antibodies turns yellow when the stop solution is added. There is no coloration when there are no antibodies present. At 450 nm, the microplate was finally read using a spectrophotometer (manually certified by ISO 9001).

Data processing and statistical analysis

The study's data, which included questionnaire responses and serological results, were entered and saved in a Microsoft Excel® spreadsheet. Statistical analyses were performed using SPSS version 16 software to ascertain the relationship between the seroprevalence of brucellosis and related risk factors. To determine potential risk factors linked to seropositive camels, the chi-square test ($\chi 2$) was utilized (20). Statistical significance was considered for each analysis conducted during this investigation, with $p<0.05$ being the threshold for significance.

Results

Seroprevalence

A total of 404 serum samples were collected from dromedary camels in the Lega Hida district of East Bale Zone. Out of these, 2 animals (0.5%) tested positive for iELISA. During the screening test using RBPT, 25 (6%) samples were found positive for *Brucella* antibodies. In the sex category, from 145 sampled male dromedary camels 1 (0.7%) was Brucella seropositive while from 259 female dromedary camels 1 (0.4%) was seropositive. From

91 young camels none was seropositive and from 243 adult dromedary camels 1 (0.4%) was seropositive, from 70 old dromedary camels 1 (1.4%) was seropositive. Out of 59 camels, 2 (3.4%) were seropositive after the introduction of new animals to the herd. From camels with an abortion history (n = 51), camels with a history of retained placenta (10), and camels with testicular swelling (8) none were found seropositive for Brucellosis. Regarding body condition, seropositive animals were in the poor (0.7%) and medium (0.6%) body condition groups, while none were positive in the good body condition group. Seropositive animals (1.2%) were only in medium size herds.

Table 1. Seroprevalence of camel brucellosis and associated risk factors in the study area.

Variables	Categories	No. of tested camel	No. of RBPT positive (%)	No. of iELISA Positive	Prevalence
Sex	*Male*	145	8(5.51%)	1	0.7
	Female	259	17(6.56%)	1	0.4
Age	Young	91	3(3.29)	0	0
	Adult	243	12(4.93)	1	0.4
	Old	70	10(14.28)	1	1.4
Abortion history	Yes	51	8(15.68)	0	0
	No	208	7(3.36)	1	0.5
History of retained placenta	Yes	10	3(30.0)	0	0
	No	249	12(4.81)	1	0.4
Testicular swelling	Present	8	4(50.0)	0	0
	Absent	137	5(3.64)	1	0.7
Introduction of new Animal	Yes	59	6(10.16)	2	3.4
	No	345	18(5.21)	0	0
Body condition	Good	98	6(6.12	0	0
	Medium	158	11(7.0)	1	0.6
	poor	148	8(5.40)	1	0.7
Herd size	Small	128	5(5.10)	0	0
	Medium	164	15(9.5)	2	1.2
	large	112	5(3.4)	0	0

Questionnaire Survey result

The majority of the respondents were male 104/120(86.7%), 51(42.5%) of the respondents were aged between 18 - 25 years, 75.8% of the respondents were illiterate and 17.5% had the elementary educational background (Table 2). Generally, the sex, age, and educational level of the respondents were the factors to compare the knowledge gap regarding public health impacts of brucellosis.

Analysis of the knowledge gap of the respondents indicated that 48 (40.0%) of camel owners were aware of the disease and ways of its transmission. From these, 19 (39.6%) and 29 (60.4%) said the disease can be transmitted during grazing watering and in the pens, respectively. Similarly, 20(16.7%) expressed zoonotic potential of the disease. 48 (40%) of the respondents said the disease is preventable. Besides, 11(22.9%), 9(18.8%), and 28 (58.3%) said that the disease is preventable by avoiding consumption of raw milk, avoiding careless handling of aborted fetuses and retained fetal membranes (RFM) and avoiding consumption of raw meat, respectively (Table 3).

All (100%) of the respondents revealed that they needed to know more information about the disease. Most individual respondents (75%) did not believe that personnel working with camel exposed to the brucella infection are at high risk of infection and the majority of the individual respondents (57.5%)

said that they want information from veterinary institutions (Table 4).

The analysis of Practice in the study area conducted on 120 respondents shows that, 50 (41.6%) of the camel owners assist the camel at the time of calving. The majority (57.5%) of the respondent cleaned their hand with soil and plant leaves after assisting; 12 (10%) and 39 (32.5%) washed their hands properly using soap and only with water, respectively. Most (67.5%) of the respondents disposed of aborted fetuses and placenta in open field, while 26 (21.7%), and 13 (10.8%) of the camel owners gave dogs and buried the aborted fetuses and placenta, respectively. The majority (86.7%) of Camel owners consume raw milk. About 90% of respondents did not separate aborted camels from other camels, whereas 12 (10%) of them separated aborted camels from other camels. The vast majority (95.8%) of the respondent migrate their camels from place to place (Table 5).

Table 2. Demographic Characteristics of Respondents.

Variables	Categories	Frequency	Present %
Sex	Male	104	86.7
	Female	16	13.3
Age	18-25	51	42.5
	26-40	42	35.0
	41-60	27	22.5
Educational Level	Illiterate	91	75.8
	Elementary	21	17.5
	High school	8	6.7
	Diploma	0	0

Table 3. Respondents' knowledge about brucellosis in the study area

Variables	Categories	Frequency	Percent (%)
Do you know brucellosis(*gatachiisa*)	*Yes*	48	40
	No	72	60
Is brucellosis (*gatachiisa*) transmittable between camels?	Yes	48	40
	No	72	60
If yes, how does brucellosis (*gatachiisa*) transmit between camels?	Shared at the time of grazing and watering	19	39.6
	Shared in the kraal	29	60.4
Do you know brucellosis (*gatachiisa*) Can infect humans?	Yes	21	17.5
	No	99	85.5
Do you know that humans can contract this disease from animals?	Yes	20	16.7
	No	100	83.3
Is brucellosis (*gatachiisa*) a preventable disease?	Yes	48	40
	No	72	60
If yes, how?	Avoiding drinking raw milk	11	22.9
	Avoiding careless handling of aborted fetuses and RFM	9	18.8
	Avoid Consumption of raw meat	28	58.3

Table 4. Respondents' Attitudes about the disease in the study area

Attitude Questions	Categories	Frequency	Percent
Do you need to know more information about the disease?	*Yes*	120	100
	No	0	0
If yes, from which source do you want information?	Meeting in the village	24	20
	Social media	27	22.5
	Veterinary institutions	69	57.5
Do you believe that you and your family working with the camels exposed to the brucella infection are at high risk of infection?	Yes	30	25
	No	90	75
Do you believe that putting delivered camels in separate kraal is important in disease prevention?	Yes	49 (40.8%)	40.8
	No	71 (59.2%)	59.2

Table 5. Respondent practice regarding brucellosis in the study area

Variables	Categories	Frequency	Percent (%)
Do you assist the camel at the time of calving?	Yes	50	41.6
	No	70	58.3
How do you save yourself after your assistance?	Washed hands properly with soap	12	10
	Washed hands only with water	39	32.5
	Clean hands with soil and plant leaf	69	57.5
How do you manage an aborted fetus and placenta?	Burying	13	10.8
	Given to dogs	26	21.7
	Disposed at the open field	81	67.5
Do you consume raw milk?	Yes	104	86.7
	No	16	13.3
Do you consume raw meat?	Yes	32	26.7
	No	88	73.3
Do you use separate *kraal* for your camel from other animal species?	Yes	78	65
	No	42	35
Do you separate aborted camels from others?	Yes	12	10
	No	108	90
Do you use mixed grazing and watering with other animal species?	Yes	24	20
	No	96	80
Do you migrate your camels from one to another areas?	Yes	115	95.8
	No	5	4.2

Discussion

The overall seroprevalence of camel brucellosis in the Lega Hida district recorded in this study was 6% by the RBPT and 0.5% by iELISA. Even though this seroprevalence is low, the seropositive animals in this study were healthy, which indicates the disease appears as a carrier and may cause Public health and productive losses. The seroprevalence of 0.5% in the present study is close to 0.4% and 0.53% from Borena Ethiopia Pastoral Areas (24), 0.9% in southeastern Somali (Filtu) and Oromia regions (Liban) (25) and 1.2% in Borena Zone of southern Ethiopia (26). Likewise, the study in the Fafen Zone of the Somali region (1.53%) by Robayo and Esubalew (27) also slightly agreed with the current study. In contrast, it was lower than the previous serological surveys showing seroprevalence of 2.43% in Babile and Jijiga (28),

4.2% from Akaki Abattoir (24), 4.1% in Afar (29), 27.8% in eastern Sudan (30), and 23.8% in Sudan (31). The difference in seropositivity of camel brucellosis between the current and previous studies might be due to, sample size, nutritional status, animal composition, presence or absence of infection foci, and immune status of the animals.

Brucellosis can occur in animals of all age groups but frequently occurs in sexually mature animals of both sexes (32). This study showed that seropositivity was 0.4% and 1.4% in adult and old age groups respectively, whereas, no positive individuals were positive in the young age group. This is in agreement with the study conducted in the Amibara district of the Afar region (21) where seroprevalence was 1.21% in adults, 12.76% in old, and 0% in young. The overall seroprevalence in male camels (0.7%) was higher than in female camels (0.4%). This is in agreement with the study by Petros and Geremo (33), which found a seroprevalence of 4.4% in male camels and 2.7% in female camels. However, our result was in contrast to the study conducted by Asim et al. (34), which reported a higher prevalence (4.46%) in males than females (1.21%). This could be due to different reasons like sample size, immunity, and reproductive status of animals. Regarding the body condition of the camels, positive animals were in poor and medium body condition (0.7% and 0.6%), respectively, and higher prevalence was seen in poor body condition. This is consistent with the results of a study conducted in Akaki abattoir (12.4 % and 4.4%) in central Ethiopia (18) and 11.3% and 1.266% in Amibara district of Afar region, Ethiopia (21). However, the present study showed that body condition was not significantly associated with *brucella* seropositivity ($p > 0.05$). The introduction of new animals into the herds has an important role in the disease appearance in the camel population. After introducing infected animals to a herd, healthy animals come into direct contact with them and become infected. This observation is similar to the study conducted in the Puntland state of Somalia (35). The herd size of the camels is also considered in this study to see the dynamics of this disease in different herd groups since it is a disease of herd importance. The prevalence of the disease was higher in medium-sized herds (1.2%) than in small (0%) and large herds (0%). However, these differences were not statistically significant. This result is in contrast to previous studies conducted in different parts of Ethiopia, 2.9%, 3.1%, and 3.3% in the Yabelo district of the Borana zone (33), 4.76%, 2.17%, and 3.45% in the Amibara district of Afar region (21), 0.94%, 1.8% and 11.9%, in Arero and Elwoye districts of Borana zone (36). These differences of observations in the studies are difficult to explain.

According to the results of the questionnaire survey, the majority (60%) of respondents did not know about the disease. This is consistent with the study in the Amibara district in the Afar region where 90% of the respondents had no previous knowledge about brucellosis (21). In this study, 40% of the participants stated that they were aware of the disease and its transmission from animal to animal. Only 16.7% of the participants knew that the disease could be transmitted from animals to humans. About 40% of respondents said the disease was preventable. Most respondents (75%) did not believe that personnel working with camels exposed to the *Brucella* infection were at high risk of infection. This is consistent with the study conducted in urban and peri-urban areas of Tajikistan (37).

A significant proportion of camel owners (41.6) in the district supported the camel in calving. Most (67.5%) of the respondents disposed of aborted fetuses and placenta in an open field. The main predisposing factor for Brucellosis is the consumption of raw milk by 86.7% of camel owners. This is in agreement with the study conducted in Marsabit County, Kenya (82.8%) (38); and a study conducted in the Amibara district of Afar (91.7%) (21). These practices may potentially support the transmission of zoonotic diseases including brucellosis in the study area. Most (90%) of the respondents did not separate

aborted camels from other camels, while the rest 10% separated aborted camels from other camels. Almost all (95.8%) camel owners allow their camels to move from place to place for grazing and watering. This is the main factor contributing to the spread of camel brucellosis, as the congregation of animals at watering points increases contact between infected and healthy animals. Thus, favoring the spread of the disease. This result is consistent with the study from Afar, Ethiopia (39). The variation across different countries and areas in terms of Knowledge, Attitude, and Practice (KAP) may be due to differences in access to formal education, previous experience with brucellosis, health education programs, and extension services, communication and collaboration between the animal and human health (40).

Conclusion

In countries where camels are bred, camel brucellosis has a serious negative impact on human health. Eradication in endemic countries is only possible through control, prevention, and surveillance. The present study showed a lower seroprevalence. Although the seropositive animals in the study appear healthy, this indicates that the disease may act as a vector and may harm productivity and public health. In addition, pastoralists are in close contact with their animals, and the consumption of raw milk and handling of aborted material is common, putting them at higher risk of infection.

Therefore, the following recommendations need focus:

- Raising awareness of animal husbandry, disease prevention, and the risk of zoonoses is very important.
- Furthermore, comprehensive research should be carried out to investigate the link between brucellosis in humans and livestock and cross-species infection.
- Restrict regulation of camel movement from one area to another to limit the disease and common usage of camel bull to serve the females in different herds.

Acknowledgments

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Ethical approval

The Hawassa University Research Ethics Review Committee ruled that no formal ethics approval was required to conduct this research. However, prior to conducting the research, informed consent was obtained from the owners of the camels and heads of household who participated in this study.

Conflict of interest

The authors declare that there is no conflict of interest.

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