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SMALL RUMINANT AND HUMAN BRUCELLOSIS: "SERO PREVALENCE, RISK FACTORS AND ASSESSMENT OF FARMERS' KNOWLEDGE, AT TITUDE, AND PRACTICES IN EJERSA LAFO DISTRICT, WEST SHEWA, OROMIA REGIONAL STATE, ETHIOPIA."

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ABSTRACT
Brucellosis is a disease of animals and humans with a great public health burden across all societal segments, particularly in developing countries and it also affects developed countries. A cross-sectional study was conducted from October 2021 to November 2022 to estimate the seroprevalence of small ruminants and human brucellosis and assess the knowledge, attitudes, and practices of farmers' in the Ejersa Lafo district, Oromia region, Ethiopia. In this study, 374 small ruminants (176 sheep, 198 goats) and 216 human samples were screened for *Brucella* antibodies using the Rose Bengal plate test (RBPT), and positive sera were confirmed by an indirect enzyme-linked immune sorbent assay (I-ELISA). A structured questionnaire was also administered to 216 respondents to assess communities' knowledge, attitudes, and practices (KAP) about brucellosis. The overall seroprevalence of brucellosis in small ruminants was 4.28% (95%CI=2.46, 6.85) and 1.34% (95%CI=0.44, 3.09) by RBPT and I-ELISA, respectively. The species seroprevalence level of brucellosis by I-ELISA was 1.70% (95%CI=0.35, 4.90), 1.01% (95%CI= 0.12, 3.60), and 0% in sheep, goats, and humans, respectively. Pregnant small ruminants were more *Brucella* seropositive than non- pregnant. The study revealed that 81.5% of the communities were unaware of brucellosis, 60.18% did not use protective measures, and 97.6% consume raw milk. Higher-educated respondents had 2.52 times more knowledge than uneducated respondents. Male individuals implement 1.08 times more preventive practices than female individuals. The study revealed a low prevalence of brucellosis in small ruminants and an absence of positive results in humans. However, the existence of the disease in small ruminants is a possible risk of spreading the disease from animal to humans. Therefore, the slaughter of positive reactors and proper hygienic practices were recommended. The study also highlights the importance of the provision of information about knowledge, attitudes, and practices regarding brucellosis as one of the major strategies for prevention and control.

KEYWORDS: Brucellosis, Human, Sero-prevalence, Small Ruminant, Ethiopia.

1. INTRODUCTION
Ethiopia is home to a significant population of small ruminants, which includes around 40 million sheep and 51 million goats (CSA, 2020a). However, despite the country's abundance of small ruminants, the sector has not been optimally utilized due to various factors, including infectious and non-infectious diseases (Adem et al., 2021). In particular, sheep and goat flocks are susceptible to infections that can cause reproductive failure and abortion, according to Radostitis et al. (2007).

Brucella, a gram-negative bacterium, causes brucellosis - a bacterial infection with a significant negative impact (Araj, 2010; Radostitis, 2007). Brucellosis is a highly contagious disease that can affect most domestic animals, including cattle, sheep, goats, and camels. There are several species of Brucella bacteria, including B. abortus, B. melitensis, B. suis, B. ovis, B. canis, B. neotomae, B. pinnipedialis, B. ceti, B. microti, and B. inopinata (Radostitis et al., 2007). The disease is found all over the world, but it is more common in underdeveloped and tropical nations. Humans can also contract brucellosis, and it is more prevalent in areas where the disease is common in animals (Norman et al., 2016).

The daily life and livelihood of communities in developing countries heavily rely on animals and animal products, which increases the risk of zoonotic diseases spreading from animals to humans (Wubishet et al., 2018). This disease is classified as a public health burden that affects all societal segments and is considered a neglected zoonotic disease by the World Health Organization (WHO, 2006).

Animals such as cattle, sheep, goats, dogs, pigs, and wild animals, can be the sources of infections and reservoirs for humans, according to Galiska and Zagórski (2013). Small ruminants can contract diseases by consuming contaminated feed or water with their discharges as suggested by Tegegn et al. (2016). The disease can spread to humans through direct or indirect contact with infected individuals or animals. The transmission of the disease to humans is primarily through the handling of an aborted fetus and the consumption of unpasteurized milk and milk products, as stated by Franc et al. (2018).Several factors can affect the chances of contracting Brucella infection. The severity of the illness depends on the reproductive health of the host, the virulence of the bacteria, and their resistance. The risk of infection can be influenced by various factors such as age, sex, breed, flock size, hygienic status, and contact with infected animals. Brucella infection is more common in adults than in young individuals, and intrinsic components such as age, sex, and reproductive status play a significant role in determining the likelihood of infection (Borba et al., 2013; Tschopp et al., 2015).

Preventing brucellosis in humans requires effective control of the disease in ruminants. A combination of measures including livestock vaccination, culling of infected animals, and improving hygienic practices to reduce the risk of transmission can help achieve this goal (Li et al., 2017). In areas with a high incidence of animal-to-human disease transmission, it is crucial to follow basic hygiene practices. Lack of awareness, engagement in high-risk behaviors, and inadequate preventive and management techniques can enhance human exposure to the disease (Lindahl et al., 2015; Li et al., 2017).

Surveys on knowledge, attitudes, and practices, commonly known as KAP, are an effective method for determining the level of vulnerability of livestock owners to diseases, as highlighted by Kansiime et al. (2014). KAP assessments provide critical and relevant data that support the investigation of potential risk factors, including disease intervention and preventive measures. Previous studies have shown that there is a direct correlation between the number of infected herds and the level of understanding about the disease among the owners (Garcia, 2013). In Uganda (Kansiime et al., 2014), Kenya (Obonyo & Gufu, 2015), and Tajikistan (Lindahl et al., 2015), research has demonstrated the importance of educating livestock owners on brucellosis for effective prevention and control of the disease.

Zoonotic illnesses pose a significant global public health threat. Brucellosis has a case fatality rate of around 1 to 2 percent, or lower. This disease is a global concern, especially in countries with poor animal health control and low animal husbandry standards, putting millions of people at risk of infection (Aune et al., 2012). The incidence of brucellosis is on the rise due to increasing international travel, trade, and migration (Sofian et al., 2008). According to Tadesse (2016), the estimated incidence rates of human brucellosis in the pastoral region of Ethiopia were 160 per 100,000 persons per year. Brucella infection is a well-known disease in small ruminants, and it's endemic in Ethiopia (Tulu et al., 2020). In sexually mature sheep and goats, the disease typically affects their reproductive tract, leading to placentitis and abortion. Brucella suis, Brucella abortus, and Brucella melitensis are believed to have the highest zoonotic potential (Radostitis, 2007; Megersa et al., 2011), making it a significant public health issue in developing countries like Ethiopia, where it has a significant impact on the economy. Brucellosis hinders the trade of animals and animal products, as well as the mobility of animals. Additionally, it results in economic losses due to reduced milk production, abortion, or breeding failure in the animal population. In people, brucellosis reduces work capacity due to illness or absenteeism (Seleem et al., 2010), making it a significant public health concern. Brucellosis is a growing issue in Ethiopia, affecting both domestic animals and humans. Although some studies have been conducted in different parts of the country (Megersa et al., 2011; Asmare et al., 2013; Sintayehu et al., 2015), there is limited information about human and small ruminants, as well as KAP (knowledge, attitude, and practices) studies related to brucellosis in the western part of Shewa, Ethiopia. Therefore, the objective of this study is to determine the seroprevalence of small ruminants and human brucellosis, identify associated risk factors, and assess the knowledge, attitude, and practices of farmers and livestock owners in Ejersa Lafo district, West Shoa Zone, Oromia region, Ethiopia.

2. MATERIALS AND METHODS

2.1. Description of the study area

The research was carried out in Ejersa Lafo district, West Shewa Zone of Oromia Regional State, Ethiopia (Figure 1), from October 2021 to November 2022. The location of Ejersa Lafo is 67 km away from Finfine and 47 km from Ambo town, Ethiopia. This district has 53,727 sheep and 15,322 goats, respectively, and 75,000 people. Ejersa Lafo has 20 *Kebeles* (the smallest administrative unit) with 71% mid-highland and 29% highland agroecology. The altitude of the district is between 2000 and 3288 meters above sea level (ELWLFRO, 2022).

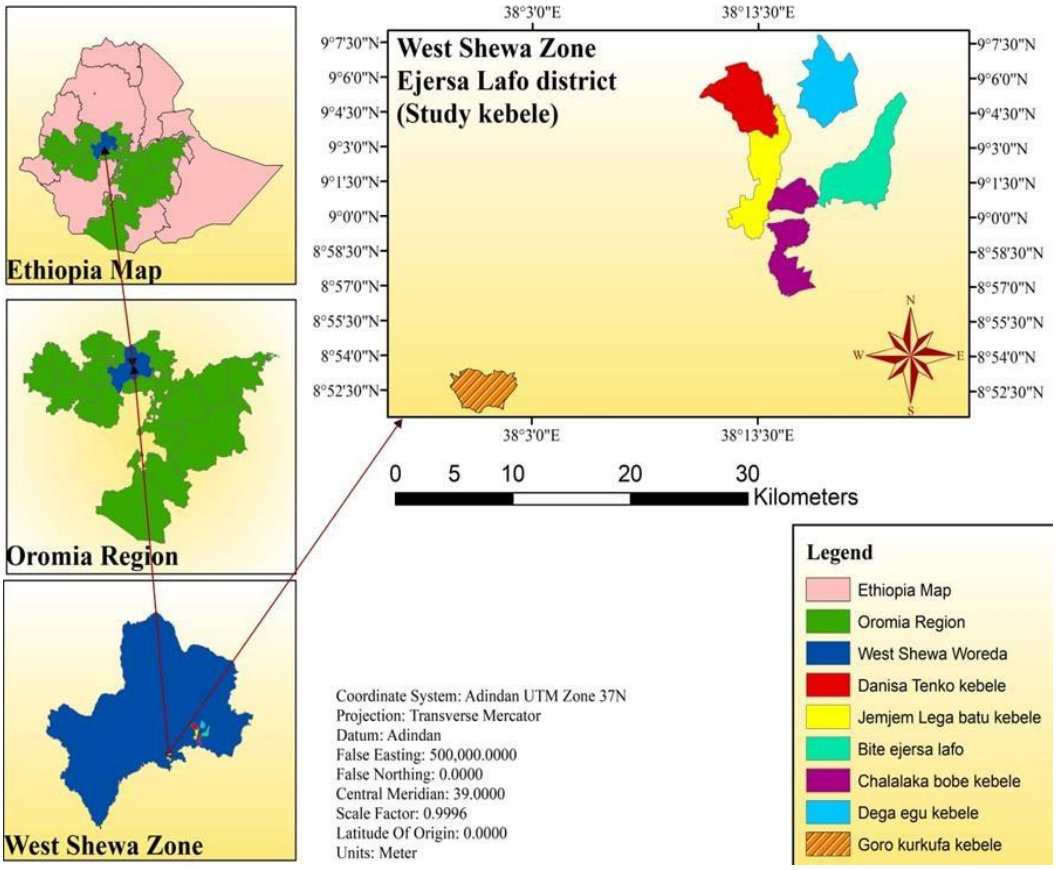

Figure 1: Map of the study area.

2.2. Study design, population, and study individuals

A cross-sectional study was performed to estimate the seroprevalence of small ruminants and human brucellosis, identify the risk factors associated with seropositivity, and assess the KAP of animal owners regarding brucellosis. All individuals above 18 years old in the district and sheep and goats greater than six months of age with no history of *Brucella* vaccination were considered as the study population. Age determination and animal level information or risk factors were obtained from the owners, like age (young, old), sex (male, female), origin (born at home, bought), flock size (<10, >10), history of abortion (yes, no), history of retained fetal membrane (yes, no), parity ((nulliparous if no birth is given, monoparous if the animal gives birth once, pluriparous if birth is given more than twice)), management (extensive, semi-intensive), water source(river, tap, pond) and gestation period (first, second and third trimester). Age was determined using herders' information and dentition was categorized as < 1 year, 1 year to 2 years, and >2 years (ESGPIP, 2009). Flock sizes were categorized as small (<7), medium (8–12), and (> 12 sheep and goats) (Margatho *et al*., 2019). Bokko (2011) defined the first trimester as 1–50, the second gestation period (51–100), and the third gestation period (101–154 days).

2.3. Sample size determination and sampling technique

The sample size was determined by using the single population proportion formula given by Thrusfield, (2005) based on a previous report of 2.09% for sheep (Gebremedhin, 2015), 2.37% for goats (Tujo, 2019), and 2.6% (Edao *et al*., 2020) for humans. A confidence level of 95% and 5% absolute precision were used to increase the sample size.

$$n = \frac{1.96^2 + P_{exp}(1-P_{exp})}{d^2}$$

Accordingly, the calculated sample size was 99 for goats, 88 for sheep, and 108 for humans. To increase precision and compensate for sample loss during processing and non-response rate, the samples were increased by two folds and the results were 198 goats, 176 sheep, and 216 humans and a total of 590 biological samples were used. The total number of samples required was distributed proportionally according to the animal population indicated in (Table 3).

To determine the required sample size of KAP, the formula of Yamane (1967) was employed at a 95% confidence level, with a 0.05 level of precision determined as follows:

$$n = \frac{N}{1+N(e)^2} \qquad n = \frac{5585}{1+5585\,(0.05)^2} = 373$$

Where: n = sample size
N= Total number of household
e = designate maximum variability or margin of error 8% (0.08 adapting be reviewing various literature), 1=designates the probability of the event occurring. Where: n = sample size

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Where: n = sample size, N= Total number of individuals in the household e = designate maximum variability or margin of error 5% (0.05), 1= designates the probability of the event occurring.

The sample size was reduced to 216 due to a lack of budget and the unwillingness of owners. The data used in this study came from a detailed household and pilot survey of 1697 households that owned sheep and goats and 5585 individuals in all selected kebeles.

Table 1: Sampling distribution for sheep and goats.

Kebeles	Sheep Population	Proportion	Sample size	Goats Population	Proportion	Sample size
Chalalaka Bobe	3600	0.17	0.17*176=30	475	0.17	0.17*198=34
Goro Kurkufa	2700	0.13	0.13*176=24	370	0.12	0.12*198=24
Jemjem Lega Batu	3000	0.14	0.14*176=25	450	0.15	0.15*198=30
Danisa Tenko	4700	0.22	0.22*176=39	125	0.04	0.04*198=11
Dega Egu	4300	0.20	0.2*176=35	232	0.07	0.07*198=14
Bite Ejersa Lafo	2653	0.12	0.12*176=23	1281	0.43	0.43*198=85
Total	20,953		176	2933		198

A multistage sampling system was used to get the required animal and human samples for the areas. The district was selected purposively based on logistics and accessibility, while *kebeles*, households, and individual animals and humans were randomly selected after having a sampling frame from the administrative team of the *kebeles*. Accordingly, 6 *kebeles* and 154 household-owning sheep and goats were selected by simple random sampling. For flock sizes under five, all animals older than six months were sampled, and for flock sizes of more than five, simple random sampling or a lottery system (after having a sampling frame by name and color from the owner) was used to obtain a maximum of five samples. A simple random sampling technique was also used to select one human from families with fewer than five members and two from families with more than five members. The necessary animal and human samples were then collected until the desired sample size was reached.

2.4. A questionnaire survey of knowledge, attitude, and practice about brucellosis

A structured questionnaire was administered to collect data about the knowledge, attitude, and practices of owners above 18 years old related to brucellosis. The question was asked in their local language after translating English to Afaan Oromo. The questionnaire covers respondents' socio-demographic (age (18–30, 31–45, >45), sex (male and female), level of education (uneducated, elementary, high school, college, and higher), occupation (farmer, student, other (merchant, keeper, preacher)), employment (employed, non-employed), family member (2–7, >7)) data. It also includes awareness regarding brucellosis causes, mode of transmission, prevention, zoonotic implications, and clinical presentations. Furthermore, parents were asked about their behavior towards their animal management, backyard slaughter, and delivery assistance, consuming raw milk or cheese, and eating raw meat. The questions consist of thirteen knowledge, seven attitude, and ten practice questions. The participants' awareness of brucellosis questions on etiology, clinical signs and symptoms, transmission, therapy, and prevention was used to test knowledge. Each response was graded on a scale of 'yes' or 'no.' Yes or correct answers were scored 1, no or incorrect answers were scored 0, and less than half of the multiple choice questions were answered correctly and were scored 0.5. The questionnaire had a scoring range of 0 (minimum) to 13 (maximum). A cutoff level of less than 60 was judged poor, between 60 and 80 as moderate, and > 80 was considered good knowledge based on bloom cutoff points (Bloom *et al.*, 1956). The attitude was assessed as positive, medium, and negative. Each response was scored as "yes" or "no," with 1 for "yes" or correct answer, and 0 for "no" or wrong answer. The scoring range of the questionnaire was 0 to 7. A cut- off level of less than 60 was considered negative, 60-80 was considered medium, and >80 was considered a positive attitude towards *Brucella*. Attitude scores for individuals were calculated and summed up to give the total attitude score. Practical questions are also calculated similarly to give overall practical scores.

2.5. Blood collection and serum separation

Blood samples of 5-7ml from each study animal were collected from the jugular vein aseptically using sterile plain vacationer tubes and needles and kept in a slanting position for 12 hours at room temperature to separate the serum. In the case of sample collection from humans, 4-5 ml of blood was withdrawn from the radial vein by health professionals using a plain vacationer tube and needle. The sera were then gently decanted into labeled sterile screw-capped tubes (Eppendorf) and transported on ice packs to the Sebeta National Animal Health Institute laboratory, Ethiopia, and stored at-20°C until analyzed.

2.6. Laboratory Analysis

2.6.1.Rose Bengal Plate Test

All the collected serum samples were tested for the presence of antibodies against brucellosis following the protocol of the OIE (Nielsen and Dunkan, 1990). To improve the sensitivity of the RBPT and minimize the discrepancies between RBPT and ELISA results, three volumes of serum and one volume of antigen (e.g., 75 µl and 25 µl, respectively) were used in place of an equal volume of each as recommended by OIE (Nielsen and Dunkan, 1990). In the human equal volume of 30 µl was used. Thus, RBPT was employed for screening purposes. Positive and negative control sera were used along with the test. After mixing the test and control sera with the antigen, the plates were gently shaken by hand for about 4 minutes. The results were interpreted according to Nielson and Dunkan (1990); "0" as negative or no agglutination; "+" barely perceptible agglutination; "+ +" fine agglutination and some clearing; and "+++" course clumping, definite with clearing. The sensitivity of RBPT was 89.6% and its specificity was 84.5% (Getachew *et al*., 2016).

2.6.2.Indirect Enzyme Linked Immune Sorbent Assay

Enzyme-linked immune sorbent assay (ELISA), has taken over as an important serological tool in the diagnosis of brucellosis because of its economy, sensitivity, specificity, rapidity, reproducibility, and easy interpretation through colorimetric end products. Thus, these commercially available ELISA test kits were used to detect antibodies against *Brucella* infection in the current study. According to Getachew *et al.* (2016), the sensitivity and specificity of I-ELISA were 96.8% and 96.3%, respectively. Before beginning the test, samples, reagents, and plates were brought to room temperature (Sadhu *et al*., 2015). The technique was performed according to the instructions from the manufacturer. In brief, serum samples and controls were added to the antigen-pre-coated micro-plate wells. The assays were then incubated at 37°C for 45 min, after which the first wash was performed the enzyme conjugate was added and incubated at 37°C for 30 minutes. All wells were washed to remove unbound materials, followed by a new incubation with the enzyme substrate. Finally, the reaction was stopped by adding 100 mL of the stopping solution. The substrate hydrolyzes the enzyme and yields a blue color. The color intensity measured by the spectrophotometric machine at 450nm is proportional to the amount of specific antibodies.

2.7. Data management and analysis

The data generated from the laboratory and the questionnaire were entered and stored in a Microsoft Excel spreadsheet (Microsoft Corporation) and analyzed by STATA version 11.0 for Windows (Stata Corp., College Station, USA).

Seroprevalence was calculated by dividing the total number of small ruminants that tested positive by the total number of tested animals multiplied by 100. The seroprevalence obtained is apparent prevalence. True prevalence = (Apparent prevalence + Sp - 1)/ (Se + Sp - 1).

Fisher's exact test was computed to test the association between explanatory variables and seropositivity to *Brucella*. Descriptive statistics such as frequency, percent, mean, standard deviation, and pie chart graphs were used, and inferential statistics namely univariable and multivariable ordinal logistic regression were conducted to analyze the association of socio-demographic factors (independent variables) with KAP (dependent variables) of respondents. Multicollinearity was diagnosed by the Variance Inflation Factor (VIF). Variables with a p-value <0.25 and VIF <10 were included in the multivariable ordinal logistic regression model to analyze the data. Assumptions of ordinal logistic regressions are:-The dependent variables measured at an ordinal level; one or more of the independent variables are either continuous, categorical, or ordinal; no multicollinearity; and the assumption of proportional odds should be met since violation of the assumption is not correctable. In all the analyses 95% confidence interval was used and a p-value of ≤ 0.05 was considered statistically significant.

2.8. Ethical clearance

The study was conducted after obtaining ethical clearance from the Ambo University ethical committee in agreement with the Ministry of Livestock and Fisheries and the animal industry. A letter of support was obtained from the administration of the Ejersa Lafo livestock and fishery resource office and woredas' health center. Informed consent was obtained from all study participants before their involvement in the study. It was informed that participants who were unwilling to participate in the study had the right to quit at any time. Confidentiality of the collected information and laboratory test results was maintained. Individual test results will be communicated with the attending physician for further management of the cases as per the routine guidelines of the hospitals or health centers. Animals from which serum samples were collected were handled under the procedures of animal welfare to minimize stress, inconvenience, and pain.

3. RESULTS

3.1. Seroprevalence of human brucellosis

A total of 216 human serum samples were tested using RBPT, and the result revealed that there was no positive case. The total number of respondents tested by age, sex, education, etc. was indicated in (table 6).

3.2. Seroprevalence of small ruminant brucellosis

From a total of 374 sheep and goats sampled, the prevalence of brucellosis in the study area was 4.28% (RBPT) and 1.34% (I-ELISA). Animal-level seroprevalence of 1.70% and 1.01% were recorded in sheep and goats, respectively (Table 4).

Table 2: Species-based seroprevalence of brucellosis.

Species		No	Total number positive					
			RBPT Positive (%)	95% CI		I-ELISA (%)	95%	CI
Small ruminant	Sheep	176	12 (6.82)	3.57	11.61	3 (1.70)	0.35	4.90
	Goat	198	4 (2.02)	0.55	5.09	2 (1.01)	0.12	3.60
	Total	374	16 (4.28)	2.46	6.85	5 (1.34)	0.44	3.09
Human		216	0			0 (0.00)		

CI= confidence Interval, RBPT= Rose Bengal Plate Test, I-ELISA= Indirect Enzyme-Linked Immune Sorbent Assay

3.3. Factors associated with *Brucella* seropositivity

Flock size, reproductive status, retained fetal membrane, history of abortion, and gestation period at abortion showed statistically significant associations ($p<0.05$) with *Brucella* seropositivity in sheep and goats. Flock size greater than ten (3.63%) were more *Brucella* seropositive than flock size less than ten (0.37%). Seropositivity increased with the gestation period in which animals in the third trimester (5.88%) were more seropositive than animals in the first trimester (3.36%). Pregnant small ruminants (4.68%) were more seropositive than non-pregnant (0.47%) small ruminants. Higher seropositivity was also detected in small ruminants with a history of purchase (2.27%) than in the animals that have no history of purchase (0.83%) but no significant difference was observed (Table 5). Results of Fisher's exact test at the species level were shown in. (Annexes 5 and 6).

Table 3: Analysis of the association between seropositivity of small ruminants' brucellosis and different variables using Fisher's exact test.

Variables	Category	Number tested	Number positive (%)	Fisher's exact test p-value
Species	Sheep	176	3 (1.70)	0.669
	Goat	198	2 (1.01)	
Sex	Female	273	4 (1.46)	1.000
	Male	101	1 (0.99)	
Age	Young(<1yr)	131	1 (0.76)	0.661
	Adult(>1yr)	243	4 (1.64)	
Origin	Purchased	132	3 (2.27)	0.356
	Born home	242	2 (0.83)	
Flock size	<=10	264	1 (0.37)	0.028
	>10	110	4 (3.63)	
Management	Extensive	269	4 (1.48)	1.000
	Semi-intensive	105	1 (0.95)	
Water source	Tap	91	1 (1.09)	1.000
	River	157	3 (1.91)	
Parity	Pond	126	2 (1.58)	0.690
	Nulliparous	96	1 (0.04)	
	Monoparous	94	1 (1.06)	
	Pluriparous	83	2 (2.40)	
Repeat breeding	Yes	72	2 (2.77)	0.284
	No	201	2 (0.99)	
Reproductive status	Pregnant	64	3 (4.68)	0.041
	Not pregnant	209	1 (0.47)	
Retained placenta	Yes	24	2 (8.33)	0.040
	No	249	2 (0.80)	
History of abortion	Yes	65	2 (3.07)	0.043

	No	208	2 (0.98)	
History of stillbirth	Yes	27	1 (3.70)	0.342
	No	246	3 (1.22)	
Gestation period	Non-pregnant	209	1 (0.48)	0.043
	First-trimester	28	1 (3.36)	
	Second trimester	19	1 (5.26)	
	Third trimester	17	1 (5.88)	

3.4. Results of a questionnaire survey for knowledge, attitudes, and practices about brucellosis

3.4.1. Socio-demographic characteristics and frequency distribution of KAP questions

Of the 216 livestock owners who participated in the questionnaire survey, the majority of respondents (71.30%) were farmers, males (64.35%) and illiterate (43.98%). The mean age of respondents was 35.69$\pm$13.98 years, ranging from 18 to 80 years, and the number of family members ranged from two to thirteen individuals (Table 6).

Table 4: Frequency distribution of socio-demographic characteristics of the respondents.

Variable	Category	Number of respondents	Percent (%)
Age	18-30 years	90	41.67
	31-45 years	75	34.72
	>45 years	51	23.61
Sex	Male	139	64.35
	Female	77	35.65
Family member	2-7 individuals	180	83.33
	>7 individuals	36	16.67
Educational status	Uneducated	95	43.98
	Elementary	49	22.69
	High school	35	16.20
	College and above	37	17.13
Employment	Non employed	208	96.30
	Employed	8	3.70
Occupation	Farmer	154	71.30
	Others	19	8.80
	Students	43	19.91

Among the study participants, 81.48% (176/216) have no awareness of brucellosis, 6.94% (15) mentioned bacteria as the cause of brucellosis, 93.05% (201/216) did not know the cause, and 66.67% of respondents have no awareness of the transmission of brucellosis from animals to humans. On the other hand, the majority of respondents had no information on how to prevent brucellosis (58.79%) and how humans get infected by brucellosis (76.85%). Most of the respondents (83.79%) did not know any of the symptoms of brucellosis in humans. Among those who had heard of brucellosis, 68.98% were through community talk or neighborhood talk, 24.53% from radio, and 6.48% were from veterinary services (Table 7).

Table 5: Knowledge of respondents about brucellosis.

Knowledge questions	Category	Number of respondents	Percent (%)
Have you encountered abortion?	Yes	167	77.32
	No	49	22.68
Do you know about brucellosis?	No	176	81.48
	Yes	40	18.52
What is the cause of brucellosis?	Bacteria	15	6.94
	Fungus	7	3.24
	Parasite	10	4.67
	Virus	3	1.38
	I don't know	181	83.79
Is brucellosis transmitted from animal to animal?	Yes	38	17.59
	No	57	26.39
	I don't know	121	56.01
Is brucellosis transmitted from a nimal to	Yes	72	33.33

human?	No	46	21.29
	I don't know	98	45.37
How do humans get infected with brucellosis from animals?	Drinking raw milk	7	3.24
	Ingestion of raw meat	12	5.55
	Contact	7	3.24
	Hand cut	6	2.77
	Blood splash on face	18	8.33
	I don't know	166	76.85
What are the clinical signs of brucellosis in animals?	Abortion	19	8.79
	RFM	9	4.16
	Emaciation	5	2.31
	Infertility and repeat	12	5.55
	Swollen tests	14	6.48
	All	32	14.81
	I don't know	125	57.87
Is brucellosis treatable in an animal?	Yes	111	51.38
	No	105	48.61
Is brucellosis treatable in humans?	Yes	44	20.37
	No	172	79.63
How is brucellosis prevented in animals	Drug	32	14.81
	Vaccine	43	19.90
	I don't know	141	65.27
How is brucellosis prevented in humans?	Cooking meat	15	6.94
	Boiling milk	3	1.38
	Treatment	20	9.26
	Abattoir slaughter	12	5.55
	All	39	18.05
	I don't know	127	58.79
What is your source of information	Neighbor	149	68.98
	Radio	53	24.53
	Veterinary service	14	6.48
Clinical signs of brucellosis in human	Fever	5	2.31
	Headache	3	1.38
	Sweating	3	1.38
	Joint pain	1	0.46
	Two sign	12	5.55
	All	11	5.09
	I don't know	181	83.79

RFM= Retained Fetal Membrane

The majority of respondents (99.07%) have a positive attitude about the necessity of hand washing after close contact with animals, vaccination of animals (93.52%), and using gloves when handling animals (90.74%) (Table 8).

Table 6: Frequency distribution of the attitudes of respondents about brucellosis.

Attitude questions	Category	Number of respondents	Percent (%)
Do you believe that brucellosis exists in this area?	Yes	77	35.65
	No	86	39.81
	Don't know	53	24.54
Would you inform the vets if your animal got sick?	Yes	214	99.07
	No	2	0.93
Do you consider the disease to be a factor affecting the production of your animals?	Yes	102	47.22
	No	114	52.77
Do you think throwing an aborted fetus or placenta into the environment transmits the disease?	Yes	84	38.88
	No	132	61.11
Do you think that using gloves when you handle animals is necessary?	Yes	196	90.74
	No	20	9.26

Do you think washing your hands is necessary after close contact with animals?	Yes	214	99.07
	No	2	0.93
Do you think that animal vaccination is necessary to prevent brucellosis?	Yes	202	93.52
	No	14	6.48

The majority of respondents (75.00%) mix their animals with other livestock. The current study showed that 60.18%, 97.67%, and 81.94% of respondents assist animal parturition by bare hand and consume raw milk and meat, respectively (Table 9).

Table 7: Practices of respondents regarding brucellosis.

Practice questions	Category	Number of respondent	Percent (%)
How do you do delivery assistance for your animals?	Bare hand	130	60.18
	Protected	23	10.64
	I don't	63	29.17
Do you wash your hands after contact with an aborted fetus?	Yes	200	92.59
	No	16	7.40
How do you manage aborted materials?	Burying	36	16.66
	Burning	3	1.38
	Open field	126	58.33
	Feed to dog	51	23.61
Do you practice backyard slaughter?	Yes	156	72.22
	No	60	27.77
Do you consume raw meat?	Yes	177	81.94
	No	39	18.05
Do you consume raw milk?	Yes	211	97.67
	No	5	2.31
Do you consume dairy products?	Yes	212	98.15
	No	4	1.85
How do you keep your sheep and goat during day time?	Mixed	151	69.90
	Separated	65	30.09
How do you keep your sheep and goat at night time?	Mixed	69	31.94
	Separated	147	68.05
Is there contact of your animals with other flocks?	Yes	162	75.00
	No	54	25.00

In the current study, 45.83% of respondents have poor knowledge, 31.02% have a negative attitude, and the majority of respondents (65.28%) have poor practices regarding brucellosis (Figure 2).

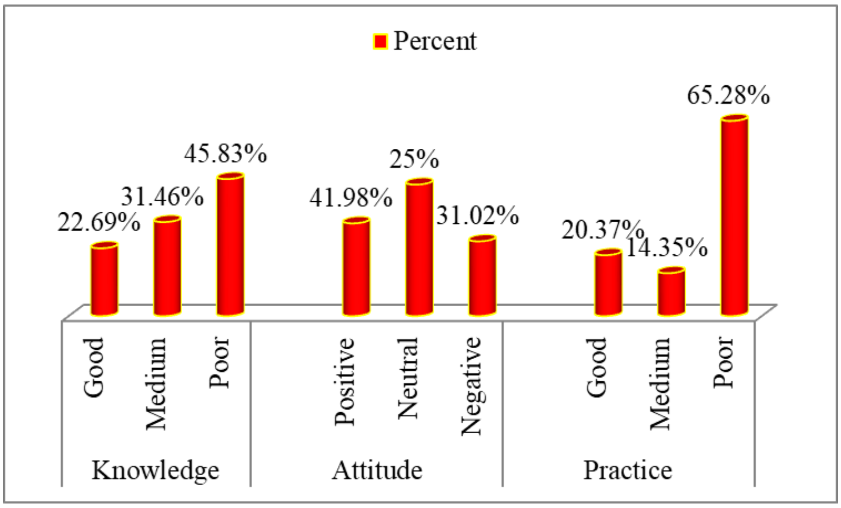

Figure 2: Summary of the frequency of overall knowledge, attitude, and practices of respondents in the Ejersa Lafo district, Oromia, Ethiopia.

3.4.2. Univariable ordinal logistic regression analysis of KAP with socio-demographic factors

Univariable ordinal logistic regression analysis indicates that The likelihood of having good knowledge and positive attitude versus the combined medium and poor KAP was 1.18 and 2.22-times higher for male individuals than their females given other factors held constant. All KAP scores of respondents had a statistically significant association with educational status (p< 0.05). Respondents with a high education level (college or higher) perform 2.05 times more preventive than uneducated respondents (Table 10).

Table 8: Results of the univariable ordered logistic regression analysis of KAP scores among study respondents of Ejersa Lafo district, Oromia, Ethiopia.

Variables	Knowledge		Attitude		Practice	
	OR	P>|z|	OR	P>|z|	OR	P>|z|
Age (Ref=18-30)						
31-45	2.01	0.017	1.41	0.225	0.78	0.450
>45	0.51	0.055	3.15	0.001	0.77	0.473
Sex (Ref= Female)						
Male	1.18	0.523	2.22	0.003	1.08	0.785
Family member (Ref=2-7)						
>7	2.05	0.036	1.32	0.402	0.66	0.298
Educational status (uneducated)						
Elementary	1.15	0.667	1.56	0.167	1.03	0.303
High school	1.74	0.131	2.43	0.019	1.9	0.001
College and above	2.79	0.006	5.51	0.000	2.05	0.000
Employment (Ref= non-employed)						
Employed	1.3	0.684	10.09	0.031	2.82	0.158
Occupation (Ref=Farmer)						
Others	1.29	0.055	3.21	0.035	3.39	0.012
Students	2.82	0.589	8.77	0.000	7.47	0.000

OR=Odd Ratio, P>|z|= P value, Ref= Reference

3.4.3. Multivariable ordinal logistic regression

Before developing the multivariable ordinal logistic regression model, the relationship between each explanatory variable and the response variable was examined. The variables with a p-value of < 0.25 were included in the multivariable ordinal logistic regression analysis. The Variance inflation factor (VIF) is used to diagnose multicollinearity, and the result showed no multicollinearity.

Accordingly, variables included in multivariable ordinal logistic regression analysis to examine their effects on knowledge of brucellosis were: age, family members and educational level (Table 11). Participants with a college and high school education had 2.52 and 1.75 times more in good knowledge versus medium and poor KAP when compared to uneducated groups. The knowledge increases with an increase in family size, in which family members of more than ten were 2.12 (95% CI = 1.06, 4.20) times more knowledgeable than family members of less than five (Table 11).

Table 9: Results of the multivariable ordinal logistic regression model for the knowledge of respondents about brucellosis.

Knowledge	OR	P>|z|	95% CI	
Age (Ref = 18-30)				
31-45	2.10	0.014	1.16	3.81
>45	0.56	0.118	0.27	1.16
Family member (Ref=2-7)				
>7	2.12	0.032	1.06	4.20
Educational status (uneducated)				
Elementary	1.04	0.899	0.53	2.05
High school	1.75	0.149	0.81	3.76
College and above	2.52	0.016	1.18	5.35

OR=Odd Ratio, CI= Confidence Interval, Ref= Reference

In multivariable ordinal logistic regression analysis for attitudes: Age, sex, educational status, employment, and occupation were included (Table 12). The multivariable ordinal logistic regression model for attitudes showed that individuals with a college and a higher level of education had five times (OR = 5.15, 95%CI = 2.10, 12.60) more positive attitudes than those uneducated (Table 12).

Table 10: Multivariable ordinal logistic regression model for attitude about brucellosis.

Attitude	OR	P>|z|	95% CI	
Age (Ref=18-30)				
31-45	2.01	0.036	1.05	3.87
>45	6.85	0.000	3.02	15.55
Sex (Ref=Female)				
Male	2.60	0.001	1.48	4.69
Educational status (uneducated)				
Elementary	2.21	0.032	1.07	4.58
High school	3.40	0.005	1.45	8.00
College and above	5.15	0.000	2.10	12.60
Employment (Non-employed)				
Employed	6.08	0.119	0.67	58.76
Occupation (Ref=Farmer)				
Others	2.04	0.162	0.69	8.59
Students	6.21	0.021	0.11	7.42

OR=Odd Ratio, CI= Confidence Interval, Ref= reference

Educational level and employment of practices fit multivariable ordinal logistic regression analysis (Table 13). The estimated odds ratio indicates that educated respondents perform 1.99 times more preventive practices for brucellosis than un educated by keeping other variables in the model constant (Table 13).

Table 11: Results of a multivariable ordinal logistic regression model for the practices of respondents in Ejersa Lafo district, Oromia, Ethiopia.

Practice	OR	P>|z|	95% CI	
Educational status (uneducated)				
Elementary	1.00	0.986	0.48	2.11
High school	1.28	0.631	0.42	2.15
College and above	1.99	0.065	0.95	4.17
Employment (Non-employed)				
Employed	2.74	0.174	0.64	11.79

OR=Odd Ratio, CI= Confidence Interval, Ref= Reference

4. DISCUSSION

In developing countries such as Ethiopia, where there is a high population of livestock and a very high portion of the human population lives in rural areas, investigating the status of brucellosis in both livestock and humans have paramount importance to safeguarding public and animal health. Brucellosis is one of the diseases that can affect the health of humans who have close contact with animals and feeding habits of raw animal products (Tsegay *et al*., 2015). The current study indicated serological evidence of small ruminant brucellosis, and KAP in respondents. The absence of seropositivity of human brucellosis in the study area may be due to the low prevalence of small ruminant brucellosis in the area.

An overall 1.3% seroprevalence of small ruminant brucellosis was in line with the previous studies conducted in the Jibat 1.29% (Tujo, 2019), 1.37% in the Somali (Mohammed *et al*., 2017), 1.5% in the Jijiga (Bekele, 2011), 1.56% in the Yabello (Dabassa *et al*., 2013), and 1.76% in the Debrezeit (Tsegay *et al*., 2015). The overall current seroprevalence was lower than the previous seroprevalence reports of 4.7% in the Jimma zone, Ethiopia (Tulu *et al*., 2020), 3.2% in Borena, Ethiopia (Edao *et al*., 2020), 3.5% in Tigray, Ethiopia (Teklue *et al*., 2013), and 4.89% in Central and North East Ethiopia (Wedajo *et al*., 2015). On the contrary, the present seroprevalence was higher than the 0.4% reported in Bahir Dar (Ferede *et al*., 2011), and 0.24% in West Hararghe (Geletu *et al*., 2021). The difference in the seroprevalence of small ruminants and human brucellosis between the current and previous studies might be related to the differences in geographical location and tests used.

In this study, flock size was significantly ($p<0.05$) associated with *Brucella* seropositivity in small ruminants which was in line with the study in Afar (Tegegn *et al*., 2016) and Jimma (Tulu *et al*., 2020), Ethiopia. Because a large flock allows great contact among animals, which creates a higher bacteria load in the environment and increases the probability of brucellosis transmission (Bruktayet and Mersha, 2016). The high seroprevalence in female than male small ruminants was also in agreement with the previous report in Bahir Dar, Ethiopia (Ferede *et al*., 2011). The higher susceptibility of female animals could be because they experience more physiological stress than males. Male animals were less susceptible to infection due to the absence of erythritol. In traditional husbandry practice, female animals are maintained in herds over a long period and have ample opportunity to acquire infections (Megersa *et al*., 2011).

Abortion, retained placenta, gestation period at abortion, and reproductive status of small ruminant brucellosis were statistically significant ($p<0.05$). In highly susceptible pregnant sheep and goats, abortion occurs in the last month of pregnancy (Radostits *et al*., 2007). Similar to the study conducted in Jimma, Ethiopia (Tulu *et al*., 2020) pregnant small ruminants (4.68%) were more *Brucella* seropositive than non-pregnant (0.47%) individuals. Susceptibility to *Brucella* infection is increased after sexual maturity and especially with pregnancy. In the uterus, allantoic fluid factors such as erythritol could stimulate the growth of the *Brucella* organism. It elevates in the placenta and fetal fluids, where it causes degeneration and necrosis of the cotyledons, leading to abortion in about the second and third trimesters of pregnancy (Radostits *et al*., 2007). Despite the low *Brucella* seropositivity in the current study, the results are associated with reproductive problems like the history of abortion and retained fetal membrane. Since statistical association might not necessarily entail causation, further investigations on the other causes of reproductive failures deserve investigation in future studies.

In the current study, the origin of animals (born at home or bought/purchased) did not show a significant association with the occurrence of small ruminant brucellosis. However, in animals with a purchase history, positive reactors were found. This finding was in agreement with (Bifo *et al*., 2020) which indicates that stock replacement from different herd locations could be one possible way of introducing the disease. Seropositivity increases with increasing age in small ruminants, but it was not statistically significant ($p > 0.05$). This finding was in agreement with the earlier reports (Lakew *et al*., 2019; Adem *et al*., 2021) and is linked to increased susceptibility to *Brucella* infection with sexual maturity. With age increasing, seroprevalence increases due to the prolonged duration of antibody responses in infected animals and prolonged exposure (Elderbrook *et al*., 2019).

In the current questionnaire survey, only 18.52% of participants reported having heard of brucellosis, which is comparable to earlier KAP studies conducted in Tajikistan 15% (Lindahl *et al*., 2015), 27.3% in Bench Maji, Ethiopia (Kenea and Megersa, 2021), and 28.1% in Tanzania (Milgo *et al*., 2022). In contrast to the current finding a high level of knowledge of 63% in northern Uganda (Nabirye *et al*., 2017) and 79% in Kenya (Obonyo and Gufu, 2015) was reported.

Brucellosis is a zoonotic disease worldwide. The most effective control method among individuals is the creation of awareness about brucellosis. It is still uncontrolled, and considerable public health problems exist in numerous developing countries (Bagheri *et al.*, 2020). In this study, 66.66% of the respondents had no information about zoonosis of brucellosis. Most of the respondents (77.32%) recognize the occurrence of abortion in their animals, but (57.87%) and (83.79%) have no awareness of the clinical signs and symptoms of brucellosis. The respondents' primary sources of information were their neighbors (68.98%), the radio (24.53%), and veterinarians (6.48%). That shows veterinarians have a limited impact on participants' awareness of zoonotic diseases. Because of the restricted role of health professionals in providing health information, there is a lack of knowledge among participants regarding brucellosis, which requires the integration of zoonotic disease experts in animal and human health (Legesse *et al*., 2018).

The respondent's awareness of the means of transmission and prevention of brucellosis was low. High proportion of respondents reported to consume raw milk, milk products, raw meat, and backyard slaughter. Moreover, 60.18% of respondents handle aborted fetuses and assist delivery without protecting their hands, which may be due to a lack of awareness and protective materials. Most respondents (81.94%) did not bury or burn the aborted material. They discarded it in the open field, which has a risk of brucellosis transmission to humans and animals. Even though most respondents drink raw milk and consume raw meat, 58.79% do not know that boiling milk can prevent brucellosis. This finding was similar to the studies reported in the Amibara district, Afar, Ethiopia (Legesse *et al*., 2018), and in the Dallo-Manna and Haranna Bulluk districts of the Bale zones (Adem *et al*., 2021).

The percentage of respondents who reported overall good, medium, and poor knowledge was 22.69%, 31.48%, and 45.83%, respectively. In agreement with the current study poor overall knowledge scores were reported in Borena, Ethiopia (Wubishet *et al*., 2018), Tajikistan (Lindahl *et al*., 2015), and Uganda (Nabirye *et al*., 2017). These poor overall knowledge scores among respondents might be due to inadequate public health promotion regarding zoonotic diseases. The average attitude scores of participants were positive, with 43.98%, 25.00%, and 31.02% of respondents having positive, neutral, and negative attitudes, respectively. Despite their lack of knowledge about brucellosis in the present study, a significant proportion of respondents had a more positive attitude towards some attitude questions, which could be due to chance or extrapolated from participants' general knowledge of other livestock diseases. This study is in line with the studies conducted in northern Uganda (Nabirye *et al*., 2017) and South Africa (Cloete *et al*., 2019). The overall practice scores of respondents were poor (65.28%) to average, with several high-risk behaviors identified in this community. Consumption of unpasteurized milk and milk products was considered a high-risk activity (Lindahl *et al*., 2015). Brucellosis KAP studies in Kenya (Obonyo and Gufu, 2015) and Jordan (Musallam *et al*., 2015) also revealed high-risk activities, including handling aborted material without protection.

The study also determined the socio-demographic characteristics of respondents regarding knowledge, attitudes, and practices. Multivariable ordinal logistic regression analysis showed respondents with a college or higher education were more knowledgeable than those with no formal education. In line with the previous studies reported in the Aseer region, Saudi Arabia (Alqahtani *et al*., 2021), and Ecuador (Ruano and Aguayo, 2017), those with higher education were 2.52 times more knowledgeable than those with no formal education. Good practices also increase with an increase in educational status because high knowledge leads to the use of protective measures or prevents handling aborted materials with bare hands and consumption of raw dairy products. Formal education can positively influence a person's ability to acquire further knowledge and is a vital component of the prevention and control of disease. In contrast to this, a study conducted in Egypt (Hegazy *et al*., 2016) found that livestock farmers had general knowledge about brucellosis.

In the present study, participants from a larger family group were more likely to have a better knowledge of brucellosis than those from smaller households, with an odd of 2.12. This finding is comparable to previous studies published in Tibet (Zeng *et al*., 2018) and Saudi Arabia (Alqahtani *et al*., 2021). A large family size shares more information within the family, which might be due to the possible presence of educated and experienced individuals in large family sizes. This finding was, in contrast, to the study of Kenea and Megersa (2021), who reported that smaller families had better knowledge of brucellosis. In this study, students perform more preventive practices than farmers because of the highly inquisitive nature of students and the possibility of possessing information-sourcing devices such as mobile phones.

Similar to the present study, previous studies conducted in Tanzania (Milgo *et al*., 2022), and Saudi Arabia (Alqahtani *et al*., 2021) showed that male respondents had more preventive practices than female individuals. Because male respondents possess tasks such as marketing and get a chance for social interaction, they were more preventive than females, especially those involved in culturally risky practices such as milking and milk handling.

Generally, disease prevalence combined with a lack of communities' knowledge, attitude, and practices about the zoonotic importance of the disease and close contact of humans with animals will create a high risk of human brucellosis. In addition, a preference for fresh or raw dairy products is a risk factor for human exposure. Since brucellosis is a disease of mature and productive animals, it causes a loss of economy in high amounts (Animal loss, production loss, cost for treatment and prevention and absenteeism) (Samaha *et al*., 2008; Alqahtani *et al*., 2021). In this study area, close contact with livestock, assisting during parturition without protective equipment, the tradition of raw milk consumption, and low awareness about the disease may facilitate the zoonotic transmission of the disease.

The limitations of this study include the inability to employ the total calculated sample sizes due to a lack of budget. Extrapolation of the results of the present study of the humans to the entire population in the study area should be done cautiously since human samples were taken from humans older than 18 years. The small number of seropositive small ruminants might have hampered to show explicitly the risk factors of *Brucella* seropositivity using the logistic regression model, suggesting the need of sampling more animals as the disease is rare.

5. CONCLUSION AND RECOMMENDATIONS

The findings of this study revealed the absence of *Brucella* infection in humans and the low seroprevalence of small ruminant brucellosis in the study area. Large flock sizes, retained placenta, history of abortion, and gestation period at abortion are significantly associated with *Brucella* seropositivity. Since the livelihood of communities mainly depends on the animals there is great close contact between animals and humans that results in a possible risk of spreading from infected animals to humans. Sexually mature sheep and goats were affected more, and this condition can greatly affect the individual and national economy due to reductions in reproductive efficiency and infertility, which contribute to great economic loss. The study showed poor knowledge and practice of respondents about brucellosis, which might result in the high transmission of brucellosis within the community.

Therefore, based on the above conclusions, the following recommendations are forwarded.Test and slaughter, Vaccination, Training the community about the zoonosis of disease and further research on human and animal brucellosis and the KAP of respondents related to brucellosis is required.

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