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A Holistic Approach to Bovine Brucellosis: Serological Tests, Knowledge, Attitudes, and Practices Evaluation, and Risk Factor Identification on Dairy Farms of Ethiopia

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ABSTRACT

Brucellosis, a contagious bacterial disease affecting animals globally presents a substantial zoonotic risk that is frequently underestimated, hinders animal trade, and endangers livestock and human health. The present study was conducted from November 2023 to June 2024 in Central and North Gondar Zone, Ethiopia. The current cross-sectional study aimed to evaluate the prevalence of brucellosis and to explore the related knowledge, attitudes, and practices within the specified region. A total of 384 serum samples were collected via random sampling from 20 dairy farms located in Ethiopia. Both local and cross-breed samples screened using the Rose Bengal Plate test and confirmed through an enzyme-linked immunosorbent assay. The seroprevalence of bovine brucellosis for both tests was 17.71% overall, with 9.62% for Central Gondar and 27.27% for North Gondar. Extensive farms exhibited notably higher odds of brucellosis compared to intensive farms, with unadjusted crude odds ratios of 3.01 and adjusted odds ratios of 2.37, respectively. Medium-sized herds also demonstrated increased odds in the multivariate analysis compared with small herds. Young respondents displayed the highest awareness levels, followed by adults and older individuals, with statistically significant differences observed across all categories. Regarding a semi-structured survey from 150 farmers on the association between sociodemographic data and knowledge, females exhibited higher awareness levels, with 117 (80.14%) responding positively. Young respondents showed a higher positive response rate of 58% compared to adults (52.7%) and the elderly (36.54%). In conclusion, these results emphasize the need for comprehensive strategies to address the factors influencing bovine brucellosis prevalence and respondent awareness.

Keywords: Brucellosis, Central Gondar, Dairy Farm, North Gondar, Seroprevalence

INTRODUCTION

Ethiopia is known as the residence of Africa's most extensive livestock numbers, due to the hosting of approximately 60.9 million cattle, 31.3 million sheep, and 32.7 million goats (Dinka and Ababa, 2011). The prevalence of animal diseases throughout any country significantly hindered the abundance of livestock and limiting their potential as reliable food sources. Despite playing a significant role in the country's economy, the productivity of each animal remained notably low, largely attributed to technical limitations and diseases such as brucellosis (Merga Sima et al., 2021). Bovine brucellosis, a globally infectious and contagious disease, constituted a significant economic burden on livestock industries in developing countries, impacting cattle production worldwide. Well-controlled in developed nations, it caused reproductive waste, economic losses, and barriers to international trade in developing countries. In dairy production, crossbreeding impeded the import of high-yielding breeds and improvements in milk production (Dahouk and Nöckler, 2011). The extensive presence of these factors rendered the disease not only endemic but also one of the foremost zoonotic threats to public health in the nation (Gashaw et al., 2022). Although bovine brucellosis significantly affected socioeconomic and zoonotic impacts, it received little attention (Bekele et al., 2002). Brucellosis, caused by *Brucella abortus* in sexually mature cattle in Africa, led to reproductive problems, such as abortion and retention of fetal membranes in cows and orchitis and epididymitis in bulls (McDermott and O'connor, 2002; Radostits et al., 2007). Brucellosis was first reported in the 1970s in Ethiopia and identified as an important livestock disease (Domenech, 1977). The infection transmitted via direct exposure to miscarried cows and their fetuses or indirectly through tainted items. It spread through polluted feed, water, and interactions with infected animals or tissues, endangering livestock handlers, vets, and lab staff. Although a dangerous sickness, no vaccine was available for human application, and the bacterium's intracellular behavior significantly hampered treatment efficacy (Qureshi et al., 2023). Addressing

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brucellosis was vital for sustaining Ethiopia's livestock industry and ensuring human well-being (Acha and Szyfres, 2001).

Crucial risk factors included the herd size, age, and gender of cattle, management practices, interactions with wild animals, environmental influences, and mixing of different species within a herd (Muma et al., 2007; Tsegaye et al., 2016). Effective management of *Brucella* in endemic regions involved vaccinating calves or heifers. Additionally, brucellosis control strategies encompassed isolating infected cattle and employing test-and-slaughter techniques (OIE, 2004).

Effective control measures encompassed surveillance, prevention of transmission, and management of the infection source, which involved practices like culling. Accurate diagnosis was pivotal and typically involved isolating and identifying *Brucella* from aborted materials, udder secretions, or tissues, alongside detecting specific antibodies using suitable serological techniques. Nevertheless, treating brucellosis was frequently challenging due to the bacterium's intracellular nature, often leading to recurrences within 3 to 6 months after halting early therapy (Khurana et al., 2021).

Brucellosis was transmitted to other cattle through direct or indirect interaction with diseased cattle or their discharges such as feeding pooled colostrum to newborn calves, and rarely, through sexual contact during artificial insemination. The disease could also spread through ingestion of contaminated feed and drinking water, as well as through birth products and uterine discharge. Mucosa/abrasions coming into contact with the fluid or tissues of aborted fetuses of diseased cattle could also serve as a source of disease in humans, especially through the ingestion of unpasteurized milk or milk products. Abattoir, farm, and laboratory workers, along with veterinarians, were recognized as at-risk groups for *Brucella* infection. Brucellosis could be eradicated through measures such as quarantining infected cattle, vaccination, and test-and-slaughter methods (Tulu, 2022). *Brucella* infection caused huge financial losses and community health concerns in many countries including Ethiopia (Tulu, 2022). This study aimed to evaluate the seroprevalence of bovine brucellosis, knowledge, attitudes, and practices (KAPs), and identify the risk factors associated with it in dairy cattle located in the North and Central Gondar Zones of Northwest Ethiopia.

MATERIALS AND METHODS

Ethical approval

The research project titled 'Major Zoonotic Reproductive Diseases and Production Problems of Current Dairy Cattle in the Milk Shade Area of Amhara Region, Ethiopia,' conducted at the University of Gondar, Ethiopia, on 12-Nov-2022, has undergone review by the institutional ethical review board of the University of Gondar for its ethical soundness. It was deemed ethically acceptable on 11/25/2022 with reference number VP/RTT/05/166/2022.

Study area

The current study took place in the North and Central Gondar Zone, located in the Amhara regional state of Ethiopia. This region is situated around 740 km north of Addis Ababa, the capital city of Ethiopia. The Amhara National Regional State falls within the tropical region, with climatic zones ranging from hot dry tropical to subtropical, temperate, and alpine (Teshome and Adamu, 2010). It is composed of 13 administrative zones and has an estimated population of 21,134,988. The geographical coordinates of the study area are approximately 12°4' North latitude and 27°2' East longitude, with altitudes ranging from 1800 to 2500 meters above sea level. The region experiences a bimodal rainfall pattern, with an average annual precipitation of 1000 mm. The short rainy season occurs in March, April, and May, while the long rainy season extends from June to September (Abdulkadir, 2019). The average yearly temperature in the location was 19.7°C. Agriculture in this area predominantly focuses on cereal crops and livestock rearing (Commission, 2008; CSA, 2011). The livestock count in the North and Central Gondar zones is estimated to comprise 2,771,701 cattle, 815,716 sheep, 1,251,867 goats, 27,248 horses, 9,695 mules, 376,841 donkeys, 3,628,832 poultry, and 227,463 beehives (CSA-Ethiopia, 2012, Figure 1).

Study population and source

A cross-sectional study was conducted on dairy farms in Ethiopia between November 2023 and June 2024. The study population consisted of cows of different breeds, encompassing indigenous, cross-breed, and exotic breeds, in the context of bovine brucellosis. The participants comprised both breeding females and replacement heifers, reared under varied management systems including intensive, semi-intensive, and extensive. To determine the occurrence of brucellosis in dairy cows, the first screening involved the Rose Bengal Plate Test, with further confirmation of positive cases through the enzyme-linked immunosorbent assay.

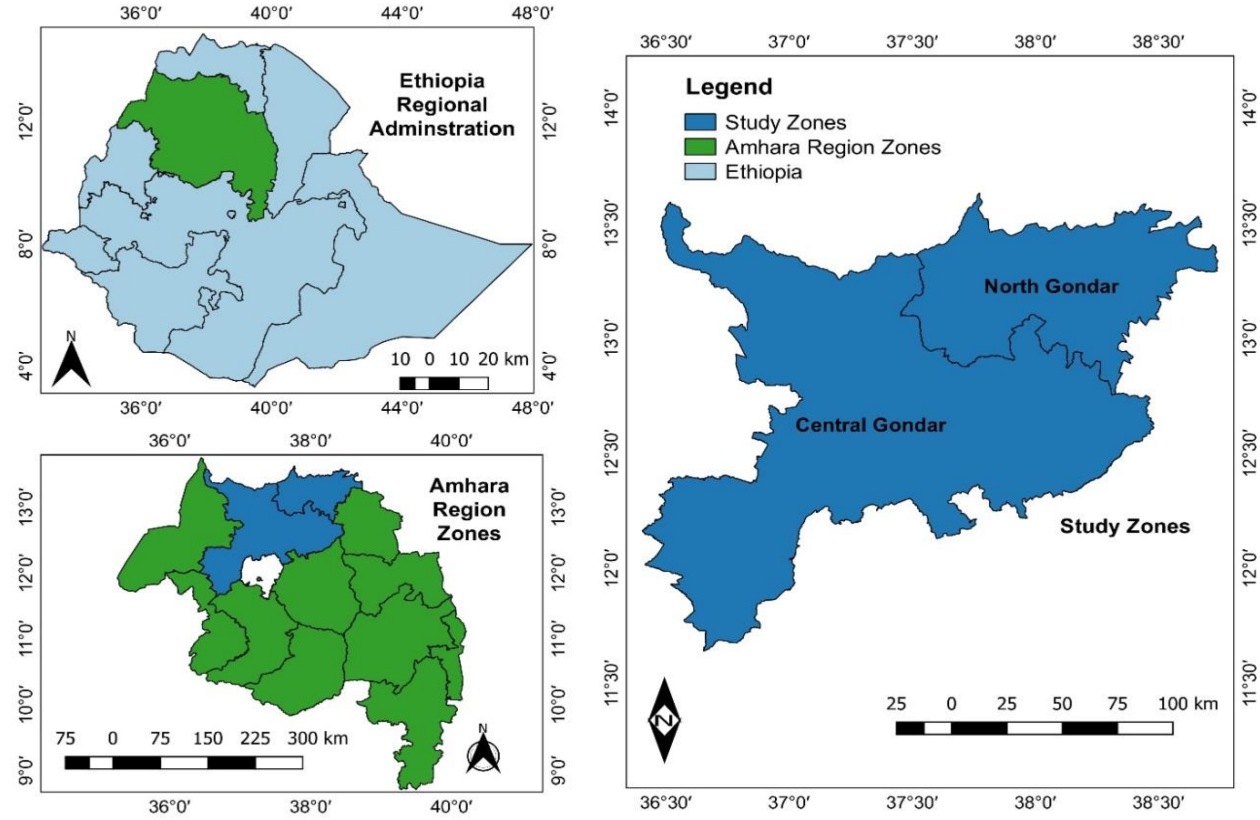

Figure 1. Map of the location of the study and dairy farms in Ethiopia

Sampling technique and sample size determination

The total number of animals needed was calculated using the formula by Thursfield (2015). The sample size was determined based on a 95% confidence level, an expected prevalence of 50%, and a desired absolute precision of 0.05.

The formula used is following:

n = ([1.96]^2 * Pexp * [1 - Pexp]) / d^2 = 384.

To enhance accuracy, the sample size was tripled and selected from various agroecological regions. Here, n represents the required sample size, Pexp signifies the expected prevalence, and d indicates the desired absolute precision.

Sample collection and processing

Experienced veterinary professionals collected information on the socio-demographic characteristics and risk factors for dairy cattle using a pretested structured questionnaire. To determine whether the language of the questions was understandable, the questionnaire was pretested on 150 dairy cattle owners in a pretest study. Pretested questionnaires were used, and the instrument's reliability and validity were confirmed in the pretest study. Around 10 mL of blood was drawn from the jugular vein of all chosen animal utilizing a standard vacutainer tube and needle. Each animal was then identified on the respective vacutainer tubes and left at room temperature overnight to facilitate clotting. The following day, the serum was separated from the clot into a different tube. These serum samples were preserved at -20°C in the Medical Microbiology Laboratory of the Faculty of Health Science at the University of Gondar until subjected to testing through the Rose Bengal Plate Agglutination Test (RBPT, Yohannes et al., 2012 a).

Rose Bengal Plate Agglutination Test and ELISA were employed as preliminary screening tests for serum samples to identify the presence of *Brucella* agglutinins. The OIE-recommended protocol was followed to screen for the presence of *Brucella* antibodies in the sampled sera. This test is generally recognized for its sensitivity, with a reported sensitivity of 97.9% (Dohoo et al., 1986). The test was conducted following the manufacturer's instructions. Prior to the test, the antigen and sera were equilibrated to room temperature. To initiate the test, 30 µL of serum was extracted from a glass slide using a micropipette. The Rose Bengal antigen bottle was adequately agitated to ensure a uniform suspension, and then one drop (30 µL) of the antigen was added. The antigen and serum were thoroughly mixed using a spreader, and the slide was rotated for 4 minutes. The test result was promptly interpreted immediately after 4 minutes rotation.

Questionnaire survey

A total of 150 semi-structured questionnaires were employed to gather data on the Knowledge, Attitudes, and Practices (KAP) concerning bovine brucellosis and the factors that contribute to the disease's prevalence in the research locations. The questionnaire consisted of three sections including part-I focused on gathering socio-demographic details about the participants; part-II aimed to evaluate factors that predispose to the disease; and part-III was dedicated to assessing the Knowledge, Attitudes, and Practices of animal owners regarding bovine brucellosis.

Statistical analysis

The data collected from field level and laboratory investigation were coded into appropriate variables and entered into a Microsoft Office Excel 2019 spreadsheet. The data were checked for errors of entry, coded, and then imported to STATA for descriptive and further analyses. All statistical analyses were performed using STATA version 14 software. Descriptive statistics involving frequency and percentage were used to determine the seroprevalence of the disease. Binary logistic regression analysis was used to identify potential risk factors associated with bovine brucellosis. First, univariable logistic regression analysis with the flock as a random effect was performed and potential risk factors (explanatory variables) with p values less than 0.25 were screened for the multivariable mixed-effect logistic regression. In statistical analysis, a p-value below 0.05 (at a significance level of 5%) was deemed to demonstrate statistical significance for both tests.

RESULTS

The prevalence study of bovine brucellosis on dairy farms explored multiple factors linked to the disease by analyzing 384 serum samples obtained from 20 farms. Among these samples, 68 (17.71%) tested positive for brucellosis based on RBPT and Indirect enzyme-linked immunosorbent assay (i-ELISA) tests. The result presented in Table 1 indicated that local breeds had a higher seroprevalence rate of 21.29% compared to exotic breeds at 13.74%. A difference in the seroprevalence rates was observed between intensive (8.82%) and extensive (22.58%) farm types. Adult cattle (>2 years) exhibited a higher seroprevalence rate of 24.85% compared to younger cattle (6 months to 2 years) at 12.33% (Table 1).

The result shown in Table 2 revealed that the seroprevalence of bovine brucellosis was higher in North Gondar (27.27%) compared to Central Gondar (9.62%). Additionally, dairy farms with poor calf management practices had a significantly higher seroprevalence rate (24.14%) compared to those with good management (7.89%). The seroprevalence rate of brucellosis for Livestock owners with a primary education level was higher (23.70%) compared to those with a secondary education or above (12.80%, Table 2).

Table 1. Seroprevalence and host risk factors for bovine brucellosis in dairy farms of Ethiopia between November 2023 and June 2024

Variables	Category	No. of Examined	No. of Positive	Prevalence (%)	(95% CI)
Breed type	Local	202	43	21.29	(0.48-0.58)
	Exotic	182	25	13.74	(0.42-0.52)
Farm type	Intensive	136	12	8.82	(0.31-0.40)
	Extensive	248	56	22.58	(0.60-0.69)
Herd size	Small	145	25	17.24	(0.33-0.43)
	Medium	142	32	22.54	(0.32-0.42)
	Large	97	11	11.34	(0.21-0.30)
Age	Young (6 month – 2 years)	219	27	12.33	(0.52-0.62)
	Adult (> years)	165	41	24.85	(0.38-0.48)
Vaccination	Vaccinated	164	18	10.98	(0.38-0.48)
	Non-vaccinated	220	50	22.73	(0.52-0.62)
Feeding status	Properly feed	144	16	11.11	(0.33-0.42)
	Non-properly feed	240	52	21.67	(0.58-0.67)
Breeding method	Natural mating	253	55	21.74	(0.61-0.71)
	AI	131	13	9.92	(0.30-0.39)

95% CI: 95% confidence interval. p values less than 0.05 were statistically significant; No. of examined: Number of examined; No. of positive: Number of positive; AI: Artificial Insemination

Table 2. Seroprevalence and environmental risk factors of bovine brucellosis in dairy farms of Ethiopia between November 2023 and June 2024

Variables	Category	No. of examined	No. of positive	Prevalence (%)	(95% CI)
Zone	Central Gondar	208	20	9.62	(0.49-0.59)
	North Gondar	176	48	27.27	(0.41-0.51)
Ventilation	Well	184	39	21.20	(0.43-0.53)
	Poor	200	29	14.50	(0.47-0.57)
Calf Management	Good	152	12	7.89	(0.35-0.45)
	Poor	232	56	24.14	(0.55-0.65)
Disposal After Birth	Yes	86	12	13.95	(0.18-0.27)
	No	298	56	18.79	(0.73-0.82)
Educational level	Primary	173	41	23.70	(0.40-0.50)
	Secondary and above	211	27	12.80	(0.50-0.60)
Space	Adequate	192	37	19.27	(0.45-0.55)
	Confined	192	31	16.15	(0.45-0.55)
House sanitation	Good	126	8	6.35	(0.28-0.38)
	Poor	258	60	23.26	(0.62-0.72)

No. of examined: Number of examined; No. of positive: Number of positive

In the multivariable analysis, a statistically significant association was observed between the seroprevalence of brucellosis with zone and herd size ($p < 0.05$, Table 3). The findings presented in Table 4 revealed a statistically significant association ($p < 0.05$) between the prevalence of brucellosis and both house sanitation and education level. The likelihood of brucellosis was 6.15 times higher (95% CI: 2.77, 13.68) in households with poor sanitation compared to those with good sanitation (Table 4).

Table 3. Univariate and multivariate mixed-effect logistic regression analysis of host factors for bovine brucellosis in dairy farms of Ethiopia between November 2023 and June 2024

Variables	Category	Univariable		Multivariable	
		COR (95% CI)	p value	AOR (95%CI)	p value
Zone	Central Gondar	Reference			
	North Gondar	3.53(2.00-6.22)	0.00*	4.48(2.42-8.27)	0.00*
Breed type	Exotic	Reference			
	Local	0.59(0.34-1.01)	0.06		
Farm type	Intensive	Reference			
	Extensive	3.01(1.55-5.85)	0.00*		
Herd size	Small	Reference			
	Medium	1.40(0.78-2.50)	0.26	2.37(1.24-4.51)	0.01*
	Large	0.61(0.29-1.31)	0.21		
Ventilation	Well	Reference			
	Poor	0.63(0.37-1.07)	0.09		
Age	Young	Reference			
	Adult	2.35(1.38-4.02)	0.00*		
Vaccination	Vaccinated	Reference			
	Non-Vaccinated	2.39(1.33-4.27)	0.00*		
Feeding status	Properly Feed	Reference			
	Non-Properly Feed	2.21(1.21-4.05)	0.01*		
Breeding method	AI	Reference			
	Natural	0.40(0.21-0.710	0.01*		

COR: Crude odds ratio; AOR: Adjusted odds ratio; 95% CI: 95% confidence interval. p values less than 0.05 were statistically significant; No. of examined: Number of examined; No. of positive: Number of Positive; AI: Artificial Insemination

Table 4. Univariable and multivariable mixed-effect logistic regression analysis of environmental risk factors for bovine brucellosis in dairy farms of Ethiopia between November 2023 and June 2024

Variables	**Category**	**Univariable**		**Multivariable**	
		COR (95% CI)	**p value**	**AOR (95%CI)**	**p value**
Space	Adequate	Reference			
	Confined	0.81(0.48-1.36)	0.42		
House sanitation	Good	Reference			
	Poor	4.47(2.07-9.67)	0.00*	6.15(2.77-13.68)	0.00*
Calf Management	Good	Reference			
	Poor	3.71(1.92-7.20)	0.01		
Disposal after birth	Yes	Reference			
	No	1.43(0.73-2.80)	0.30		
Educational level	Primary	Reference			
	Secondary and above	0.47(0.28-0.81)	0.01*	0.33(0.19-0.58)	0.00*

COR: Crude odds ratio, AOR: Adjusted odds ratio; Referenence: In the provided result table, the term Reference: Serves as a point of comparison for the other categories within each variable. It is the baseline or default category against which the other categories are compared to determine the association between those categories and the outcome of interest.

An Area Under the Curve (AUC, Figure 2) value of 0.7084 indicated the overall performance of the model in distinguishing between the positive and negative classes. This suggested that the model had a moderate level of discrimination ability. Raising the probability cutoff beyond 0.5 in Figure 3 generally elevated the bar for categorizing an observation as positive.

The survey depicted in Table 5 revealed a predominance of female respondents (80%) compared to males (20%). Among the respondents, the 'adult' age group (35-52 years) constituted the largest segment at 50.67%, followed by the 'young' category (18-35 years) at 34.67% and the 'old' category (52 and above) at 14.67% (Table 5). In Table 6, the majority of respondents (62.67%) disagreed with being concerned about the impact of bovine brucellosis on cattle health and productivity. A significant portion (31.33%) neither agreed nor disagreed, while only 6.00% agreed. A considerable number of respondents (46.00%) agreed with having awareness regarding the symptoms and transmission of bovine brucellosis. 42.00% disagreed, and 12.00% neither agreed nor disagreed.

In Table 7, a majority of respondents (61.33%) correctly identified that the primary causative agent of bovine brucellosis was a bacterium called *Brucella*, while 38.67% answered incorrectly. A significant portion of respondents (68.67%) correctly identified that bovine brucellosis is typically transmitted among cattle through contact with infected reproductive fluids or tissues, with 31.33% answering incorrectly. A majority of respondents (61.33%) incorrectly identified common symptoms of bovine brucellosis in cattle as fever, abortion, and decreased milk production, while 38.67% answered correctly.

Among females, 57.53% acknowledged transmission among cattle, while 42.47% did not. In comparison, males exhibited a higher affirmative response rate, with 75% acknowledging transmission. This gender-based divergence, although present, did not exhibit significant statistical variance ($\chi^2 = 0.49$, P = 0.49). When considering age groups, distinct patterns in knowledge levels were evident. Among the younger participants, 76% were aware of common symptoms, while 84% recognized the impact on cattle productivity. These figures contrasted with the responses from adult participants, where 91.67% acknowledged the zoonotic transmission and 93.75% understood its impact on cattle productivity. The statistical analysis underscored significant differences between the age groups, particularly concerning the common symptoms and impacts of bovine brucellosis ($\chi^2 = 20.48$, $p < 0.05$ and $\chi^2 = 7.80$, P = 0.02, respectively, Table 8).

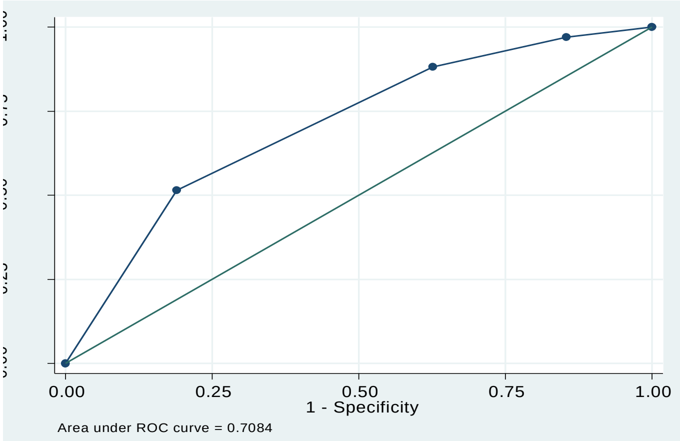

Figure 2. The area under the receiver operating characteristic (ROC) curve the performance of diagnostic tests and the predictive accuracy of various factors in the context of bovine brucellosis management and control strategies

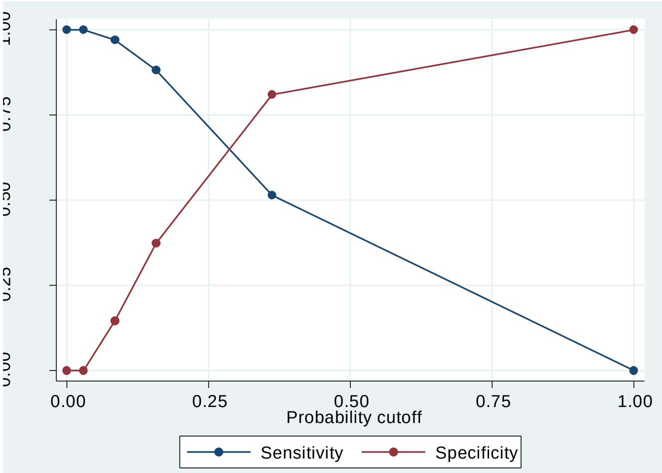

Figure 3. Illustration explaining the relationship between setting a probability cutoff in classification models and its impact on model sensitivity

Table 5. Socio-demographic characteristics of respondents (n = 150) in Ethiopia between November 2023 and June 2024

Variables	Category	Frequency (%)
Gender	Male	30(20)
	Female	120(80)
Age	Young (18-35)	52(34.67)
	Adult (35-52)	76(50.67)
	Old (52 and above)	22(14.67)
Education Level	No formal education	74(49.33)
	Primary school	66(44.00)
	Secondary school and above	10(6.67)
Occupation	Farmer	56(37.33)
	Veterinary professional	8(5.33)
	Agricultural worker	27(18.00)
	Student	59(39.33)
Herd Size	Small	64(42.67)
	Medium	59(39.33)
	Large	27(18.00)
Experience with Livestock	Novice (less than 1 year)	59(39.33)
	Intermediate (1-5 years)	64(42.67)
	Experienced (5+ years)	27(18.00)
Access to Veterinary Services	Easily accessible	27(18.00)
	Somewhat accessible	59(39.33)
	Not accessible	64(42.67)
Body Condition	Good	33(22)
	Poor	117(78)

Table 6. Respondents' attitude toward bovine brucellosis (n = 150) in Ethiopia between November 2023 and June 2024

Attitude queries	Response	Frequency (%)
Q1. How concerned are you about the impact of bovine brucellosis on cattle health and productivity?	Agree	9(6.00)
	Neither agree nor disagree	47(31.33)
	Disagree	94(62.67)
Q2. What is your level of awareness regarding the symptoms and transmission of bovine brucellosis?	Agree	69(46.00)
	Neither agree nor disagree	18(12.00)
	Disagree	63(42.00)
Q3. Do you believe that bovine brucellosis poses a significant threat to public health?	Agree	104(69.33)
	Neither agree nor disagree	36(24.00)
	Disagree	10(6.67)
Q3. In your opinion, what are the main challenges in preventing and controlling bovine brucellosis in cattle populations?	Agree	112(74.67)
	Neither agree nor disagree	20(13.33)
	Disagree	18(12.00)
Q4. How likely are you to seek veterinary assistance or report suspected cases of bovine brucellosis in your cattle?	Agree	69(46.00)
	Neither agree nor disagree	18(12.00)
	Disagree	63(42.00)
Q5. Are you satisfied with the current level of government support and policies aimed at preventing and controlling bovine brucellosis?	Agree	17(11.33)
	Neither agree nor disagree	34(22.67)
	Disagree	99(66.00)

Table 7. Respondents' knowledge level on bovine brucellosis (n = 150) in Ethiopia between November 2023 and June 2024

Knowledge queries	Response	Frequency (%)
Q1. Is the primary causative agent of bovine brucellosis a bacterium called *Brucella?*	Yes	92(61.33)
	No	58(38.67)
Q2. Is bovine brucellosis typically transmitted among cattle through contact with infected reproductive fluids or tissues?	Yes	103(68.67)
	No	47(31.33)
Q3. Are common symptoms of bovine brucellosis in cattle fever, abortion, and decreased milk production?	Yes	58(38.67)
	No	92(61.33)
Q4. Can bovine brucellosis be transmitted from cattle to humans, causing a zoonotic infection?	Yes	43(28.67)
	No	107(71.33)
Q5. If you answered "Yes" to the previous question, which of the following are clinical signs of Bovine brucellosis?	Abortion	23(15.33)
	Decreased milk production	53(35.33)
	Joint swelling	74(49.33)
Q6. Does bovine brucellosis impact cattle productivity and reproduction by causing abortion, reduced fertility, and decreased milk production?	Yes	116(77.33)
	No	34(22.67)
Q7. Do potential economic consequences of bovine brucellosis outbreaks in a cattle herd include loss of productivity, treatment costs, and trade restrictions?	Yes	114(76.00)
	No	36(24.00)
Q8. Can biosecurity measures such as quarantine, disinfection, and testing help prevent the spread of bovine brucellosis within a cattle herd?	Yes	143(95.33)
	No	7(4.67)
Q9. If you answered "Yes" to the previous question, which of the following factors were associated with the occurrence of Bovine brucellosis in your flock?	Contact with infected animals	136(90.67)
	Contaminated feed or water sources	3(2.00)
	Poor biosecurity practices	11(7.33)

Table 8. Association between demographic variables with knowledge level of participants in bovine brucelosis in Ethiopia between November 2023 and June 2024

Variable	Characteristic	Response									
		Transmission among cattle		**Common symptoms**		**Zoonotic transmission**		**Availability of vaccines**		**Impact on cattle productivity**	
		Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)
Gender	Female	84(57.53)	62(42.47)	117(80.14)	29(19.86)	55(37.67)	91(62.33)	50(34.25)	146(65.75)	127(86.99)	19(13.01)
	Male	3(75)	1(25)	1(25)	3(75)	2(50)	2(50)	1(25)	3(75)	3(75)	1(25)
	Statistics	χ^2= 0.49 P=0.49		χ^2=7.05 P=0.00		χ^2=0.25 P=0.61		χ^2=0.15 P= 0.70		χ^2=0.48 P=0.49	
Age	Young	29(58)	21(42)	38(76)	12(24)	11(22)	39(78)	8(16)	42(84)	42(84)	8(16)
	Adult	39(52.7)	9(18.75)	44(91.67)	4(8.33)	16(33.33)	32(66.67)	27(43.75)	21(43.75)	45(93.75)	3(6.25)
	Old	19(36.54)	33(63.46)	36(69.23)	16(30.77)	30(57.69)	22(42.31)	16(30.77)	36(69.23)	43(82.69)	9(17.3)
	Statistics	χ^2=20.48 P=0.000		χ^2=7.80 P=0.02		χ^2=14.44 P=0.00		χ^2=18.05 P=0.00		χ^2=3.10 P= 0.21	
Education	No education	39(52.7)	35(47.3)	61(82.43)	13(17.57)	25(33.78)	49(66.22)	23(31.08)	51(68.92)	64(86.49)	10(13.51)
	Primary school	40(60.61)	26(39.39)	50(75.76)	16(24.24)	26(39.39)	40(60.61)	24(36.36)	42(63.64)	57(86.36)	9(13.64
	2nd and above	8(80)	2(20)	7(70)	3(30)	6(60)	4(40)	4(60)	6(60)	9(90)	1(10)
	Statistics	χ^2=3.02 P=0.22		χ^2=1.41 P=0.49		χ^2=2.67 P=0.26		χ^2=0.61 P= 0.74		χ^2=0.10 P=0.95	
BCS	Good	24(72.73)	9(27.27)	20(60.61)	13(39.39)	13(39.39)	20(60.61)	11(33.33)	22(66.67)	28(84.85)	5(15.15)
	Poor	63(53.85)	54(46.15)	98(83.76)	19(16.24)	44(37.61)	73(62.39)	44(36.36)	77(63.64)	102(87.18)	15(12.82)
	Statistics	χ^2=3.77 P=0.05		χ^2=8.22 P=0.00		χ^2=0.04 P=0.85		χ^2=0.01 P=0.93		χ^2=0.12 P=0.73	
Season	Dry	60(60)	40(40)	81(76.42)	25(23.58)	44(41.51)	62(58.49)	34(32.08)	72(67.92)	91(85.85)	15(14.15)
	Wet	27(61.36)	17(38.42)	37(84.07)	7(15.91)	13(29.55)	31(70.45)	17(38.36)	27(61.64)	39(88.64)	5(11.36)
	Statistics	χ^2=0.29 P=0.59		χ^2=1.09 P=0.29		χ^2=1.89 P=0.17		χ^2=0.59 P=0.44		χ^2=0.20 P=0.65	
Access	Accessible	19(57.58)	14(42.42)	27(81.82)	6(18.18)	7(21.21)	26(78.79)	12(36.36)	21(63.64)	25(75.76)	8(24.24)
	Somewhat	33(58.93)	23(41.07)	46(82.14)	10(17.86)	27(48.21)	29(51.79)	16(28.57)	40(71.43)	52(92.86)	4(7.14)
	Not accessible	35(57.38)	26(42.62)	45(73.77)	16(26.23)	23(37.7)	38(62.3)	23(3770)	38(62.3)	53(86.89)	8(13.11)
	Statistics	χ^2=0.03 P=0.98		χ^2=1.47 P=0.48		χ^2=6.42 P=0.04		χ^2=1.19 P=0.55		χ^2=5.26 P=0.03	

χ^2: chi-squared; P: p-value; BCS: Body Condition Score

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DISCUSSION

Brucellosis, a zoonotic disease prevalent in many developing regions, inflicted significant losses on the livestock industry and small-scale livestock keepers. This disease, capable of transmission from animals to humans, posed a substantial public health risk (Franc et al., 2018). The present research revealed that the combined prevalence of *Brucella* antibodies, as detected through both RBPT and ELISA tests, stood at 27.27% in North Gondar and 9.62% in Central Gondar areas within the Amhara region. The overall prevalence of bovine brucellosis in dairy farms across the Central and North Gondar zones of the Amhara region was calculated to be 17.71%.

The current investigation unveiled a heightened prevalence compared to certain preceding studies. This overall seroprevalence of 17.71% exceeded figures documented in previous research conducted in Ethiopia, such as 0.4% in the Oromia Special Zone encircling Addis Ababa by Bifo et al. (2020), 3% in Bishoftu Town, Oromia by Waktole et al. (2018), and 0.6% in selected cities of the Central Highlands of Ethiopia by Getahun et al. (2023). Nielsen (2018) reported an overall prevalence of 0.14% in the North Gondar Zone, contrasting with the higher prevalence found in the current study.

The detection of *Brucella* antibodies relied solely on the Complement Fixation Test, revealing a prevalence of 5.7% in selected districts of the Afar National Regional State, Ethiopia as documented by Negash and Dubie (2021). Individual animal-level prevalence of 0.06% and herd-level prevalence of 0.8% were reported through c-ELISA in dairy farms within Addis Ababa by Edao et al. (2018). Asmare et al. (2013) reported an overall farm-level prevalence of 10.6% in Ethiopia. Additionally, Mekonnen et al. (2010) found a prevalence of 4.9% in Western Tigray, while Mussie et al. (2007) reported 4.63% in the Bahir Dar milk shed, similar with the current study's results.

Degefu et al. (2011) reported prevalence of 1.38% in the Jigjiga Zone, which was lower that current study results. Alehegn et al. (2017) found 4.9% around Gondar Town, and Yayeh (2003) found 0.14% in North Gondar Zone. Notably, the current found prevalence of 17.71% which was higher the previous reports of Molla (1989) at 8.2% in the Arsi area and Yohannes et al. (2012b) at 8.1% in and around Addis Ababa. Factors contributing to this increase may include geographical location and specific demographics of the studied population. Differences in farming practices, animal husbandry methods, and biosecurity measures among regions or populations could contribute to varying levels of *Brucella* infection (Wolff et al., 2017). Utilizing more accurate or sensitive diagnostic techniques in the current study may have led to a higher detection rate compared to studies that relied on less sensitive methods. Changes in these herd management practices, breeding practices, herd size; presence of wildlife, geographical location, feed and water quality, presence of aborted fetuses, human factors, lack of vaccination, climate conditions over time could potentially impact the spread and high prevalence of the disease.

Similar to present findings, Bekele et al. (2002) documented a prevalence of 18.4% in selected farms and ranches in South Eastern Ethiopia. In the Arsi region, Molla (1989) reported a prevalence of 16.8%, while Taye (1991) identified 14.2% at Abernosaranch. Surrounding Addis Ababa, Gebremariam (1985) found an 18.4% prevalence in dairy farms. Taye (1991) and Yirgu (1991) observed a prevalence of 19.5% in East of Ethiopia. Moreover, urban and peri-urban dairy farms displayed a prevalence of 16.9%. Taken together, these results indicate a consistent prevalence pattern across different regions and types of dairy farms in Ethiopia. In contrast, the current findings demonstrate a lower prevalence compared to earlier studies. For example, Reshid (1993) documented a prevalence of 38.7% in and around Addis Ababa. Mekonnen et al. (2010) observed a prevalence of 24.1% in Western Tigray, while Corbel (2006) noted a 33% prevalence in commercial and breeding farms, with Asmare et al. (2013) reporting a prevalence of 20.0%. When comparing the current research outcomes from Ethiopia with studies primarily conducted in African nations, the present results indicate a higher prevalence compared to previous findings across Africa. In South Africa, Kolo et al. (2019) documented a prevalence of 5.5% in animals that were tested via RBPT. Subsequent confirmation through I-ELISA revealed an overall animal-level prevalence of 1.20% in Bangladesh, as reported by Hassan et al. (2014). In Caquetá state, Motta-Delgado et al. (2020) identified a prevalence of 3.23%. Furthermore, Nahar and Ahmed (2009) reported a prevalence of 4.5% in the Mymensingh District in Bangladesh. Conversely, the current seroprevalence results in Ethiopia exhibit a lower prevalence compared to other African countries. For instance, Angara et al. (2004) found a prevalence of 24.9% in the Kuku Dairy Scheme in Sudan, with c-ELISA as a confirmatory test subsequent to RBPT screening. Maiga et al. (1995) noted a prevalence of 19.7% in Mal, while Kabagambe et al. (1988) identified a prevalence of 25.7% in Rwanda. Understanding these variations can offer valuable insights for developing targeted interventions and control measures to effectively manage disease prevalence across diverse geographical regions (Jagapur et al., 2013).

The current findings held significant importance in identifying various associated risk factors. Dairy farms situated in North Gondar showed notably higher odds of bovine brucellosis compared to those in Central Gondar. In the univariate analysis, the odds ratio (COR) was recorded at 3.53 (95% CI 2.00-6.22), which increased to 4.48 (95% CI 2.42-8.27) during the multivariate analysis after accounting for other factors. The examination revealed that extensively categorized farms demonstrated significantly higher odds of bovine brucellosis compared to intensively managed farms in both univariate (COR = 3.01, 95% CI 1.55-5.85) and multivariate (AOR = 2.37, 95% CI 1.24-4.51) analyses, emphasizing the impact of farm management practices on disease prevalence.

Herd size also played a role, with medium-sized herds showing increased odds in the multivariate analysis (AOR = 2.37, 95% CI 1.24-4.51) compared to small herds. Factors such as age (2.35, 95% CI 1.38-4.02), vaccinations (2.39, 95% CI 1.33-4.27), feeding status (2.21, 95% CI 1.21-4.05), and breeding methods (0.40, 95% CI 0.21-0.71) all displayed

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significant associations with bovine brucellosis in the analyses, highlighting their importance in disease transmission and control. Animals in natural mating may exhibit a higher seropositivity for *Brucella* infection compared to those animals contributed in artificial insemination, likely due to close confinement and exposure to diseased animals.

In the study that examined awareness levels of brucellosis across different demographic factors, several key findings emerged. Females displayed higher awareness across all categories compared to males, particularly excelling in understanding common symptoms, zoonotic transmission, vaccine availability, and the impact on cattle productivity. Age groups were significantly linked to awareness levels regarding transmission among cattle, common symptoms, zoonotic transmission, vaccine availability, and the impact on cattle productivity. In terms of education, individuals with primary school education demonstrated the highest awareness levels, followed by those with no education and secondary education, with no significant associations identified between education levels and awareness levels. Cattle with poor body condition scores exhibited higher awareness, with significant associations in understanding common symptoms and vaccine availability. Awareness levels remained consistent throughout the year, with no significant differences between dry and wet seasons. Access to information did not notably impact awareness levels, except for a marginally significant effect on understanding zoonotic transmission and cattle productivity impacts. These findings collectively highlight the necessity for targeted educational interventions to enhance brucellosis awareness, particularly among males, older individuals, and those with lower educational attainment.

Furthermore, cattle situated in environments with inadequate house sanitation exhibited significantly higher odds of contracting bovine brucellosis compared to those in areas with acceptable house sanitation. After conducting a multivariable analysis with adjustments for other factors, the odds ratio increased to 6.15, highlighting a strong and significant correlation between the quality of house sanitation and the prevalence of bovine brucellosis. A similar trend was observed in calf management practices concerning bovine brucellosis. Cattle under inadequate calf management displayed notably higher odds of contracting the disease compared to those under proper management practices. The impact of educational attainment on bovine brucellosis prevalence was also examined. In the univariable analysis, it was noted that livestock owners with a secondary education or higher exhibited significantly reduced odds of being affected by bovine brucellosis compared to those with a primary education level. Following a multivariable analysis considering other variables, the odds ratio decreased further to 0.33, indicating a robust inverse relationship between higher educational levels and the prevalence of bovine brucellosis. This association was also reflected in the relatively good hygienic conditions on the farms and the practices implemented to dispose of aborted materials to prevent animal contact (Getahun et al., 2023).

There were no significant differences in transmission rates between females and males. Females displayed significantly higher rates of common symptoms compared to males. No significant variance was detected in zoonotic transmission between females and males. Vaccine availability remained consistent across genders. Females had a greater impact on cattle productivity compared to males. Notably, there was a significant difference in transmission rates among different age groups, with younger individuals exhibiting higher rates. Young individuals also displayed more common symptoms compared to adults and older individuals. A significant distinction was observed in zoonotic transmission among age groups, with younger individuals having a higher potential. Vaccination availability varied significantly among age groups, with young individuals having a more substantial impact on cattle productivity compared to adults and older individuals. No significant differences were noted based on education levels ($\chi^2 = 3.02$, $P = 0.22$). Common symptoms did not show significant differences based on education levels. Zoonotic transmission did not present a clear pattern based on education levels. Vaccine availability appeared consistent across education levels. The impact on cattle productivity varied slightly based on education levels, but these differences were not statistically significant. There was a notable difference in transmission rates between individuals with good and poor BCS. Individuals with poor BCS exhibited significantly more common symptoms. No significant variation in zoonotic transmission was evident based on BCS. Vaccine availability remained consistent across BCS categories. Individuals with poor BCS had a higher impact on cattle productivity. There were no significant differences in transmission rates based on the season. However, the dry season showed slightly higher common symptoms than rainy season. No clear pattern emerged based on the season for zoonotic transmission. Vaccine availability appeared consistent across seasons. The dry season had a slightly higher impact on cattle productivity, however these differences were not statistically significant. No significant differences in transmission rates were observed based on accessibility. There was no clear pattern based on accessibility levels.

CONCLUSION

The data presented in this study indicated a significant association between gender and knowledge of common symptoms related to bovine brucellosis, specifically fever, abortion, and decreased milk production in cattle. The questionnaire survey conducted in this study revealed a notable association between gender and awareness of bovine brucellosis symptoms. 80.14% of females were informed compared to 25% of males. Concerning age, 58% of young respondents, 52.7% of adults, and 36.54% of older individuals were knowledgeable about brucellosis transmission, indicating a strong correlation. In Ethiopia, brucellosis presented a substantial public health concern, notably in Central Gondar (9.62%) and North Gondar (27.27%), with an overall seroprevalence of 17.71%, highlighting a high prevalence of bovine brucellosis antibodies. Future research on bovine brucellosis could explore genetic susceptibility factors in cattle breeds for resistant breeding, develop advanced diagnostic tools, analyze transmission dynamics, adopt a One Health approach, assess vaccine efficacy, investigate antimicrobial resistance, evaluate economic impacts, analyze farm biosecurity, explore zoonotic risks, and consider the effects of climate change to enhance prevention and management of the disease.

DECLARATIONS

Competing of interests

There are no conflicts of interest.

Availability of data and materials

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethical consideration

The animal study was reviewed and approved by Institutional Review Board of University of Gondar. Written informed consent was obtained from the owners for the participation of their animals in this study.

Authors' contributions

Mastewal Birhan was responsible for the conception of the study and prepared the initial draft of the manuscript. Mastewal Birhan collected the blood samples and questionnaire data, analyzed the data, and supervised the study's conduct. Mastewal Birhan has read, revised, improved, and approved the final manuscript. Mastewal Birhan approved the submitted version of the manuscript.

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