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The Ethiopian Veterinary Journal (*Ethiop. Vet. J.*) is a multidisciplinary peer-reviewed journal intended to promote animal health and production of national and regional/international importance. The journal publishes review articles, original research articles, short communication as well as technical notes in English. Under special circumstances, articles in Amharic may be considered for publication.

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Knowledge, attitude, and practices regarding rabies among residents of Mekdella district, South Wollo, Ethiopia

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Abstract

Rabies, a highly fatal neglected tropical disease that affects warm-blooded animals, was the subject of a cross-sectional study conducted from January 2021 to April 2021. The study aimed to assess the level of knowledge, attitudes, and practices (KAPs) regarding rabies and associated risk factors among the communities of Mekdella district. A semi-structured questionnaire was administered to a total of 384 households randomly selected from six kebeles. Factors associated with KAP were evaluated using the logistic regression model. The findings revealed that about 60% and 57% of respondents have good knowledge and implement preventive practices, respectively. However, the majority of respondents were unaware of the causative agent of rabies (55.7%) and rabies post-exposure prophylaxis (65.9%). Although 88.3% of the respondents knew about vaccination as a means of rabies prevention, only 20.1% of the respondents had their dogs vaccinated. About 53.9% of respondents would wash the wound with water and soap if bitten by rabid animals. Educated respondents were eight times (AOR = 7.7; 95% CI: 2.5-23.7) and three times (AOR = 3.1; 95% CI: 1.2-8.0) more likely to have good knowledge and practice preventive measures against rabies, respectively, compared to illiterate participants. Respondents who were trained about rabies were nine times (AOR = 9.7; 95% CI: 1.4-66.4) and five times (AOR = 4.5; 95% CI: 1.2-16.3) more likely to have good knowledge and implement preventive practices, respectively, than the non-trained ones. Being previously exposed to rabies made respondents four times (AOR = 3.9; 95% CI: 1.8-8.3) and two times (AOR = 2.3; 95% CI: 1.2 - 4.3) more likely to have good knowledge and implement preventive practices regarding

rabies. This study revealed the vast gaps in the communities' knowledge on rabies and poor practices in the prevention and control of rabies and post-exposure prophylaxis. It underscores the importance of community involvement in improving rabies prevention and control, fostering a sense of responsibility and empowerment.

Keywords: Ethiopia; Knowledge and practice; Mekdella; Prevention and control; Rabies.

Introduction

Rabies is characterized by an acute encephalitis illness caused by the rabies virus of the genus *Lyssavirus* in the family of *Rhabdoviridae*. It affects all mammals and is present in all continents except Antarctica. Following the onset of clinical symptoms, rabies is almost always fatal (WHO, 2017; Aklilu *et al.*, 2021). People are usually infected following a deep bite or scratch from an animal with rabies, and transmission to humans by rabid dogs accounts for 99% of the cases. Africa and Asia have the highest rabies burden in humans and account for 95% of rabies deaths worldwide.

Studies conducted in different parts of Ethiopia reported an overall good knowledge, attitude, and practices (KAP) score among the communities about rabies ranging from 49.0% to 85.7% (Alie *et al.*, 2015; Abdela *et al.*, 2017; Abdela and Teshome, 2017; Gebremeskel *et al.*, 2019; Bihon *et al.*, 2020). A study in the Amhara region for example reported that 61%, 72%, and 45% of the respondents had adequate knowledge, desirable attitude, and good practice scores, respectively (Bahiru *et al.*, 2022), while 56.1%, 56.2%, and 61.3% of the study participants of Mekele city had a good level of knowledge, attitude, and practice on the prevention and control of rabies, respectively (Hagos *et al.*, 2020).

Domestic dogs are considered to be the primary sources of human rabies in Africa. Retrospective studies of rabies using data collected from 1990 - 2000 and 2015 - 2019 indicated that dogs were responsible for maintaining and disseminating rabies in Ethiopia and that they were the primary culprits of human rabies cases (Yimer *et al.*, 2002; Aklilu *et al.*, 2021). Furthermore, a separate study found that of the 655 animal bite cases recorded in the Bishoftu, Lemuna-bilbilo, and Yabelo districts, 96.5% were caused by dogs (Beyene *et al.*, 2018).

Rabies is a severe problem in Ethiopia, with thousands of people infected each year according to the Centers for Disease Control and Prevention (CDC, 2017). Several studies documented a significant number of human rabies exposure cases in different regions of Ethiopia over several years (Yibrah and Damtie, 2015; Teklu *et al.*, 2017; Yizengaw *et al.*, 2018; Gebru *et al.*, 2019). The estimated death toll is around 2,700 annually, which is one of the highest in the world (CDC, 2017). However, the true number of rabies deaths is likely even higher because many cases go unreported and Ethiopia lacks sufficient diagnostic laboratories to confirm all cases (CDC, 2017; Bihon *et al.*, 2020). According to the reports of Jemberu *et al.* (2013), an annual estimated rabies incidence in the North Gondar zone was 2.33 cases per 100,000 in humans, 412.83 cases per 100,000 in dogs, 19.89 cases per 100,000 in cattle, 67.68 cases per 100,000 in equines, and 14.45 cases per 100,000 in goats.

Rabies can be controlled and prevented with vaccination, however few places in Ethiopia offer life-saving human rabies post-exposure prophylaxis, and most people don't have the means to make it to a major hospital to get treated (CDC, 2017). Moreover, people's awareness about what to do if bitten by dogs is low, and they may contact local traditional healers for treatment instead, losing precious time, and increasing the danger of infection and death (CDC, 2017; WHO, 2017).

The World Health Organization, the World Organisation for Animal Health, the Food and Agriculture Organization, and the Global Alliance for Rabies Control have set a global target to eliminate human rabies deaths entirely by 2030 (WHO, 2017; Broban *et al.*, 2018). To achieve this ambitious goal, several strategies are being considered, including strict quarantine measures, elimination of stray dogs, control of rabies in wildlife, dog registration programs and raising awareness about rabies in communities.

While Mekdella district began eliminating stray dogs and offering prophylactic rabies vaccinations in October 2015, there is a need for more quantitative data on rabies in the area both in humans and in animals. Furthermore, there is a need to more understanding of how much the community knows about rabies and the importance of preventive measures. No prior studies have been conducted in this district, despite records of death of humans and animals due to rabies in the area. Therefore, this study aims to assess the community's knowledge, attitudes, and practices regarding rabies and to identify associated risk factors in Mekdela district of South Wollo zone.

Materials and methods

Study area

The study was conducted in the Mekdella district of the Amhara regional state of Ethiopia (Figure 1). The district has a latitude of 11.55°N, a longitude of 38.28°E, and an elevation ranging from 700 to 3600 meters above sea level. The area receives an annual rainfall of 891.23 mm and an average temperature of 16.5 °C. According to the Mekdella district administration development and statistical planning report, the total human population of the district is estimated to be 142,654. The district has an estimated 131,090 bovines, 24,872 equines, 126,406 ovine, 97,651 caprines, 145,643 poultry, and 16,500 pet population (Mekdella District Statistical Planning Office).

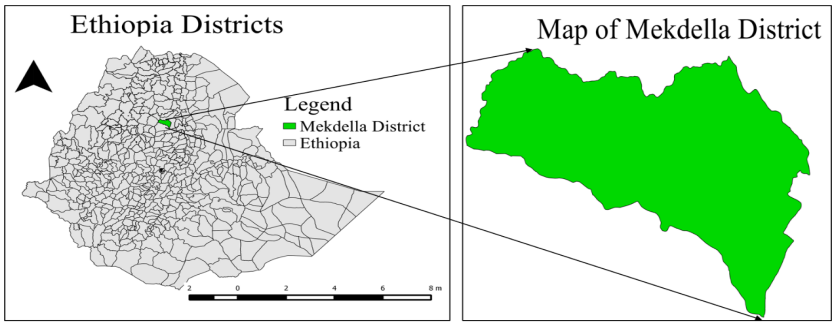


Figure 1. Map showing the study area.

Study design and population

A cross-sectional study design was employed from January 2021 to April 2021. The study population was household heads who had lived as permanent residents in randomly selected kebeles of the Mekdella district (Genatit, Deferge, Kibitiya, Gonderochi, Kebele 01 and 02). A systematic random sampling method was used to select study participants from households that had lived for more than six months in a Kebele.

Sample size determination and sampling techniques

The required sample size for this study was estimated by considering 50% of the population knowing about rabies since there were no studies on KAPs related to rabies in the study area. Thus, the sample size was calculated ac-

cording to Thrusfield (2018) using a 95% confidence interval and 0.05 absolute precision; 422 respondents were selected as study subjects, with a 10% non-response rate.

A simple random sampling procedure was employed to select kebeles. Of the 32 kebeles in the district, six were randomly selected using the lottery method. Then, 70 households were further selected from each kebele using the systematic random sampling method. In the absence of an eligible respondent in a given household, the next household was substituted.

Questionnaire survey methods

A semi-structured questionnaire survey was used to capture details of the household characteristics that were used to assess the socio-demographic factors (sex, age, religion, education status, occupation, and family size) and social and environmental factors (dog ownership, source of information, and awareness), and disease-related factors (training, awareness, and family exposure). Additional questions include knowledge of rabies, especially on the disease, mode of transmission, species affected, and means of prevention and control, as well as practices about rabies prevention strategies, including willingness to vaccination.

The questions were pre-tested to ensure the questionnaire's applicability and revised accordingly. One adult person from each household (more than 18 years old and who lived more than 6 months as a permanent resident in the study area) was interviewed face-to-face. The questionnaire was first developed in English and then translated into Amharic (local language) for appropriateness and ease of approach to the study participants.

Data management and analysis

The data were checked for completeness, clarity, consistency, and accuracy. Then the data were cleaned, coded, and entered into a Microsoft Excel spreadsheet 2016 and analyzed using SPSS version 25. Descriptive statistics were used to present results in tables and figures. Binary logistic regression was used to show the association between independent variables with dependent variables. The knowledge and preventive practice of the respondents were determined by computing knowledge and practice-related questions, respectively. KAP questions were given scores and dichotomized into good and poor KAP levels using a cut-off of 50% of the highest score possible (Bahiru *et al.*, 2022).

Binary knowledge and practice questions received a score of 1 for a correct response and 0 for an incorrect response. Attitude questions were given 0 for I don't know the response, 1 for an incorrect response to the question, and 2 for the correct answer to the question. Respondents who had a score of 50% or more were thought to have adequate knowledge, good practice, and a desirable attitude. In comparison, those who received a score of less than 50% were thought to have inadequate information, poor practice, and an undesirable attitude (Hagos *et al.*, 2020; Bahiru *et al.*, 2022). Variables having *p*-value< 0.2 in the univariable logistic regression were fitted into the multiple logistic regression models. Adjusted odds ratio (AOR) with a 95% Confidence Interval (C.I) was computed to assess the presence and strength of associations. Variables having a *p*-value less than 0.05 in multiple logistic regressions were considered as significantly associated with the dependent variables.

Data quality assurance

Before the study began, a pre-test was performed on 20 veterinary and paraveterinary professionals in the district to validate the questionnaires. Each questionnaire was checked for incompleteness, missed values, and unlikely responses and then manually cleaned upon such indications. The data were cross-checked for consistency and accuracy.

Results

Socio-demographic characteristics

Out of the 422 heads of household interviewed, 38 individuals (9%) were discovered to have incomplete data, and as a result, they were excluded from the analysis. The majority of the respondents, 307 (79.9%) were male. Among the respondents, nearly half, specifically 186 individuals (48.5%), fell within the age range of 18 to 35. A slight majority of the participants, comprising 207 individuals (53.9%), identified themselves as Muslim, while the second largest religious group was Orthodox, with 177 individuals (46.1%). Concerning educational status, 150 (39.1%) of the participants were illiterate, followed by 140 (36.5%) primary school, 48 (12.5%) secondary school, and 46 (11.9%) higher education. Among interviewed households, 64.3% (247), 16.9% (65), and 11.9% (46) were farmers, unemployed, and government employees, respectively (Table 1).

Table 1. Socio-demographic information of the study participants.

Variables		No. of respondents (%)	Knowledge		Practice	
			Good	Poor	Good	Poor
Sex	Male	307 (79.9)	191	116	182	125
	Female	77 (20.1)	38	39	37	40
Religion	Muslim	207 (53.9)	127	80	113	94
	Orthodox	177 (46.1)	102	75	106	71
Educational status	Illiterate	150 (39.1)	65	85	66	84
	Primary school	140 (36.5)	90	50	80	60
	Secondary school	48 (12.5)	35	13	35	13
	Higher education	46 (11.9)	39	7	38	8
Occupation	Jobless	65 (16.9)	33	32	33	32
	Farmer	247 (64.3)	138	109	135	112
	Merchant	26 (6.8)	20	6	16	10
	Employed	46 (11.9)	38	8	35	11
Age	18-35 years	186 (48.5)	115	71	101	85
	36-55 years	161 (41.9)	91	70	90	71
	> 55 years	37 (9.6)	23	14	28	9
Family size	≤3	271(70.6)	160	111	156	115
	4-6	101 (26.3)	61	40	55	46
	>6	12 (3.1)	8	4	8	4

Knowledge of respondents related to rabies

Figure 2 illustrates that the community of Mekdella district exhibited a favorable level of knowledge regarding rabies, with 60% of the population demonstrating good knowledge on the subject. The majority of respondents (59.1%) had no awareness of rabies, and 55.7% of respondents didn't know the causative agent. However, the majority of the respondents knew the means of transmission of rabies (83.6%), and the disease could not easily be treatable once clinical signs were manifested (89.6%). Besides, the majority of the respondents (65.9%) were unaware of post-exposure prophylaxis for rabies. In

contrast, 88.3% of the respondents were aware of dog vaccination as a means of rabies prevention, as shown in Table 2.

Table 2. Knowledge of the community of Mekdella district related to rabies.

Questions	Yes (%)	No (%)
Do you know the causative agents of rabies?	170 (44.3)	214 (55.7)
Do you know the means of transmission of rabies?	321(83.6)	63 (16.4)
Is rabies easily treatable after the onset of clinical signs?	40 (10.4)	344 (89.6)
Do you know about post-exposure prophylaxis?	131 (34.1)	253 (65.9)
Can we prevent rabies by vaccination of dogs?	339 (88.3)	45 (11.7)
Do you know the vaccination interval for dogs?	110 (28.6)	274 (71.4)
Does castration decrease the incidence of rabies?	294 (76.6)	90 (23.4)

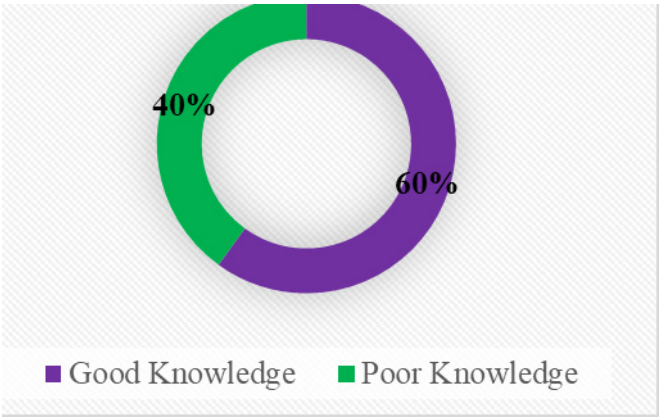


Figure 2. Overall knowledge status of respondents after computing knowledge-related questions.

The majority of respondents (71.6%) believed that almost all animals are affected by rabies and almost half of the respondents (49%) also believed that all animals could transmit rabies to humans. In addition, more than half of the respondents (59.4%) responded that both bite and contact with saliva were the means of rabies transmission (Table 3).

Table 3. Respondent's knowledge of rabies-affected species, causes, and its transmission to humans.

Questions	Response	Number (%)
Which species are affected by rabies?	Dog only	29 (7.6)
	Wild animals	16 (4.2)
	Dog and humans	21 (5.5)
	Human and domestic animals	43 (11.2)
	All animals	275 (71.6)
Which animals transmit rabies to humans?	All animals	188 (49)
	Dog and domestic animals	26 (6.8)
	Dog and cat	67 9 (17.4)
	Dog only	103 (26.8)
Means of transmission of rabies?	Infected meat	10 (2.6)
	Contact with saliva	74 (19.3)
	Bite	72 (18.8)
	Bite and contact with saliva	228 (59.4)
Clinical signs of rabies?	Behavioural change	148 (38.5)
	Salivation	184 (47.9)
	Hydrophobia	52 (13.5)

Community attitudes toward rabies

Fewer numbers of respondents (7%) believed the consumption of rabid animal meat could be a medicine for rabies. However, 52.6% disagreed with this idea. Nearly seven percent of respondents believed that burning and smoking rabid animals could be a medicine for rabies. However, 51.8% disagreed with this idea. The majority of respondents believed that traditional healers were a solution for rabies (73.9%), whereas about 91.6% of respondents also would seek health professional support if rabid animals bite them (Table 4).

Table 4. Attitudes of the community toward rabies.

Attitude Related Variables	Agree (%)	Disagree (%)	Don't know (%)
Rabid animal meat consumption as a medicine for rabies	27 (7)	202 (52.6)	155 (40.4)
Burning of rabid animals and inhalation of the smoke as a medicine for rabies	26 (6.8)	199 (51.8)	159 (41.4)
Traditional healers could be a solution for rabies	284 (73.9)	85 (22.2)	15 (3.9)
Seeking advice from a health professional during a dog bite	352 (91.6)	26 (6.8)	6 (1.6)

Preventive practices of the participants toward rabies

Overall, the preventive practices of respondents towards rabies indicated that 57% had good practices (Figure 3). Out of 384 respondents, 23.9% had dogs, and most of the dogs (76.1%) were kept free-ranging. The majority of the respondents from the community do not practice vaccination of dogs against rabies (79.9%). The majority of respondents take immediate action (killed/tied) on rabid animals (94.8%), seek medical help when humans are bitten by rabid animals (67.2%), and practice washing the wound with soap and water after a dog bite (53.9%) (Table 5).

Table 5. Preventive practices of the respondents toward rabies.

Practice related variables	Yes (%)	No (%)
Do you have dogs?	92 (23.9)	292 (76.1)
Do you manage your dog indoors?	113 (29.4)	271 (70.6)
Would you take immediate action on the rabid animals (killed/tied)?	364 (94.8)	20 (5.2)
Do you wash wounds with water and soap after a bite by a rabid dog?	207 (53.9)	177 (46.1)
Do you take persons to the health centers immediately after first aid?	258 (67.2)	126 (32.8)
Have you had your dog vaccinated for rabies?	77 (20.1)	307 (79.9)



Figure 3. Overall preventive practices status of respondents after computing practice related questions.

About one-third of the respondents, 115 (29.9%), visited traditional healers, 11 (2.9%) used holy water, while the majority, 258 (67.2%), preferred health centers for rabies treatment. About half of the respondents, 206 (53.6%), know about stray dog control to prevent rabies, and 143 (37.2%) killed stray dogs to prevent and control rabies (Figure 4).

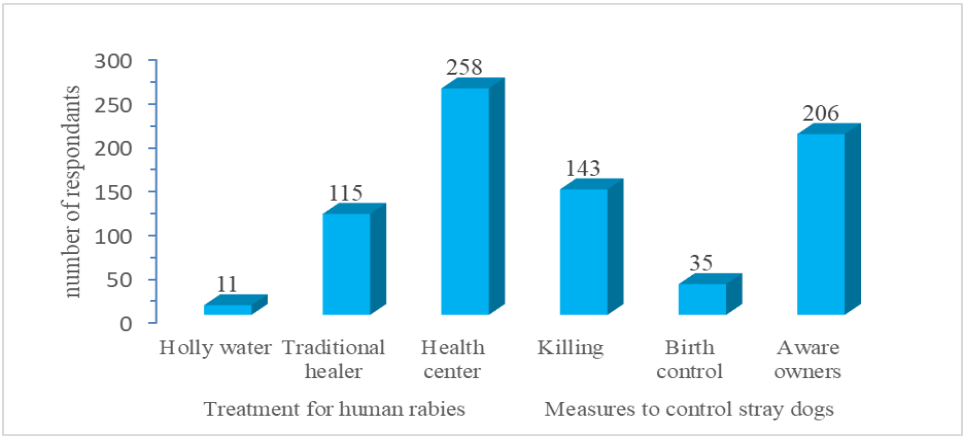


Figure 4. Measures for control of stray dogs and practices after a dog bite.

Univariable and multivariable logistic regression results of knowledge of rabies

After controlling the other variables, logistic regression was carried out to evaluate the association between independent variables and knowledge (Table 6). Respondents having a higher level of education were eight times (AOR = 7.7; 95% CI: 2.5-23.7) more likely to have good knowledge than that of illiterates. Respondents who had awareness were twelve times (AOR = 12.4; 95% CI: 6.4-24.2) more likely to have good knowledge than those who didn't have awareness of the disease. Respondents who gained health-related training were ten times (AOR = 9.7; 95% CI: 1.4-66.4) more likely to have good knowledge than those who didn't gain health-based training. Participants who had family exposure to rabies were four times (AOR = 3.9; 95% CI: 1.8-8.3) more likely to have good knowledge than those who didn't have family exposure. Other factors, suchas sex, age, religion, marital status, family size, and occupation, were not significantly associated with the knowledge of this disease (Table 6).

Table 6. Factors associated with the knowledge of respondents.

	Factors	Knowledge		COR (95%CI)	AOR (95% CI)
		Good	Poor		
Sex	Male	191	116	Ref.	Ref.
	Female	38	39	0.59 (0.4-0.9)	0.79 (0.4-1.7)
Educational	Illiterate	114	116	Ref.	Ref.
	Primary school	40	20	2.35 (1.5-3.8)	3.15 (1.7-5.9)
	Secondary school	30	18	3.52 (1.72-7.2)	4.69 (1.8-12.4)
	Higher education	45	1	7.29 (3.1-17.3)	7.68 (2.5-23.7)
Occupation	Jobless	29	36	Ref.	Ref.
	Farmer	142	105	1.23 (0.7-2.1)	1.69 (0.8-3.9)
	Merchant	17	10	3.23 (1.2-9.1)	2.24 (0.6-8.6)
	Employed	41	4	4.61 (1.9-11.4)	2.86 (0.8-9.8)
Awareness	No	90	137	Ref.	Ref.
	Yes	139	18	11.76 (6.4-20.6)	12.39 (6.4-24.2)
Dog ownership	No	166	126	Ref.	Ref.
	Yes	63	29	1.65 (1.0-2.7)	0.43 (0.2-0.9)
Training	No	198	153	Ref.	Ref.
	Yes	31	2	11.98 (2.8-50.8)	9.65 (1.4 -66.4)
Family-exposure	No	152	133	Ref.	Ref.
	Yes	77	22	4.53 (2.6-8.1)	3.91 (1.8-8.3)

Univariable and multivariable logistic regression results of practice toward rabies

Univariable and multivariable logistic regression was made by controlling other factors to identify the associations between independent factors and preventive practices toward rabies. Respondents with higher levels of education were three times (AOR = 3.1; 95% CI: 1.2-8.0) more likely to have good preventive practices for rabies disease than illiterates. Dog owners were four times (AOR = 4.4; 95% CI: 2.2-16.3) more likely to exercise good preventive practice compared to those who didn't have dogs. Respondents who gained health-related training were five times (AOR = 4.5; 95% CI: 1.2-16.3) more likely to have good preventive practice than those who didn't gain health-based training. Participants who had family exposure to rabies were two times (AOR = 2.3; 95% CI: 1.2-4.3) more likely to have good preventive practices than those who didn't have family exposure. Other factors, like sex, age, occupation, and awareness, were not significantly associated (P>0.05) with the preventive practice of respondents (Table 7).

Table 7. Factors associated with the preventive practice of respondents.

Factors		Practice		COR (95%CI)	AOR (95%CI)
		Good	Poor		
Sex	Male	182	125	Ref.	Ref.
	Female	37	40	0.64 (0.4-1.1)	0.69 (0.4-1.3)
Education	Illiterate	66	84	Ref.	Ref.
	Primary school	80	60	1.97 (1.1-2.7)	1.75 (1.2-2.9)
	Secondary school	35	13	3.43 (1.7-6.9)	2.599 (1.1-5.9)
	Higher education	38	8	6.04 (2.6-13.8)	3.11(1.2-8.0)
Age	18-35 years	101	85	Ref.	Ref.
	36-55 years	90	71	1.07 (0.7-1.6)	0.6-1.7)
	> 55 years	28	9	2.62 (1.2-5.9)	2.15 (0.9-5.4)
Occupation	Jobless	33	32	Ref.	Ref.
	Farmer	135	112	1.17 (0.7-2.0)	0.91 (0.4-1.9)
	Merchant	16	10	1.6 (0.6-3.9)	0.89 (0.3-2.8)
	Employed	35	11	3.09 (1.3-7.1)	2.11 (0.8-5.7)
Awareness	No	117	110	Ref.	Ref.
	Yes	102	55	1.74 (1.15-2.7)	1.0 (0.6-1.8)
Dogs	No	140	152	Ref.	Ref.
	Yes	79	13	6.59 (3.5-12.4)	4.44 (2.23-16.3)
Training	No	189	162	Ref.	Ref.
	Yes	30	3	8.57 (2.6-28.7)	4.48 (1.2-16.3)
Family exposure	No	140	145	Ref.	Ref.
	Yes	79	20	4.09 (2.4-7.0)	2.27 (1.2-4.3)

Discussion

In this study, more than half of the respondents (60%) had good knowledge regarding rabies, which was in line with those reported by residents of the Amhara region (61.3%) (Bahiru *et al.*, 2022). Slightly lower results (56.1% and 51.9%) were reported from Mekelle city and Dedo district of the Jimma zone (Abdela *et al.*, 2017; Hagos *et al.*, 2020), and higher results (64.1% and 85.7%) were reported at Bahir Dar city and Kombolcha town (Guadu *et al.*, 2014; Gebremeskel *et al.*, 2019). More than half of the respondents (55.7%) didn't know the causative agents of rabies, and this report disagrees with lower findings of 22%, 32.4%, 39.3%, 32.4%, and 21.6% in Munisa district, Dedo district, Gondar

city, Dessie city, and Debretabor city, respectively (Serebe *et al.*, 2014; Alie *et al.*, 2015; Gebeyaw and Teshome, 2016; Abdela *et al.*, 2017; Abdela and Teshome, 2017). Almost 59.4% of respondents in the current study replied that both bite and saliva contacts were the means of transmission of rabies; however, a lower finding of 9.8% was reported in the Dedo district and Gondar city (Serebe *et al.*, 2014; Abdela *et al.*, 2017). The variation in the knowledge status of the current study compared to other studies might be due to the variation in educational status, low community awareness, and limited disease training status.

About two-thirds of respondents (71.6%) replied that all animals are affected by rabies. This is in line with other findings reported in Addis Ababa (71.9) (Ali *et al.*, 2014) and Kombolcha town (72.9%) (Ali *et al.*, 2014; Gebremeskel *et al.*, 2019). In this study, only 34.1% of respondents were aware of post-exposure prophylaxis. However, higher results of 39.6% and 65.5% were reported in and around South Gondar and Dedo districts, respectively (Abdela *et al.*, 2017; Bihon *et al.*, 2020). Most of the respondents (73.9%) in this study believed in traditional healers. This is in agreement with reports from South Gondar (81.5%) (Bihon *et al.*, 2020) and North Gondar (84%) (Jemberu *et al.*, 2013; Bihon *et al.*, 2020). Studies conducted in Addis Ababa, Dessie city, and Araba Minch reported lower (58.3%, 46%, and 42.45%) beliefs in traditional healers, respectively (Ali *et al.*, 2014; Gebeyaw and Teshome, 2016; Tamirat *et al.*, 2016). The preference for traditional practices might arise from many factors, including easy access to medicinal plants, limited health centers, and low treatment costs in the study area.

In this study, the respondents believed that eating rabid animals' meat (7%) could be a medicine for rabies. This belief was also reported in Kombolcha (48.2%) (Bihon *et al.*, 2020) and South Gondar (10.6%) (Gebremeskel *et al.*, 2019). This indicates the perception gap between functional treatments and traditional beliefs. This belief revealed that the community in the study areas has limited knowledge of rabies post-exposure treatment and requires intensive awareness training.

The current finding revealed that more than half of the respondents (57%) had good preventive practices against rabies. A similar finding was reported by Hagos *et al.* (2020) in Mekelle City (61.3%), but a higher finding in Kombolcha town (79.9%) (Gebremeskel *et al.*, 2019). Almost half of the respondents (53.9%) would wash the wound with water and soap if bitten by rabid ani-

mals. A higher number of respondents practiced washing wound with water and soap (92.4%) in Kombolcha town (Gebremeskel *et al.*, 2019), than in South Gondar (8.9%) (Bihon *et al.*, 2020). Poor preventive and control practices in the current study might be associated with the characteristics of the respondents, including the fact that most lived in rural areas, their educational status, and low community awareness.

In the present study, although more than 80% of the respondents knew that rabies could be prevented by vaccinating dogs, only 20.1% of them had their dogs vaccinated. This indicated that dog vaccination practice is very poor in the area. Poor and non-existent dog vaccination practices were reported in the Dabat district, and good practices were reported in Gondar town (Jemberu *et al.*, 2013), and Dessie city (36%) (Gebeyaw and Teshome, 2016). Lower coverage of rabies vaccines in the current study area might be due to a lack of access and low awareness of rabies vaccination. Thus, raising awareness about dog vaccination and improving access and affordability of the vaccine need to be done to control the disease.

In this study, 41.9% of informants obtained information related to rabies from informal sources. This indicated the need for extensive public education about rabies to increase dog-associated rabies awareness. Many scholars have mentioned raising the community awareness level as an essential tool to control rabies (Yimer *et al.*, 2002; Jemberu *et al.*, 2013; Rine *et al.*, 2017; Broban *et al.*, 2018).

Conclusion

This study showed considerable gaps in the community's knowledge of the cause, mode of transmission, and host range of rabies and poor practice in prevention and control methods for dog rabies and post-exposure prophylaxis. Therefore, veterinarians and health professionals should pay due attention to the periodic education of the community on rabies and improve the accessibility and affordability of the rabies vaccine for dogs and post-exposure prophylaxis for humans.

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Ethical consideration

The study protocol was reviewed and approved by the Ministry of Agriculture, Ethiopia, in collaboration with the Food and Agriculture Organization of the United Nations (FAO). Before conducting the research, the participants were informed of the study's objectives and benefits. Oral informed consent was obtained from each participant after informing them about the objectives, the benefits of the study, and the rights of the study participants. Only voluntary participants were enrolled in the study. They gave verbal consent for inclusion in the study because they were unable to write and read. These consents were taken in the presence of a third independent party.

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Declaration

The authors declare that they have no conflict of interest in this work.

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Prevalence of bovine mastitis, risk factors, changes in milk composition and bacterial isolation in and around Modjo town, central Ethiopia

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Abstract

A cross-sectional study was conducted on 316 lactating dairy cows from November 2021 to May 2022 in and around Modjo. The main objectives of this study include estimating the prevalence and isolation of aerobic bacterial pathogens and observing compositional changes in mastitic milk. To this end, mastitis was diagnosed both clinically and using California mastitis test (CMT). Isolation of major aerobic pathogens and alterations in milk composition were determined using their respective standard techniques. Accordingly, the overall prevalence of mastitis observed in the study was 72.8%, of which 9.5% was clinical and the rest 63.3% subclinical mastitis. Quarter level prevalence was 45.2%. Among the potential risk factors considered, the presence of teat/udder injury, poor udder and farm hygiene showed higher prevalence of mastitis ($p < 0.05$) compared to the corresponding categories. The most predominant bacterial pathogen isolated was *Staphylococcus aureus* (11.25%), followed by *E. coli* (10%), *Micrococcus* species (10%) and *Pseudomonas aeruginosa* (10%). Protein, solid non-fat (SNF), pH and freezing point measurements showed statistically significant difference ($p < 0.05$) between milk from mastitic and non-mastitic udders. However, fat and density were not statistically different ($p > 0.05$). In conclusion, mastitis was highly prevalent and clinically important disease of dairy cows in the study area and hence deserves attention to minimize its impact. Cows' udder and farm should be kept clean as much as possible to reduce the prevalence of mastitis. Some of these bacterial isolates can be the real causes of mastitis. However, studies should be conducted to establish causal relationship.

Keywords: Bacteria; Bovine mastitis; Ethiopia; Milk; Modjo; Prevalence.

Introduction

Ethiopia is believed to have the largest livestock population in Africa. The total cattle population of the country is estimated at 61.5 million. Of this total cattle population, female cattle constitute about 55.7 percent, and the remaining 44.3 percent are male cattle (CSA, 2019).

Even though cow milk is a vital source of nutrition for many people in Ethiopia, the country has long failed to satisfy the demand for milk and milk products through domestic production. Multiple factors contribute to this, one of the major reasons being the high prevalence of mastitis in dairy cows in the country (Fekadu, 1995).

Mastitis, one of the most economically important diseases for the dairy industry worldwide, continues to cause serious problems through reduced milk production (Korhonen and Kaartinen, 1995; Bradley, 2002). It can also pose a risk to human health (Al-Majali *et al.,* 2008), as bacterial contamination of milk from the affected cows may render it unsafe for human consumption (Quinn *et al.,* 1999).

Mastitis (Greek, "mastos" = breast + "itis" = inflammation), defined as inflammation of the parenchyma of the mammary glands, is a complex multi-etiological disease characterized by physical, chemical and usually bacteriological changes in milk, and pathological changes in the mammary gland tissues (Radostits *et al.,* 2007).

In dairy cattle, mastitis can have an infectious or non-infectious etiology, although the vast majority is of bacterial origin (Anon, 2001). Mastitis continues to be the most economically important disease of the dairy industry, accounting for about 38% of the total direct losses (Albenzio *et al.,* 2002).

There are two main types of bacteria that cause mastitis: contagious and environmental. Contagious bacteria, such as *Staphylococcus aureus* and *Streptococcus agalactiae*, spread easily between cows. Environmental bacteria, such as coliforms and some streptococci, live in the milking environment and can infect cows during milking. The risk of mastitis is likely to increase with poor milking sanitation, low barn sanitation, lack of teat dipping after milking, use of lubricants during milking, and lack of treatment of existing infection in milking cows of various age groups (Radostits *et al*., 2007).

Mastitis can be classified as clinical or subclinical. Clinical mastitis cases are characterized by the presence of one or more of the following signs such as abnormal milk, udder swelling and systemic signs including elevated temperature, lethargy and anorexia. In subclinical mastitis there are no visible changes in the milk or the udder, but milk production decreases, bacteria are present in the milk and composition of the milk is altered. For every case of clinical mastitis there are 20-40 times as many cases of subclinical mastitis (Eriskine, 2001). Subclinical mastitis in the mammary gland is detectable only by determining high somatic cell count (SCC) in milk or by bacterial culture (Quinn *et al.,* 1994).

Even though subclinical mastitis isn't as overt as clinical mastitis, it causes the greatest financial losses to the dairy industry. It is estimated to cause about 70% of the total losses. These losses, however, are difficult for producers to appreciate since they are associated with decreased milk production caused by the effects of chronic inflammation of the mammary gland without observable clinical signs (Radostits *et al.,* 2007).

One consequence of mastitis in dairy cows is a change in the composition of milk (Gianneechini *et al.,* 2002). An increase in pathogenic bacteria in milk is accompanied by an increase in somatic cell count and changes in milk physicochemical properties (Santos *et al.,* 2004; Rerk *et al.*, 2008).

Studies conducted in different parts of the country have shown that mastitis is a widespread problem in Ethiopia, with prevalence ranging from 6% to 74.7% (Biffa *et al*., 2005; Sori *et al.,* 2005; Mekbib *et al.,* 2010; Zeryehun *et al.,* 2013; Belachew, 2016; Abebe *et al.,* 2017; Tesfaye and Abera, 2018). However, there is a need for more information on the occurrence of bovine mastitis in and around Modjo town. Moreover, while these earlier studies explored the prevalence, the associated risk factors, and the bacteria involved in mastitis, there is a need for more information on the effect of mastitis on milk composition in the study area.

Therefore, the objectives of this study were to estimate the prevalence of mastitis, identify associated risk factors, isolate aerobic bacteria from mastitic milk, and study changes in milk composition in cows with mastitis in the study area.

Materials and methods

Study area description

The study was conducted in Modjo town from November 2021 to May 2022. Modjo is the administrative center of Lume wereda, located in the East Shewa Zone of the Oromia Region, Ethiopia. It is located at 73 km south east of Addis Ababa at an altitude of 1,777m above sea level. It is located at 8.36°N latitude and 39.7°E longitude. The monthly mean minimum and maximum temperature for Modjo town ranges from 8.5°C-13.5 °C to 25.6-30.8 °C, respectively (CSA, 2012).

Study animals

The study animals were Holstein-Zebu crossbred milking cows in selected small -and large-scale dairy farms and villages in Modjo town and the surroundings. The study animals were classified as young adults (3–6 years), and adults (≥7 years) based on their dentition (Wakeman and Pace, 1983). The herd size was categorized as few (< 10) and many (≥ 10). The number of parities was classified as few (1-3) and many (≥ 4). The stage of lactation was categorized as early (1-3 months), mid (4-6 months) and late (≥ 7 months).

Study design and sampling strategy

A cross-sectional study was conducted from November 2021 to May 2022. Lume Wereda agriculture office provided a list of dairy farms.Then, study farms and households were selected by simple random sampling, and all lactating cows in the selected farms were included in the study. This was done until the desired sample size was met.

Sample size determination

The desired sample size for the study was calculated using a 95% confidence interval, 5% absolute precision and 40.1% (Birhanu *et al.*, 2017) expected prevalence, as follows (Thrusfield, 2005):

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

Where, P_{exp} was expected prevalence, d absolute precision and n sample size. Accordingly, the calculated sample size was 221 animals. However 316 lactating cows were included in the study to increase precision and make the data representative.

Data collection

Data on the risk factors like age, parity, stage of lactation, udder hygiene, farm hygiene, presence of injury on the skin of udder/teat, and previous mastitis history were collected in a properly designed format.

Clinical examination

The udder was examined through visual inspection and palpation to detect possible abnormalities like swelling or decrease in size, pain, swelling of supra mammary lymph nodes, disproportional symmetry of teats, and blindness. Likewise, the milk was examined for discoloration and the presence of clots, flakes, blood, and watery secretions to detect clinical mastitis.

California mastitis test

The California mastitis test was carried out according to the method described by Quinn *et al*. (1999). A squirt of milk, about 2 ml from each quarter was placed in each of four shallow cups in the CMT paddle. An equal amount of the commercial reagent (Immuncell 56 Evergreen Drive, Portland, ME 04103) was added to each cup. A gentle circular motion was applied to the mixtures in a horizontal plane for 15 seconds. The result was scored from 0-3 and Score 0 (N) was considered negative and T (Trace), 1, 2 and 3 were considered positive for subclinical mastitis according to Quinn *et al*. (1999).

Milk sample collection

Milk samples from both forms of mastitis were collected according to the National Mastitis Council (NMC) (1990). The teats were cleaned with 70% ethyl alcohol, and approximately 10 ml of milk were collected in to a sterile test tube after discarding the first 3 milking streams. Then, samples were placed in racks for ease of handling and transported in an ice to Addis Ababa University, College of Veterinary Medicine and Agriculture, Microbiology Laboratory, and on arrival, it was inoculated on a standard bacteriological media for isolation and characterization of bacteria (Biru, 1989; NMC, 1990) and for milk composition analysis the milk was taken to Ethiopian Meat and Dairy Industry Development Institute (EMDIDI).

Bacteriological isolation

Milk samples were bacteriologically examined according to the procedures employed by Quinn *et al.* (1999). A loopful of milk samples collected from each infected quarter was inoculated separately on MacConkey agar and blood agar enriched with 5% defibrinated sheep blood. The inoculated plates were then incubated aerobically at 37 °C for 24 to 48 hours.

The identification of the bacteria on the primary culture was made on the basis of colony morphology, hemolytic characteristics, and Gram stain reaction, including the shape and arrangements of the bacteria, as well as catalase and O-F tests. Staphylococci were identified based on catalase test, growth characteristics on mannitol salt agar and purple agar, and tube coagulase test. Identification of streptococci was made according to growth characteristics on Edward's media, catalase test, hydrolysis of Esculin, and CAMP test. Gram negative isolates grown on MacConkey agar were identified based on growth characteristics on MacConkey agar, oxidase reaction, catalase test and the "IMViC" (Indole, Methyl red, Voges-Proskaur and Citrate) test (Quinn *et al*., 1999).

Chemical analysis of milk

Milk samples collected from both mastitis positive and negative cows (controls) were brought to Ethiopian Dairy and Meat Industry Development Institute (EDMIDI) milk chemistry laboratory and analyzed using Lactoscan Ultrasonic milk analyzer machine according to the manufacturer's (Milkotronic Ltd, Nova Zagora, Bulgaria) instructions. The pH was measured by a digital pH-meter, as described by Aggad *et al.* (2010).

Data analysis

Data were entered into Microsoft Excel sheet and analyzed using STATA software version 13. The Chi-square test was used to assess the association of factors with mastitis; two samples mean comparison *t*-test was used to compare means in milk composition of mastitis positive and negative cows. A difference was taken as significant at *p*-value less than 0.05 and the confidence level was held at 95%.

Results

Prevalence of mastitis

Out of 316 cows examined, 230 (72.8%) were positive for bovine mastitis, of which 30 (9.5%) were positive for clinical and 200 (63.3%) were positive for subclinical mastitis, as presented in Table 1. Out of 1264 quarters, 36 (2.85%) were blind teats and non-functional. Out of 1228 quarters with functional teats, 555 (45.2%) were mastitis positive, as presented in Table 2.

Table 1. Prevalence of clinical and subclinical mastitis.

Forms of mastitis	Number of cows examined	Number positive	Prevalence (%)
Clinical	316	30	9.5
Subclinical	316	200	63.3
Total	316	230	72.8

Table 2. Prevalence of mastitis at quarter level.

Quarter	Number of quarters examined	Number positive	Prevalence (%)	Blind teats	Prevalence (%)
Left front	304	139	45.72	12	3.79
Right front	306	140	45.75	10	3.16
Left rear	310	147	47.42	6	1.89
Right rear	308	129	41.88	8	2.53
Total	1228	555	45.2	36	2.85

Association of risk factors with prevalence

The potential risk factors considered for the occurrence of bovine mastitis were age, herd size, number of parity, stage of lactation, udder hygiene, farm hygiene, presence of injury on the skin of the udder/teat, and previous mastitis history. A statistically significant association ($p<0.05$) was observed in cows with poor udder and farm hygiene and cows with teat injury, as presented in Table 3.

Risk factors like udder hygiene, presence of teat/udder injury, and previous mastitis showed significant association ($p<0.05$) with the prevalence of clinical

mastitis. Udder and farm hygiene showed significant association (p<0.05) with the prevalence of subclinical mastitis, as presented in Table 4.

Table 3. Association of overall prevalence of bovine mastitis with some potential risk factors.

Risk factors	Nº of cows examined	Nº positive (%)	X^2	*p* value
Age				
Young adult (3-6 years)	206	149 (47.15)	0.0618	0.804
Adult (≥7 years)	110	81 (25.63)		
Herd Size				
Few (<10)	152	106 (33.54)	1.3736	0.241
Many (≥10)	164	124 (39.24)		
No of Parity				
Few (1-3)	148	104 (32.91)	0.8886	0.346
Many (≥4)	168	126 (39.87)		
Stage of lactation				
Early (1-3 months)	120	89 (28.16)		
Mid (4-6 months)	90	65(20.57)	0.1933	0.908
Late (≥7 months)	106	76(24.05)		
Udder hygiene				
Good	131	72(22.78)	35.8835	0.000
Poor	185	158(50)		
Farm hygiene				
Good	115	51(16.14)	73.8081	0.000
Poor	201	179(56.65)		
Teat/udder injury				
Absent	288	205(64.87)	4.2230	0.040
Present	28	25(7.91)		
	190	136(43.04)	0.3498	0.554
	126	94(29.75)		

X2 : Chi-square

Table 4. Association of clinical and subclinical mastitis with some risk factors.

Risk factors	Nº of cows examined	Clinical mastitis			Subclinical mastitis		
		No positive (%)	X2	*p* value	No positive (%)	X2	*p* value
Age							
Young adult (3-6 years)	206	16 (5.06)	2.053	0.152	133 (42.09)	0.4121	0.521
Adults (≥7 years)	110	14 (4.43)			67 (21.20)		
Herd size							
Few (<10)	152	10 (3.16)	2.895	0.089	96 (30.38)	0.0022	0.962
Many (≥10)	164	20 (6.33)			104 (32.91)		
No of parity							
Few (1-3)	148	14 (4.43)	0.000	0.984	90 (28.48)	0.7371	0.391
Many (≥4)	168	16 (5.06)			110 (34.81)		
Stage of lactation							
Early (1-3 months)	120	6 (1.90)	4.795	0.091	83 (26.27)	3.0186	0.221
Mid (4-6 months)	90	10 (3.16)			55 (17.41)		
Late (≥7 months)	106	14 (4.43)			62 (19.62)		
Udder hygiene							
Good	131	4 (1.27)	10.801	0.001	68 (21.52)	12.478	0.000
Poor	185	26 (8.23)			132 (41.77)		
Farm hygiene							
Good	115	6 (1.90)	3.847	0.050	45 (14.24)	45.424	0.000
Poor	201	24 (7.59)			155 (49.05)		
Teat/udder injury							
Absent	288	20 (6.33)	24.582	0.000	185(58.54)	1.2492	0.264
Present	28	10 (3.16)			15(4.75)		
Previous mastitis							
No	190	10 (3.16)	9.925	0.002	126 (39.87)	1.8763	0.171
Yes	126	20 (6.33)			74 (23.42)		

X2: Chi-square

Bacterial isolates

A total of 80 milk samples from distinct and strong positive quarters for CMT were cultured. From 80 milk samples cultured, 58 (72.5%) yielded single bacterial colony, 7 (8.75%) mixed colonies and 15 samples had no growth. In this study, *Staphylococcus aureus* was the most frequently isolated species

(11.25%), followed by *Escherichia coli* (10%), *Micrococcus* species (10%) and *Pseudomonas aeruginosa* (10%), as presented in Table 5.

Table 5. Bacterial species isolated from bovine mastitis in the study area.

Bacteria	Frequency	Prevalence (%)
Staphylococcus aureus	9	11.25
Escherichia coli	8	10
Micrococcus species	8	10
Pseudomonas aeruginosa	6	7.5
Coagulase negative *Staphylococcus* (CNS)	5	6.25
Streptococcus agalactiae	5	6.25
Enterobacter aerogenes	5	6.25
Klebsiella pneumonae	4	5
Streptococcus disgalactiae	2	2.5
Streptococcus uberis	2	2.5
Staphylococcus intermedius	2	2.5
Bacillus species	2	2.5
Mixed growth	7	8.75
No growth	15	18.75
Total	80	100

Chemical analysis of milk composition

A total of 80 milk samples (50 from distinct and strong CMT positive and 30 from CMT negative cows) were analyzed using Lactoscan Ultrasonic milk analyzer machine for fat, protein, solid non-fat (SNF), density, and freezing point of the milk. The pH meter was used to measure pH. The difference in pH, protein, SNF, and freezing point showed statistically significant difference ($p< 0.05$) between mastitis-positive and negative milk. Mastitis-positive milk had higher pH value than negative milk. Protein and SNF content of mastitis positive milk were lower than negative milk, as presented in Table 6.

Table 6. Variations in milk composition between mastitis positive and negative samples.

Analytes	Mastitis	Mean ± SE	95% CI	*p* value
pH	Positive	6.316 ± 0.061	6.194 - 6.439	0.000
	Negative	5.897 ± 0.041	5.813 - 5.980	
Fat	Positive	4.330 ± 0.281	3.914 - 4.747	0.092
	Negative	3.867 ± 0.207	3.293 - 4.441	
Protein	Positive	2.966 ± 0.042	2.844 - 3.087	0.000
	Negative	3.278 ± 0.060	3.190 - 3.363	
SNF*[1]	Positive	6.924 ± 0.168	6.587 - 7.261	0.000
	Negative	7.836 ± 0.118	7.595 - 8.077	
Freezing point	Positive	-0.493 ± 0.009	-0.513 - 0.473	0.000
	Negative	-0.550 ± 0.007	-0.564 - 0.536	
Density (g/ml)	Positive	1.023 ± 0.002	1.029 - 1.030	0.162
	Negative	1.029 ± 0.001	1.028 - 1.030	

SNF: Solid not fat; SE: Standard error; CI: Confidence interval

Discussion

In the present study the overall prevalence of mastitis was 72.8%, which was comparable with previous studies of Mekbib *et al.* (2010) (71.0%) in Holeta and Zeryehun *et al.* (2013) (74.7%) in Addis Ababa, Ethiopia. However, it was higher than the findings of Sori *et al.* (2005) and Abunna *et al.* (2013) who reported 57.7% in Sebeta and 52.27% in Addis Ababa, respectively.

The prevalence of clinical mastitis in the current study (9.5%) was comparable with previous findings of Delelesse (2010) (10.3%) and Mekbib *et al.* (2010) (10%) in Holeta, Ethiopia, but lower than the reports of Workineh *et al.* (2002) (25.1%) and Zeryehun *et al.* (2013) (19.6%) in Addis Ababa. The prevalence of subclinical mastitis in this study was comparable with Fesseha (2021), who reported 71.02% in Modjo, Ethiopia, but higher than the findings of Adugna (2008), who reported 18.9% in Dire Dawa and Haramaya University Dairy farm and Gizat (2004), who reported 17% in and around Bahir Dar. The variations in prevalence may exist as a result of differences in the management of farms, especially with respect to the prevailing sanitary conditions at the time of investigation, agro-climatic variation, selection criteria and number of animals. Mastitis is a complex disease involving interactions of various factors

such as husbandry practices and environmental conditions (Radostits *et al.,* 2007).

The quarter level prevalence in the current study (45.2%) was comparable with Mekbib *et al.* (2010), who reported 44.9% in Holeta. However, it was lower than findings by Kifle and Tadele (2008), who reported quarter prevalence rate of 63.1%. There was no significant difference (p>0.05) among the quarters in occurrence of bovine mastitis.

In this study, subclinical mastitis has been found to be higher than clinical mastitis. This could be attributed to the little attention given to subclinical mastitis while treating clinical cases. Moreover, farmers may need to be better informed about the impact of the silent cases of mastitis (Karimuribo *et al.*, 2006).

The presence of injury on the skin of the udder/teat, udder, and farm hygiene showed statistically significant (p< 0.05) association with the occurrence of bovine mastitis. These findings show a strong agreement with the findings of Biffa *et al.* (2005), who reported association of bovine mastitis with presence of injury and Zeryehun *et al.* (2013), who reported association of bovine mastitis with udder hygiene. This shows that poor udder and farm hygienic condition and udder injuries may lead to increased mastitis prevalence and, consequently, result in reduced milk quality and quantity .

The most frequent bacterial species isolated from mastitis-positive milk was *S. aureus*. Radostits *et al.* (2007) asserted that *S. aureus* is well adapted to survive in the udder. It usually establishes a mild sub clinical infection of long duration from which it is shed in milk, facilitating transmission to healthy animals mainly during milking. In the present study, the frequency of isolation of *E. coli* was quite high, and it could be due to poor hygiene condition, as it infects udder through teat canal (Sumathi *et al*., 2008). 18.75% of milk samples from CMT- positive cows did not yield any bacterial growth. This might be due to the elimination of the infection although the SCC has still not declined (Sandholm, 1995). Anaerobes and, fungi may also cause mastitis, and it is probably more rarely caused by non-infectious causes (Hogan *et al*., 1999). The predominant isolation of *Staphylococcus aureus* (11.25%) in the present study showed an agreement with previous reports of Mekbib *et al.* (2010), Zeryehun *et al.* (2013), Atyab *et al.* (2006), and Fadlelmoula *et al.* (2007), who reported higher prevalence of *Staphylococcus aureus*.

The pH, total protein, solid not fat, and freezing point showed significant difference ($p < 0.05$) between CMT-positive and negative samples. The pH was significantly ($p < 0.05$) higher in CMT-positive milk; the result was in agreement with Riaz *et al.* (2012), Batavani *et al.* (2007), Charjan *et al.* (2000) and Haggag *et al.* (1991) and in disagreement with Ogola *et al.* (2007) who reported non-significant increase in pH of positive milk. These change were due to the increase in permeability of the mammary epithelial cells that lead to the transfer of components from blood to milk, such as citrates, bicarbonates that cause elevated pH levels (Harmon, 1994) and due to reduction in lactose contents as the lactic acid formation is minimum (Tanveer *et al.*, 2005). The milk pH of non mastitic cows in this study decreased from normal pH range, and this might have resulted from formation of lactic acid due to unwanted delay to analyze milk samples immediately after collection.

Total milk protein content was significantly ($p<0.05$) lower in milk from positive animals than negative animals; this result was in agreement with that of Jones (2006) and but was in disagreement with the results of Sonea *et al.* (2009) and Auldist and Hubble (1998), who reported significant elevation of milk protein due to mastitis. This study showed a decrease in the protein content of milk from mastitic cows. This might be due to high increase in the activity of proteolytic enzyme (plasmin) that causes extensive destruction of milk proteins in the udder before milk removal (Khan and Khan, 2006).

Solid not fat (SNF) was significantly ($p<0.05$) lower in mastitis-positive milk compared with normal milk. Decrease in SNF in positive milk depends on the destruction that occurs due to the invasion of pathogens to the mammary tissue causing a decrease in synthetic activity of mammary gland (Benchedly *et al.*, 2009). The result was in accordance with findings of Riaz *et al.* (2012), Ahmed *et al.* (2007) and Malek *et al.* (2013) but disagreed with Leitner *et al.* (2004) and Sefinew *et al.* (2013), who reported that mastitis had no significant effect on the solid not fat content of milk.

In this study, the freezing point of mastitic milk significantly increased when compared with normal milk. This might be attributed to significant decrease in protein and SNF content of the milk. The chemical composition of milk had influence on freezing point, as Navrátilová *et al.* (2006) found correlation between freezing point and chemical composition of milk.

Conclusion

Bovine mastitis is the major constraint to the agricultural sector in general and dairy production in particular. The present study revealed that bovine mastitis was highly prevalent disease in the study area. Cows kept in farms with poor hygiene and hence contaminated udder and teat/udder injuries were predisposed to mastitis than those kept in relatively clean farms. As the study revealed, subclinical mastitis was more prevalent than clinical mastitis. Also, milk composition was affected in cows with mastitis, and a variety of aerobic bacterial pathogens were isolated from milk of mastitic cows. These pathogens could be the cause of mastitis. Most of these pathogens were contagious and hence, might easily contaminate other cows in the herd. Dairy production and health extension programs should give special attention to controling bovine mastitis as it is a serious challenge for the dairy industry in the study area. Improved sanitary measures with particular emphasis to udder/teat and farm hygiene should be implemented in all dairy farms. Regular screening and early treatment of mastitic cows can reduce dispersion of pathogens within the herd. In this regards, antibiotic sensitivity tests are recommended before treatment to obtain maximum efficacy. Farms should avoid as much as possible and pay attention to wounds on the udder/teats and get immediate veterinary assistance before it leads to clinical mastitis.

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Non-biological factors affecting bovine tuberculosis control and prevention in dairy cattle: knowledge, attitude, and practices (KAP) of dairy farmers in Ethiopia

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Abstract

Bovine tuberculosis (bTB) is a serious animal health problem in Ethiopia, ranking among the top three livestock diseases. If conditions that favor the transmission and persistence of bTB are not addressed, the situation will get worse; hence bTB control should be priority. However, interventions are influenced by several "non-biological" factors. A cross-sectional study was conducted to assess farmers` knowledge, attitude, and practice (KAP) using a structured questionnaire administered to 307 respondents. A multi-stage sampling procedure was employed to select study subjects. Accordingly, 55% of participants knew about bTB; of which 36.4% knew basic information, and the remaining (18.6%) knew nothing except the name bTB. Less than 2.0% of the farmers knew about zoonotic importance of bTB. The knowledge among dairy farmers varied depending on farm size, milk-shed (MS), training received, veterinary consultation, and years of farming experience. Using multivariable analysis, farmers from medium and large-scale farms knew more about bTB than those from small scale farms, by a factor of 2.8 and 7.7, respectively. Similarly, farmers who had been farming for more than 6 years and farmers from Selale MS had higher odds of being knowledgeable about bTB, by 5.7 and 10.4 times compared to other MS, respectively. Only 12% of participants were aware of the test and slaughter control method. Likewise, only 18% of the farmers tended to avoid buying cattle from risky sources. The finding revealed a lack of knowl-

edge on the production loss incurred (12%) and the probability of human infection (1.9%); instead, a substantial proportion of farmers (25%) believed that bTB infection could affect the dairy market. In conclusion, the present study highlighted knowledge, attitude, and practice gaps that, if addressed using tailor-made training, might assist in reducing the consequences of the disease.

Keywords: Bovine tuberculosis; Control; Farmers; KAP; Milk-shed.

Introduction

Although bTB is still present in some industrialized countries, currently, it is a disease that mostly affects low-income countries lacking the resources to apply expensive test and slaughter schemes (Bemrew *et al.,* 2015). Recently, bovine tuberculosis has been identified as one of the top three, high-priority, livestock diseases in Ethiopia (Lakew *et al.,* 2022). The existence of potential associated factors would make it conducive for the spreading and persistence of bTB (Ayele *et al.,* 2004; Regassa *et al.,* 2008; Girmay *et al.,* 2012) and making control of bTB priority.

Bovine tuberculosis is a poorly studied and widely underreported zoonosis but is believed to be a significant contributor to animal and human losses (Grace *et al.,* 2012; Liverani *et al.,* 2013). In Ethiopia, the number of *M. bovis* infections in humans has been reported to be low despite it likely to be a substantial and high health risk because of the high prevalence of the disease in livestock (Ayele *et al.*, 2004; Kemal *et al.,* 2019).

According to the World Health Organization (WHO), there is no single intervention that can effectively control bTB on its own, unless able to block all routes of transmission of the disease (WHO, 2023). Conventionally, a typical strategy for bTB control in domesticated animals involves regular tests and slaughter of infected animals However, beyond the high cost incurred, these interventions are influenced by several "non-biological" factors (Ciaravino *et al.,* 2017). For instance, farmers are the actors who regularly interact with veterinarians, and who comply with or resist the legislative basis and biosecurity practices recommended or enforced for disease control. They own the animals which succumb to infection, determine their husbandry and welfare, buy and sell them, present them for disease testing, and take risks of financial loss for affected animals (Robinson, 2017). Hence, the knowledge, attitudes, and

behaviors of dairy farmers is a key factor for successful disease control and surveillance systems (Pfeiffer, 2006; Brennan *et al.,* 2016).

Most high-income countries have successfully controlled bTB based on test-and-slaughter programs, alongside slaughterhouse surveillance, and trade and movement restrictions of affected herds (Napp *et al.,* 2019; OIE, 2019). A test-and-slaughter control method is impractical to adopt in low- income countries due to extensive socioeconomic significance where replacement of equivalent breeding stock might be excessively unaffordable (McCrindle and Michel, 2007). Therefore, the need for evaluation of potential control strategies is critical to minimize the impact of the disease (Lakew *et al.,* 2022). In the past, social factors have not been often given enough attention in the implementation of animal health programs. Inversely, the impact of social factors on public health interventions is well known, and these aspects have been accounted for in several human medicine studies (Berkman *et al.,* 2014; Bach *et al.,* 2017).

Recently, the situation has changed, and social factors have become more relevant for the control of animal diseases. Studies have shown that it is crucial to understand how the different actors involved think and act since their attitudes and behaviors affect the effectiveness and sustainability of such programs (Catley *et al.,* 2012; Enticott *et al.*, 2015; Brunton *et al.,* 2018).

Even though bovine tuberculosis is an endemic disease in Ethiopia, less attention was given to its control because of lack of awareness, policies, and resources. Measuring farmers' knowledge, attitudes and practices (KAPs) is a significant step when developing and implementing disease control and prevention strategies (Balkhy *et al.,* 2010; Brennan *et al.,* 2016).

The knowledge, attitudes, and practices (KAPs) of farmers regarding bovine tuberculosis have not yet been adequately investigated in Ethiopia. Many of the previous studies were fragmented, as they were conducted by different scholars to address only some specific problems. These studies were noted to have limitations either to produce a more comprehensive and representative pooled country level picture, or to generate detailed information that could remarkably impact the application and effectiveness of interventions against bTB, for one or another reason including, differences in the scope of study objectives, the methodology used, the target population and geographic coverage. As a result, additional all-inclusive investigations on the potential source of infec-

tion, public health and economic burden of the disease, the transmission ways, and its control measures are essential to attain a comprehensive and accurate insight into the subject (the gaps in knowledge, attitude and practice) which, if addressed, could help policy makers and researchers to design effective and adaptable intervention strategies. With this understanding, a cross-sectional study was designed to assess farmers' knowledge and attitude associated with bovine tuberculosis and to identify farm practices that potentially resulted in the disease remaining endemic in selected milk-sheds of Ethiopia, where most commercial dairy farms are found.

Material and methods

Study setting and sampling procedure

A multi-stage sampling procedure was applied for the study. Taking the level of intensification of dairy farming into consideration, the four milk-sheds (MS) of Asela-MS, Debre-birhan-MS, Selale-MS and Others-MS were selected for their substantial dairy farm number, different socio-economic and cultural features, facilities, operational safety, and convenience. Since farmers` knowledge of bTB and its possible determinants may vary in different areas, the study was conducted in four different settings. The Selale-MS covers the areas from Addis Ababa, following the major highway, to Muke-turi; Debrebirhan-MS extends from Addis Ababa to the city of Debre-birhan in the north; Asela-MS spans the area from Addis Ababa through Bishoftu and Adama to the town of Asela. Others-MS consists of commercial dairy farms from various regions of Ethiopia (Diredawa, Bahirdar, Gondar, Hawasa, Kombolcha, Harbu /South Wollo). The number of selected farms from each MS ranged between 35 and 108 depending on the number of commercial dairy farms (semi-intensive and intensive dairy farms) with good road access. With regard to herd size, a balanced number of small, medium, and large-scale farms were selected. The classification of herd size into small (1–3 dairy animals), medium (4 − 10 dairy animals), and large farms (more than 10 dairy animals) was made according to the classification made by an FAO report (Sharma *et al.,* 2003). Hence, as part of the survey, we interviewed 111 large-scale, 101 medium-scale and 95 small-scale commercial farmers. A simple random sampling technique was applied to choose dairy farms from each milk-shed.

Number of the study participants was calculated using the formula previously described

$N = (Z^2 P(1-P))/d^2$

Based on the 24.1% awareness of dairy farmers and using 95% level of confidence (CI) and 5% precision in the estimates, the total sample size was determined as 282 dairy farms. To account for an assumed small design effect, we aimed at 307 farmers.

The participants were identified from different randomly selected villages along the study MS, based on their availability and willingness to participate.

Data collection and interview procedure

Using standardized set of questions, we gathered socio-demographic data on determinants that could influence the knowledge, attitude and practice of farmers regarding bTB. This included the age, gender, educational level, participants` role in the farm, training received, veterinary consultation and farming experience of the participants.

Knowledge and attitude on bTB were assessed by asking whether the respondents had heard or knew about bTB or not. The farmers who knew about bTB were further asked some additional questions related to bTB detection, its impacts, transmission, the potential source of infection for their herds, and control measures, through a set of structured questions. Their practices relevant to preventing their herds from getting bTB infection, frequent measures they had taken on reactors and the priority they give to bTB intervention compared to other diseases were also assessed. Furthermore, information on farm size, animal husbandry practices, history of exposure to bTB, their prevailing knowledge and practices towards bTB management, and animals entering and exiting the herd were collected.

The questionnaire was pretested, and interviewing method was validated to have a good insight into commercial dairy farming in the study area. During the pre-test, the questions were evaluated to make sure that the farmers understood them correctly. Using enumerators, dairy farm managers or other persons responsible for the selected farms were questioned in their local language. The enumerators also filled out an observation checklist during the visit. At the beginning of the interview the farmers were informed about the purpose of the study.

Data analysis

The final data were recorded and analyzed using SPSS 16.0. Descriptive statistics were generated for each variable of interest. Percentages and their 95% confidence intervals (95% CI) were calculated. Characteristics of the respondents in terms of age, gender, level of education and farming experience were summarized and presented as percentages and frequency. The selection of variables to be included in the multivariable model was carried out in two steps. Initially, a univariable logistic regression model was built to identify important covariates or determinants that are at least moderately associated with farmers' knowledge, attitudes, and practices, where candidate variables with $p \leq 0.2$ were selected for further analysis based on the Wald test. The multicollinearity between pairs of explanatory variables was assessed by a correlation matrix. The significance of this association was examined using the Chi square test. In the case of a pair of variables with a significant association ($p < 0.05$), the variable judged as the most biologically plausible was used as a candidate in the multivariable analysis. All variables that passed the previous 2 steps were incorporated into the final multivariable logistic regression model. A manual stepwise selection approach was used for the selection of variables in the model using backward variables selection techniques to keep only variables with $p < 0.05$ in the final model.

Results

Demographic characteristics of the respondents

The socio-demographic profile of the respondents is summarized in Table 1. Almost two-thirds (65%) of the study participants were found to be between the ages of 31 and 60 years. Male respondents constituted 69.4%, of which 59.6% (127/213) engaged actively on major dairy farming practices. Only 27.7% of respondents were currently unmarried. Regarding occupational status of the participants, 43.3% (133/307) were owners of the dairy farm, followed by employed staff (31.3%; 96/307). However, a notable percentage, 25.4% (78/307) was unemployed family members.

Table1. Socio-demographic characteristics of study participants.

Variable	Category	Frequency	Percent
Age (years)	18-30	71	23.1
	31-45	98	31.9
	45-60	101	32.9
	>60	37	12.1
Gender	Female	94	30.6
	Male	213	69.4
Marital status	Married	222	72.3
	Unmarried	85	27.7
Occupational status	Employed	96	31.3
	Unemployed/family	78	25.4
	Owner	133	43.3
Role in the farm	Actively engaged	179	58.3
	Supportive staff	116	37.8
	Partime/occasionally	12	1.6

Farmers` awareness on bovine tuberculosis

Nearly 45% of the dairy farmers were not aware of bTB or never heard the name of the disease. Thirty six point four percent of the farmers reported that they knew some fundamental information about bTB, and the remaining 18.6% of the farmers had heard about bTB but needed to learn more in detail. The association between independent variables and knowledge of participants on bTB is shown in Table 2. MS, herd size (scale of the farm), previous experience, training and veterinary consultation had a significant association with participants` awareness of bTB. In Selale MS, more farmers 80% (67/83), knew about bTB than Debre-birhan MS (75%) or other MS (34%). The most common knowledge reported was that bTB could affect cattle and it could be transmitted from infected animals to healthy ones. The source of knowledge was mainly through training (46%), previous experience in dairy farming, and consultation from veterinarians (52%).

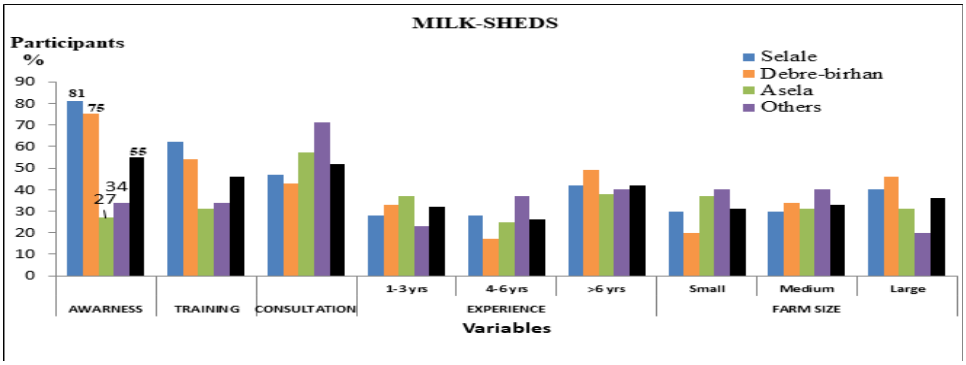


Figure 1. Determinants along the study milk-sheds.

Table 2. Variables having association with farmers' knowledge towards bTB.

Variable	N	X^2-Value	df	*p* value
Milk shed	307	76.34	3	0.000
Educational level	307	24.94	2	0.000
Herd size	307	42.2	2	0.000
Training	307	67.98	1	0.000
Veterinary consultation	307	10.22	1	0.001
Previous dairy farming experience	307	28.55	2	0.000

Multivariable logistic regression analysis revealed that farmers` knowledge of bTB in medium and large-sized farms was almost 2.8 and 7.7 times higher than that of small-sized dairy farms, respectively (Table 3). Dairy farmers who had >6 years of farming experience and farmers from Selale MS had significantly better knowledge about bTB (odds 5.7 and 10.4 times higher in the case of more experienced farmers and farmers from Others MS).

Table 3. Results of multivariable analysis of selected determinants having association with farmers' knowledge towards bTB

Determinants	OR	95% CI	*p* value
Farmers from Selale MS compared to Others MS	10.4	3.8-28.9	0.000
Farmers from Debre birhan MS compared to Others MS	5.8	2.2-15.7	0.001
Medium size (4–10 dairy animals) farms compared to small size (1–3 dairy animals) farms	2.8	1.4-5.9	0.005
Large size (>10 dairy animals) farms compared to small size	7.8	3.6-16.5	0.000
Farmers with 3 - 6 years of farming experience compared to ≤3 years of experience	3.0	1.3-6.8	0.008
Farmers with >6 years of farming experience compare to ≤3 years of experience	5.7	2.8-11.5	0.000

Farmers` knowledge regarding bTB control measures, ways of transmission, its impacts and diagnosis

About 38.8% (117/301) of the participants knew at least some control and prevention methods, of which 30% (36/117) were aware of test and slaughter method as a typical strategy for bTB control and prevention. Likewise, 46% of them preferred to refrain from buying cattle from unknown or potentially infected sources. Farmers` knowledge on bTB control was significantly associated with market sheds, farm size and previous farming experience ($p < 0.05$).

Concerning its modes of transmission, 42.3% (130/307) of participants had some knowledge of how bTB spreads. Knowledge on ways of bTB transmission is summarized in Figure 2. Participants from Selale MS, as well as participants from large-scale dairy farms, had more level of knowledge on bTB transmission ($p < 0.05$). Similarly dairy farming experience had a significant association with disease transmission knowledge.

Less than 2% (1.88%) of the respondents knew the possibility of human infection from infected animals. Only 1.6% of participating farmers knew the skin test as common diagnostic tool for bTB. Similarly, majority of the respondents didn't know its impact on milk and meat production loss (Figure 2).

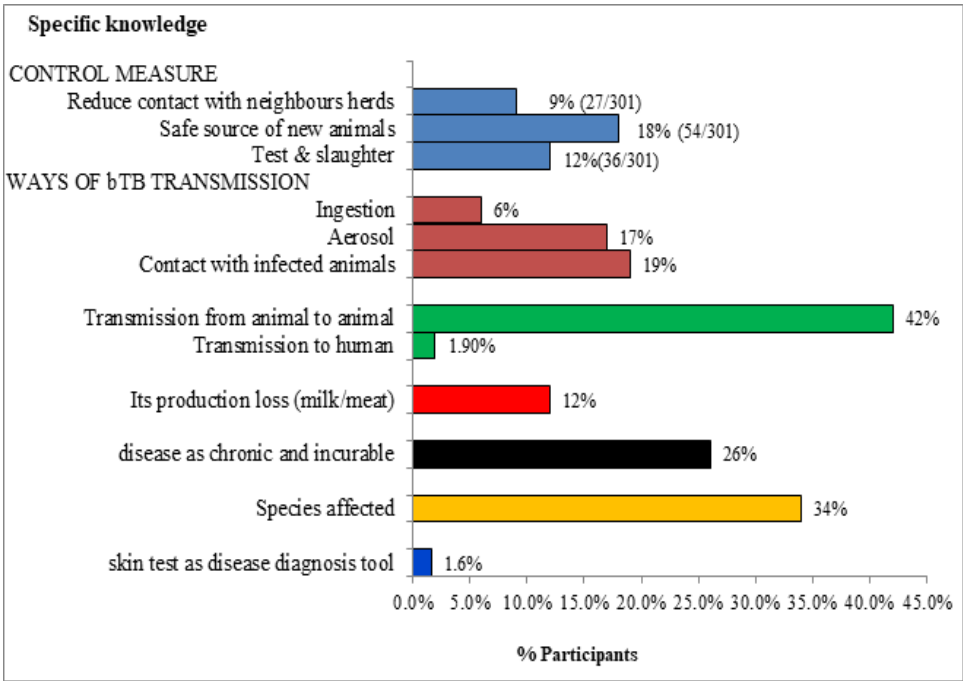


Figure 2. Detail knowledge of participants on bTB control measures, transmission ways, and feature of the disease.

The number of respondents who mentioned aerosol as a mode of bTB transmission, compared to those who mentioned contact with infected animals, was approximately 3 times higher in trained participants compared to those not receiving training (Table 4).

Table 4. The influence of farmers` previous training exposure on their response to the type of mode of transmission.

Farmers' knowledge about mode of transmission	N	OR	CI	*p* value
Contact with infected animal	59	-	-	-
Aerosol	51	3.02	1.27, 7.14	0.012
Ingestion	20	4.17	1.10, 15.78	0.036

Attitudes and practices towards bTB

Results for the responses to questions relating to farmers' attitudes and practices are presented in Tables 5 and 6. Despite only 12% of participants being aware of the production loss incurred by bTB infection, one fourth of the farmers (25%) believed that bTB infection could negatively impact the dairy markets.

Surprisingly, almost none of the respondents (0.98%) believed the test and slaughter method was a feasible and applicable control and prevention measure. Approximately one-fifth of the farmers (22%) thought that improving the community's awareness of bTB is an important element for effective prevention and control. Similarly, 16.3% of respondents mentioned that government or other legal bodies should provide disease free heifers for sale in order to avoid purchasing of cattle from unknown or potentially infected sources. Only few farmers were aware of skin test as a diagnostic tool to identify infected or non-infected animals, and, none of them reported it as a reliable test.

Roughly 21% (64/307) of the farmers listed new animal entry, neighboring dairy farms, and people, equipment and vehicles entering from infected sources as potential sources of infection for their herds, of which introduction of new animals (62.5%) taking the largest share.

Univariable analysis revealed that trained farmers' perception was 12.8 times higher than non-trained farmers, perceiving new animal entry as a primary source for the introduction of the disease into their farms. Farmer's recommending nothing to do to bTB reactors or to sell reactors to others was 11.8 times higher in untrained farmers, while those advised slaughter of reactors for meat was 4.95 times higher in trained farmers.

Among those who knew about bTB, only 1.6% of dairy farmers considered bTB as a prioritized animal health problem, which made majority of the farmers (54%) to do nothing as control and prevention measures. On the other hand, mastitis (50.8%) and abortion (35.5%) were their concerns to tackle. Almost none of the farmers used skin test to screen new entry animals to protect their herd from getting bTB infection; instead, 26.5% of farmers preferred not to buy cattle from unknown or potentially infected sources.

Table 5. Results of univariable model for the association between farmers' attitude and previous training exposure (N=307).

Attitudes	Response	OR	95% CI	*p* value
The potential source of infection for your herds				
	Don`t know	-		
	Neighboring herds	5.03	2.11-11.96	0.001
	Contaminated utility from infected sources	2.9	1.09-8.16	0.032
	New animal introduction	12.8	6.40-25.82	0.000
What do you suggest to prevent or control bTB country wise?				
	Government should provide disease free heifers for sale	2.88	1.51-5.49	0.001
	Strict animal movement restriction governed by rules and regulations	15.85	3.48-72.2	0.000
	Awareness to dairy farmers about the disease	3.66	2.04-6.57	0.000
What do you recommend to reactors?				
	Slaughter for meat	-	-	0.001
	Slaughter & bury/burn	2.59	1.17-5.74	0.019
	Sell	4.95	1.81-13.51	0.002
	Nothing	11.88	1.39-101.33	0.024
In your openion, how could bTB affect your farming?				
	Reduce milk production	-	-	0.48
	Loss from culling animals	1.11	0.275-4.51	0.88
	Marketing	1.65	0.71-3.85	0.24

Despite their knowledge and attitude about control and prevention measure (test and slaughter), the majority of the farmers with reactor cattle (77.8%) did nothing to control/prevent the disease, while some (22.2%) sold the reactors (Table 6).

Table 6. Farmers' practices relevant for control and prevention of bTB

Variable		Frequency	Percentage
Have ever your animal tested for bTB (n=307)			
	Yes	36	11.7
What measure did you take on reactor animals? (n=9)			
	Nothing done	7	77.8
	Sale	2	22.2
Which disease/problem is your priority of concern to intervene in your farm? (n=307)			
	Mastitis	156	50.8
	Abortion	109	35.5
	Bloating	35	11.4
	bTB	7	2.2
What preventive measure you usually take to protect your farm from getting bTB infection? (n=307)			
	Nothing done	218	71
	Screening test/skin test	2	0.6
	Not buying cattle from unknown/risky sources	87	28.3

Discussion

Non-biological factors, such as farmers' knowledge, attitudes, and practices, may remarkably impact the effectiveness of disease control interventions, including against bovine tuberculosis (bTB) (Zahedi *et al*., 2014; Ciaravino *et al.,* 2017; Robinson, 2017).

In the present study, 55% of participants knew something about bTB. Comparable results were reported from Ethiopia in Bahir Dar City (57.2%) (Hailu *et al.,* 2021) and from commercial dairy farmers in Zimbabwe (65.0%) (Mosalagae *et al.,* 2011). However, several lower awareness levels were reported from previous studies conducted in different locations and times in Ethiopia: 13.9% (Kidane *et al.,* 2015), 24.1% (Bihon *et al.,* 2021), 35% (Ameni and Erkihun, 2007), 45.6% (Kuma *et al.,* 2013) and 37.1% (Tigre *et al.,* 2011). This variation may be due to differences in composition of study participants. Inclusion

of participants in the present study from the vicinity of Addis Ababa, from intensive dairy farms with long years of experience in farming, participants with access to veterinary consultation, various trainings and experience sharing visits might have contributed to the observation of better awareness about bTB among farmers in the current study.

In the current study, only 12% of participants were aware of the test and slaughter method. Llikewise, only 18% of the farmers tended to avoid buying cattle from risky sources (potentially infected sources or areas with previous reports of bTB). Consistent with our findings, Kao *et al*. (1997) stated that majority of dairy farmers didn't know that a typical strategy for bTB control involve regular field tests and the slaughter of infected animals. The fact that only small proportion of the farmers paid due care to the health status of herds from which they source replacement animals demonstrated the high risk of spread of bTB from infected herds in the study area. It is well known that introduction **of new animals** is one of the significant risk factors for disease entry into dairy herds (Massó Sagüés *et al.,* 2019). Mekonnen *et al*. (2019) reported movement of infected animals as the most critical source for the spread of bTB. The same authors also stated that the absence of restriction of infected animal movements and quarantine were considered to be the main barriers to the control of bTB.

Contrary to the well-established knowledge about the economic and zoonotic importance of bTB as a significant contributor to animal and human losses (Grace *et al.,* 2012; Liverani *et al.,* 2013). our result revealed a lack of knowledge on the production loss incurred and the probability of human infection by bTB. Instead, a substantial number of farmers (25%) believed that bTB infection could badly affect the dairy business by disturbing their milk markets. In other reports, the majority dairy farmers do not know about tuberculosis in cattle; rather, farmers' knowledge was limited to human tuberculosis, and they had strong perception that tuberculosis was a disease that 'only' humans could get. This poor awareness may be attributed to farmers` perceptions of the benefits of bTB control and prevention, which was believed to be mainly commercial, as bTB was not considered having an impact on public health nor a disease causing production loss (Ciaravino *et al.,* 2017). Furthermore, farmers mainly perceived the control of bTB as an imposition rather than a necessary activity to protect their animals (Ciaravino *et al.,* 2017).

Among the dairy farmers who were aware of bTB, a very small percentage (1.6%) considered it a prioritized animal health issue, leading to a lack of interest in taking preventive and control actions. All these gaps may exacerbate the public health and economic burden of bTB not only on the community but also on the country

Skin test are a common diagnostic tool for bTB, but only 1.6% farmers were aware of it. Actually, farmers do not want to have any bTB-infected animal in their herd, but they want to be sure that the test-positive animal is truly infected. In the present study none of the participants reported skin test as reliable. Even veterinarians and farmers expressed strong uncertainties about the reliability of skin test results (Ciaravino *et al*., 2017). According to our findings, therefore, in the absence of a common conscience on the conventional bTB diagnostic tool and a clear policy to compensate for culled animals, almost none of the respondents (0.98%) thought that test and slaughter method was a feasible and applicable way to control bTB. Instead,16.3% and 22.0% of respondents mentioned that educating the community about bTB and providing healthy heifers from reliable sources may be key factors for a successful bTB prevention and control plan, in a similar pattern reported by another scholar (Kawsar, 2022).

Conclusions

The present study assessed knowledge, attitudes, and practices (KAPs) of the study participants and some important issues that could remarkably impact the application and effectiveness of interventions against bTB in the specific area we examined. MS, farm size, previous years of experience, training received, and veterinary consultation had a significant association with awareness towards bTB. Hence, training and regular consultation with veterinarians could boost the farmers' understanding of bTB. The enhanced knowledge could then help farmers change some of their practices for the better. A tailored education or training program could be developed to raise the farmers' awareness and encourage them to adopt bTB prevention practices.

The scope of this study is limited to commercial dairy farms in the selected milk-producing areas of Ethiopia. To gain a more comprehensive and accurate insight into the subject, further and broader studies are needed to examine the gaps in farmers' knowledge and attitude in both commercial and extensive dairy farming systems.

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Prevalence of bovine schistosomiasis and associated risk factors in Bahir Dar, northwest Ethiopia

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Abstract

A cross-sectional study was conducted from December 2022 to March 2023 to estimate the prevalence of bovine schistosomiasis and associated risk factors in Bahir Dar town, northwest Ethiopia. A total of 298 cattle were selected randomly, and subsequent fecal samples were obtained for coprological examination. *Schistosoma* eggs were examined using sedimentation technique. A logistic regression model was employed to assess the association between risk factors and prevalence of schistosomiasis. Of the total of 298 fecal samples examined, 63 (21.1%) were found positive for *Schistosoma bovis* eggs. In the study, sex, breed, and management system were not significantly associated ($p > 0.05$) with the occurrence of bovine schistosomiasis. On the contrary, prevalence of bovine schistosomiasis showed significant variability ($p < 0.05$) amongst the different age and body condition groups. The study disclosed a significantly higher risk of bovine schistosomiasis in old cattle (AOR= 3.2; 95% CI: 1.4 - 7.0) and cattle with poor body condition (AOR=3.9; 95% CI: 1.5 - 10.2). The findings indicated a higher prevalence of *Schistosoma bovis* infection among older cattle and those with poor body condition. Therefore, it is recommended that husbandry-related risk factors be incorporated into disease control and prevention strategies.

Keywords: Bahir Dar; Bovine schistosomiasis; Coprology; Risk factors.

Introduction

Ethiopia has the largest livestock population in Africa, with an estimated 70 million cattle, about 42.9 million sheep, 52.5 million goats, 2.15 million horses, 10.80 million donkeys, 0.38 million mules, about 8.1 million camels, and 48.9 million poultry (CSA, 2020). Cattle play a vital role in the country's economy,

providing traction power for crop cultivation, as export commodities, manure, food security, and wealth accumulation. However, the livestock sector is not fully exploited due to several constraints, including malnutrition, traditional husbandry practices, poor genetic makeup, and prevailing diseases (Jabbar *et al*., 2007; Negassa *et al*., 2011).

Parasitic diseases are one of the major constraints of animal health and production throughout tropical and sub-tropical countries (Juyal and Singal, 2011). Among the challenges of cattle production, trematode parasites, especially schistosomiasis, are one of the most prevalent infections of ruminants in different parts of the world, including Ethiopia (Urquhart *et al.,* 2003; Ali *et al.,* 2006; Yeneneh *et al*., 2012).

Cattle infected with *Schistosoma bovis* develop a syndrome characterized by liver damage, roughness of the hair coat, pale mucous membrane, severe emaciation, and inferior reproductive performance that results in a significant economic downturn and a threat to public health (Hambali *et al.,* 2016). Schistosomiasis is a significant economic burden for livestock farmers in Ethiopia. The parasite can cause mortality, retarded growth, poor productivity, low milk yield, and increased susceptibility to other parasitic or bacterial diseases (Marquardt *et al*., 2000; Lefevre *et al.,* 2010). Therefore, it is essential to control schistosomiasis to protect livestock health and productivity.

Schistosoma bovis, a parasitic trematode, is among the significant causes of parasitic infections in cattle in Ethiopia. The prevalence of the parasite has been reported to vary widely across the country, with studies in Kemissie (Ameni *et al*., 2001), Fogera (Chane *et al.,* 2012; Mengistu *et al*., 2012), Debre Tabor (Tsega and Samuel, 2015), Dangila (Alemayehu and Asrat, 2015), and around Bahir Dar (Samuel *et al*., 2016). The absence of recent surveys on schistosomiasis in cattle within the current study area, coupled with the recognized economic significance of the disease, underscores the imperative to undertake an investigation aimed at elucidating the present status of this issue in the area. This information will be essential for developing effective control strategies. Therefore, the objectives of this study were to estimate the prevalence of bovine schistosomiasis in Bahir Dar town and assess the major risk factors for the occurrence of schistosomiasis in the study area.

Materials and methods

Study area

The study was conducted from December 2022 to March 2023 in Bahirdar town, northwest Ethiopia. Bahir Dar is found 570 km away from Addis Ababa. It is located at 11° 29'N latitude and 37°29'E longitude with an altitude of 1500-2300 m.a.s.l., annual rainfall of 1200-1600 mm, and mean annual temperature of 29.5° C. The landscape is marked by the presence of Lake Tana, which drains a watershed of about 3,000 $km^{2,}$ and areas adjacent to Lake Tana and Abay River have poor drainage and over flooding during the wet months.

Study population

In this study, cattle from different areas of three kebeles of Bahir Dar town were included. The major risk factors considered included breeds (local and cross), management systems (intensive, semi-intensive, and extensive), age, body condition, and sex. The animals were classified into three age categories: young (< 2 years old), adult (2- 5 years old), and old (> 5 years old) on the basis of dentition, as described by Pope (2008). The body condition of the animals was classified into three groups: poor, medium, and good based on visual assessment as described by Debont *et al.* (2005).

Study design

The study had a cross-sectional study design in which the study subjects were sampled once during the study period to assess the prevalence and associated risk factors of bovine schistosomiasis in Bahir Dar town, northwest Ethiopia.

Sample size determination and sampling of animals

The desired sample size was calculated according to Thrusfield (2005) with a 95% confidence level, a 5% desired absolute precision, and an expected prevalence of 26.3% (Samuel *et al.,* 2016).

$$n= (1.96)2\ p\ (1\text{-}pexp)/d2$$

Where: n= required sample size, 1.96=the value at 95% confidence interval,
p= expected prevalence of schistosomiasis, and
d= desired absolute precision (0.05).

$$n = (1.96)2 * 26.3\% (1- 26.3\%)/0.0025= 298$$

Hence, a total of 298 cattle were chosen through a simple random sampling technique for the purpose of estimating the prevalence of the disease.

Cattle of different breeds, sexes, and age groups in three kebeles were included in the study. Multi-stage sampling was employed to select cattle for the field survey. In the first stage, kebeles (Kebele Nine, Sebatamit, and Zenzelima) were selected based on practical logistic and demographic considerations. In the second stage, households encountered during the field survey were systematically selected, and then cattle were randomly selected for the study.

Sample collection and laboratory procedure

Fresh fecal samples were collected directly from the rectum of the animals in the field using gloves. The samples were filled into universal sampling bottles, preserved in 10% formalin to prevent the hatching of the eggs before examination and closed with screw tops. It was placed in ice box and transported to Bahir Dar University Parasitology laboratory. Subsequently, the samples underwent concentration through the standard sedimentation technique, as outlined by Urquhart *et al*. (2003). Briefly, approximately 3 grams of feces were placed into a centrifuge tube, to which 40 ml of water was added, and the mixture was thoroughly blended. The mixture was then passed through a tea strainer into another centrifuge tube and allowed to settle for 15 minutes. Following this, supernatant was poured off, and the sediment was mixed again. This process was repeated three times until the supernatant became clear. Finally, the sediment was transferred using a pipette onto a clean microscope slide for examination under a microscope at 10x magnification. A slide was considered positive if eggs that were oval to spindle-shaped, with a central bulge and a terminal spine on one side, were detected. The slides were considered positive when oval to spindle shaped centrally bulged eggs with terminal spine on one side of egg was identified (Urquhart *et al*., 2003).

Data analysis

The data was collected and entered into an Excel spreadsheet. The spreadsheet was then transferred to the Statistical Package for Social Sciences (SPSS) software version 20 for analysis. The data were then summarized using descriptive statistics. A univariable logistic regression analysis was used to assess

the association of prevalence with individual risk factors for bovine schistosomiasis. Variables with p value ≤ 0.25 in the univariable logistic regression analysis with no multicollinearity were entered into the multivariable logistic regression model. Collinearity between variables was also checked by standard error and model fitness assured by Hosmer and Lemeshow test and Omnibus test (Dohoo *et al.,* 2003). In all analyses, statistical significance was set at $p <$ 0.05, with a confidence level of 95%.

Results

Overall prevalence of bovine schistosomiasis

The current study revealed that out of the total 298 faecal samples examined in the study area, 63 (21.1%) were found to be positive for *S. bovis* eggs (Table 1).

Prevalence of bovine schistosomiasis by risk factors

The outcomes of the univariate logistic regression analysis are detailed in Table 1. The model indicates a nearly equivalent prevalence of schistosomiasis in males (20.4%) and females (21.7%). Likewise, there was no statistically significant difference (p > 0.05) in the prevalence of bovine schistosomiasis between the local breed (23.9%) and cross-bred (15.5%) cattle. However, within this model, age, body condition score, and management system exhibited significant associations with the prevalence of the disease ($p < 0.05$).

A multivariable logistic regression model was applied to ascertain the association between the prevalence of schistosomiasis, as detailed in Table 2. The results indicated significant associations ($p < 0.05$) between the prevalence of bovine schistosomiasis and both age and body condition score. Specifically, the model revealed a significantly elevated risk of bovine schistosomiasis in older cattle (AOR = 3.2; 95% CI: 1.4 - 7.0; $p = 0.004$) and in cattle with poor body condition (AOR = 4.6, 95% CI: 1.8 - 11.7; $p = 0.005$) (Table 2).

Table 1. Univariable logistic regression analysis for potential risk factors associated with the prevalence of bovine schistosomiasis in cattle in the study area.

Variables		No. Examined	No. Infected	Prevalence (%)	COR (95% CI)	*p* value
Breed	Local	201	48	23.9	1[a]	
	Cross	97	15	15.5	0.6 (0.3 - 1.1)	0.098
Sex	Female	161	35	21.7	1[a]	
	Male	137	28	20.4	0.9 (0.5 - 1.6)	0.784
Age	Young	86	11	12.8	1[a]	
	Adult	121	23	19.0	1.6 (0.7 - 3.4)	0.237
	Old	91	29	31.9	3.2 (1.5 -6.9)	0.003
Body condition score	Good	70	6	8.6	1[a]	
	Medium	119	24	20.2	2.7 (1.1 - 6.9)	0.041
	Poor	109	33	30.3	4.6 (1.8 -11.7)	0.001
Management	Intensive	30	2	6.7	1[a]	
	Semi-intensive	54	10	18.5	3.2(0.6-15.6)	0.154
	Extensive	214	51	23.8	4.4 (1.1-19.0)	0.049

1[a]: Reference; COR: Crude odds ratio; CI: Confidence interval

Table 2. Multivariable analysis to identify the strengths of factors associated with the prevalence of bovine schistosomiasis in the study area (n=298).

Variables		No examined	No. positive	Prevalence (%)	AOR (95% CI)	*p* value
Age	Young	86	11	12.8	1[a]	
	Adult	121	23	19.0	1.6 (0.7 - 3.6)	0.242
	Old	91	29	31.9	3.2 (1.4 - 7.0)	0.004
Body condition score	Good	70	6	8.6	1[a]	
	Medium	119	24	20.2	2.3 (0.9 - 6.0)	0.097
	Poor	109	33	30.3	3.9 (1.5 - 10.2)	0.005
Management	Intensive	30	2	6.7	1[a]	
	Semi-intensive	54	10	18.5	3.3 (0.6 - 17.4)	0.152
	Extensive	214	51	23.8	4.1 (0.9 - 18.9)	0.067

1[a]: Reference; AOR: Adjusted odds ratio; CI: Confidence interval

Discussion

The study revealed a 21.1% overall prevalence of *Schistosoma bovis,* a figure comparatively lower than prevalence reported in some prior studies. For instance, the prevalence documented in this investigation was notably lower than the 37.3% reported by Habtamu and Mariam (2011) in Bahir Dar area, and the 27.13% reported by Gebre (2010) in Dembia district. However, it exceeds the prevalence reported in other studies, such as the 10.17% by Chanie *et al*. (2012) in South Gondar zone, the 4.59% by Abebe *et al.* (2011) in Agaro and the 10.11% in Jimma area, 11.5% by Alemayehu and Asrat (2015) in Dangila district, and 7.6% by Mihret and Samuael (2015) in Debre Tabor. Disparities in the prevalence could be attributed to various factors, such as irrigation practices, agroecology, types of animal management, climatic variations, and other geographical influences both on the parasite and its snail vector. Yihunie *et al*. (2019) mentioned that agro-ecological factors, such as temperature, moisture, humidity, availability of large permanent water bodies, and irrigation practices are crucial to sustain schistosome life cycle. Additionally, Hansen and Perry (1994) highlighted that a critical factor influencing the spread of bovine schistosomiasis is the prevalence of intermediate hosts and their capacity to thrive and reproduce in specific environmental conditions, particularly in proximity

to substantial, permanent bodies of water. Marshlands and stagnant water sources, including creeks, ponds, and swamps situated near rivers, lakes, and irrigation areas, are identified as principal factors for transmission of bovine schistosomes. This emphasizes the importance of these aquatic environments in the life cycle of the disease, serving as critical hubs for the infection's propagation (Urqhart *et al*., 2003).

The prevalence of *S. bovis* infection exhibited no statistically significant difference ($p > 0.05$) among male (20.4%) and female (21.7%) cattle. This pattern aligns with the results reported by Mihret and Samuel (2015), who found a prevalence of 33.1% in females and 27.1% in males. This might be due to the absence of difference in grazing behavior, animal management and grazing land for the two sexes. Nonetheless, our findings diverge from the outcomes of studies conducted by Kebede *et al.* (2008) and Gebre (2010), where higher prevalence rates were recorded in male cattle. The present study suggests, in contrast, that both sexes face an equal risk of contracting bovine schistosomiasis.

In the present study, a markedly elevated prevalence of bovine schistosomiasis was observed in older cattle (31.9%), in comparison to adults (19.0%) and young cattle (12.8%). Multivariable logistic regression analysis revealed that older cattle exhibit a threefold higher likelihood of contracting bovine schistosomiasis (AOR = 3.2; 95% CI: 1.4 - 7.0; $p = 0.004$) when compared to their younger counterparts. These findings align closely with previous reports in the country (Mengistu *et al.,* 2012; Merawe *et al*., 2014; Yihunie *et al.,* 2019). The increased prevalence of bovine schistosomiasis in older cattle is postulated to be linked to husbandry practices that elevate the risk of environmental exposure. Moreover, older cattle, with potentially compromised immune systems, are more susceptible to infection. In contrast, a study by Gebre (2010) reported a higher prevalence of 17.6% in cattle below two years of age in Dembia district. The reasons for the discordance between these studies are not entirely clear, but potential contributing factors might include variations in parasite load in the environment and/or in the intermediate host, differences in animal management systems, or disparities in age definitions applied across studies.

The current study indicated that cattle with poor body condition exhibit a fivefold increased likelihood of schistosomiasis infection (AOR = 4.6, 95% CI: 1.8 - 11.7; $p = 0.005$) compared to those with a good body condition score. This finding aligns with the outcomes of previous studies that reported a notably higher prevalence of bovine schistosomiasis in cattle with poor body conditions (Abebe

et al., 2011; Melkamu, 2016; Samuel *et al.*, 2016; Shiferaw and Deressa, 2017). Marquardt and Greive (2000) and Mihret and Samuel (2015) indicated that acquired immune status of animals with poor body condition and that are weak become suppressed and prone to become more vulnerable to parasitic infestations. On the other hand, Urqhart *et al.* (2003) and Hansen and Perry (1994) have documented that bovine schistosomes exploit the nutrition of their hosts and undermine their immune capabilities that could lead to several adverse effects, such as anorexia, weight loss, and emaciation. On a similar report, Niaz *et al.* (2010) mentioned that *S. bovis* infection can result in weight loss, poor weight gain, and weak acquired immunity (Niaz *et al.*, 2010). Hence, the poor body condition of animals might have resulted as a result of schistosomiasis and/or it might result in higher exposure to *S. bovis* infection.

The highest prevalence of bovine schistosomiasis was observed in animals under an extensive management system (23.8%) as opposed to those in semi-intensive and intensive management systems. This outcome aligns with findings from other studies conducted in the country (Gebre, 2010; Lulie and Guda, 2014; Samuel *et al.*, 2016). Extensive management practices commonly involve allowing cattle to graze in open areas, thereby increasing their exposure to contaminated water sources. In contrast, animals maintained under intensive management systems are typically housed in pens or stalls, effectively reducing their likelihood of encountering contaminated water.

Conclusions

In conclusion, the study establishes prevalence of bovine schistosomiasis in the study area, with an overall prevalence of 21.1%. The findings underscore the influence of various factors including age and body condition. Specially, older cattle, and cattle with poor body condition were more likely to be infected with the parasite. The significance of these factors underscores the crucial importance of incorporating husbandry-related risk factors into disease control and prevention interventions.

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The impact of repeated skin testing and slaughter on bovine tuberculosis control in Holeta Dairy Farm: an update on subsequent prevalence and herd demographic changes

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Abstract

Bovine tuberculosis (bTB) is a serious animal health problem in Ethiopia, ranking among the top three livestock diseases. If the factors contributing to the spread and persistence of bTB are not managed, the situation may get worse. Therefore, prioritizing bTB control is essential. A retrospective study was conducted to evaluate the effect of repeated skin testing and slaughter on subsequent bTB prevalence and herd demographic changes in Holeta dairy farm. A total of 810 Friesian (F) * Boran (B) crosses, and pure Boran animals were involved in the study; all animals on the farm, except calves ≤6 months of age, were tested using single intradermal comparative cervical tuberculin test (SICCTT). On the basis of 21.3% prevalence in the first-round test in December 2014, three successive rounds of test and slaughter were conducted. During this period, the prevalence of bTB infection exhibited an oscillating pattern. It initially declined to 8.4% (n = 496) during the second-round test but then experienced resurgence, reaching 24.8% (n = 503) in the third-round. Finally, the prevalence decreased to 5.4% in the fourth-round test. The time interval between successive SICCTT tests varied from 0.95 years to 1.84 years. The test and slaughter intervention resulted in the culling of a substantial number of cows (n=342). With an increased culling rate, the average age of the herd and the average number of lactations per cow decreased. Similarly, animal entries and exits also influenced the breed composition of the herd. During the first

round test, over 63% of the herd was composed of 50% HF*B crosses, followed by pure Boran, and 75% HF*B crosses, respectively. However, between the first and fourth test rounds, the proportion of Borans declined to 5%, while high-grade (75% HF*B) animals increased almost fivefold. In conclusion, the findings of this study showed the effect of prolonged time interval between consecutive tests which might result in failure of test and slaughter intervention, hence, due attention should be given on maintaining the recommended regular time interval (2-6 months) during consecutive test and slaughter procedures. Finally, the study recommends further study on assessing other alternative control strategies such as test and segregation methods to reduce the economic impact of culling of bTB reactor animals at a farm level.

Keywords: Bovine tuberculosis; Disease control; Prevalence; Reactor; Test-and-slaughter.

Introduction

The intensification of livestock farming is emerging as a response to the rising need for milk and dairy products due to swift population growth and urbanization (Demissie *et al*., 2014; Greentumble, 2016). Unfortunately, this intensification of livestock production is associated with a rise in bTB, particularly in peri-urban areas

Researches in Ethiopia have identified risk factors conducive to the spreading and persistence of bTB (Ayele *et al.,* 2004; Regassa *et al.,* 2008; Girmay *et al*., 2012;).

Bovine tuberculosis (bTB) is among the top three serious animal health problems in Ethiopia (Lakew *et al.,* 2022). The costs of bTB are mainly related to livestock production losses, including increased mortality and lower milk and meat production. So, the best control method of the disease in domesticated animals is regular skin test- and-slaughter or test- and-segregation of infected herds (Marshet, 2020). This prevents the disease from spreading beyond the herd, while the slaughter of diseased animals removes the infection from the herd. However, implementing this method poses significant challenges in most low-income countries due to the inseparable connection between the value of cattle and the socio-cultural system, as well as the poor savings of the rural people (Adeyemo and Silas, 2020; Michel *et al*., 2004). Valuable breeding stock may easily be lost through slaughter, as well. This can be of extensive

socio-economic significance in non-industrial nations where the replacement of equivalent breeding stock might be excessively unaffordable (McCrindle and Michel, 2007). Despite the lack of a national control program, some localized efforts have been undertaken in Ethiopia to control bTB at the farm level.

Cows are culled from the herd either voluntary, in which farmers have the freedom to choose which cows to remove based on factors like the availability of replacement heifers, land availability, and market prices (*Grandl et al.,* 2019; Adriaens *et al.,* 2020; Rostellato *et al.*, 2021), or involuntary in which animals are culled due to reasons such as disease, injury, infertility, or death, without the farmer's choice (De Vries, 2013, 2017; Zehetmeier *et al.*, 2014). Decisions on voluntary culls are made to maximize profit. Usually, involuntary culling is not effective, resulting in all cows eventually leaving the herd and the replacement expenditures are excessive (Hadley *et al.,* 2006). The lack of replacement cows, their high expenses, or the unavailability of suitable replacements often forces farmers to prolong the calving interval for existing cows. Consequently, disease-prone, high-risk cows are retained in an effort to manage overall culling rates, resulting in reduction in milk production, reproduction, or genetic improvement (Hadley *et al.,* 2006; Orpin and Esslemont, 2010). Grandl *et al.* (2019) indicated that a large number of cows are removed from the herd early in lactation mainly because of diseases. Likewise, the impact of test- and-slaughter control option for bTB also goes beyond its direct economic losses (Caminiti *et al.,* 2016), since removal or slaughter of infected animals had unintended consequences on herd demography. This affects the dairy production response and profitability (Hadley *et al.,* 2006).

The single intradermal comparative cervical tuberculin test (SICCTT) is the most commonly used diagnostic test for bTB in live animals (Marassi *et al.,* 2013). Unfortunately, the SICCTT has limitations in terms of low sensitivity and specificity, which means some infected animals might be missed (false negatives) and some non-infected animals might be culled needlessly (false positives) (De la Rua-Domenech *et al.,* 2006; Lahuerta-Marin *et al.,* 2016, 2018; Nunez-Garcia *et al.*, 2018). This is a critical issue because infected animals can remain undetected and continue spreading the disease.

Despite some limitations, Holeta dairy farm has been practicing a test-and-slaughter program to control and eliminate bTB infection from its herd. Therefore, the study was designed to evaluate the effect of repeated skin testing and

slaughter on the subsequent prevalence of bTB, and identify factors that affect its success. Additionally, the study examined the impact of this control strategy on herd demographics over five years.

Materials and methods

Farm description

Holeta dairy farm under Holeta Agricultural Research center, was established in 1977 for genetic improvement by animal breeding research unit. The farm contains a breeding unit, an animal health unit, a feed production unit, and an animal husbandry unit with two sub-units, namely, herd management and milk production sub-units.

The foundation stock, Boran cattle brought from southern Ethiopia, was inseminated with Friesian semen from worldwide sire (WWS) to produce first and second-generation crossbred offspring. Fifty percent Boran-Friesian (BO*F) (50%F1) crosses were produced from Boran dams inseminated with Friesian semen, and the other BO*F (50% F1) were back-crossed with pure Friesian semen to produce the 75% first generation (BOFF). Crossing 50% males with 50% females and 75% males with 75% females produced later generations. Beside herders, a teaser bull was reared with cows for heat detection. Cows were bred using artificial insemination by qualified technicians. Cows that failed to come into heat were checked for pregnancy 60 days after service.

Cattle at the farm are grouped based on breed, pregnancy, lactation stage, and age, and uniform feeding and management are used for all animals within each group. Natural grazing, hay, and concentrate supplement constituted the primary feed supply. Concentrate is supplemented based on body weight, productivity, and physiological categories. Milking cows, heifers, and calves are supplemented with concentrate mixture at a rate of 4, 1-1.5, and 0.25-1kg per day, respectively, depending on the availability of the concentrate mixture. All cows have unrestricted access to fresh drinking water. Calves are allowed to suckle their dam immediately after birth for about four days to receive colostrum. Weighing and ear tagging are completed within 24 hours after birth. After four days, calves are moved to calf-rearing pens and fed whole milk for 98 days from bucket feeding, except the 50% F1 calves, which suckled their dams. Weaned calves are then transferred to a group pen (calves housed in pens of 15- 20 calves) and kept indoors until six months of age. Milking is conducted by milking machine twice daily (early morning and evening).

Study design and skin testing

A retrospective study was conducted to investigate the five-year (2014-2019) trajectories of bTB and demographic change of the study herd practicing irregular test and slaughter control program. In each testing round, all animals in the herd (except the suckler/rearing calves and replacement heifer calves ≤ 6 months of age) were tested using SICCTT. Cows were classified as reactors, doubtful/inconclusive, and negative. Reactors were culled and not involved in the later retests. There were a total of four testing rounds on the herd, during which all reactors were removed. The time interval between two consecutive test rounds varied from 345 to 672 days.

An animal entry is defined as the number of new animals entering the study herd and receiving their first SICCTT at a given screening test round. Self-sourced replacement heifers and outsourced Boran heifers as damline, were the main entry animals. Exit is defined as animals that had been tested by SICCTT in a penultimate test round (the second to last test) but lost from the study herd at a given screening test round. Animals might exit from the herd either due to culling or death.

The SICCTT was used as described in OIE manual (OIE, 2019). Two sites on the right side of the skin of the middle third neck of the animal, 12cm apart, were shaved; the skin thickness was measured with calipers, and 0.1 ml of avian purified protein derivatives (PPD-A) and 0.1 ml of bovine (PPD-B) antigens were injected. After 72 hours, the same researcher measured skin thicknesses at the injection sites again.

A positive reactor for *M. bovis* was defined as the relative increase in skin thickness at the injection site for PPD-B minus the skin thickness at PPD-A injection site was ≥ 4mm, inconclusive/doubtful if >2mm - <4mm, and negative if <2mm. A positive reaction to *M. avium* was interpreted if ▲A>4mm and negative if ▲A<4mm.

Data analysis

Data were entered into an Excel spreadsheet (MS Excel) and analyzed using Stata 14 (STATA Corp. Ltd). Descriptive statistics were generated for each variable of interest. Percentages and their 95% confidence intervals (95% CI) were calculated to determine the prevalence of bTB. Pearson's Chi-square test was used to evaluate differences in proportions.

Results

Effects of skin test and slaughter

The dairy farm conducted four rounds of SICCTT tests and slaughtered positive reactors. Of 502 animals tested in the first-round in December 2014, about 21.3% of the herd was SICCTT positive. On the basis of this result, three successive rounds of testing were conducted, and their prevalences were determined Table 1 presents the time interval among consecutive test rounds and results of the tests. In this study, the prevalence of bTB was neither reduced steadily along successive test rounds nor fell below 1% or nearly eliminated from the herd. The prevalence of bTB had declined to 8.4 % (n = 496) in the second round test. However, a resurgence of bTB occurred in the third-round skin test (n = 503; 24.8% prevalence was reported). In the meantime, the prevalence remarkably decreased to 5.4% in the fourth-round test (Figure 1). In general, reactor animals were identified in all test rounds although the proportion of positive cattle varied along test rounds.

Table 1. Results of the four consecutive test and slaughter rounds applied to the study farm.

Test rounds	Number of animals tested	Test interval (days)	Percent positive
Test-1	502		21.3
Test-2	521	459	8.4
Test-3	503	672	24.8
Test-4	498	345	5.4

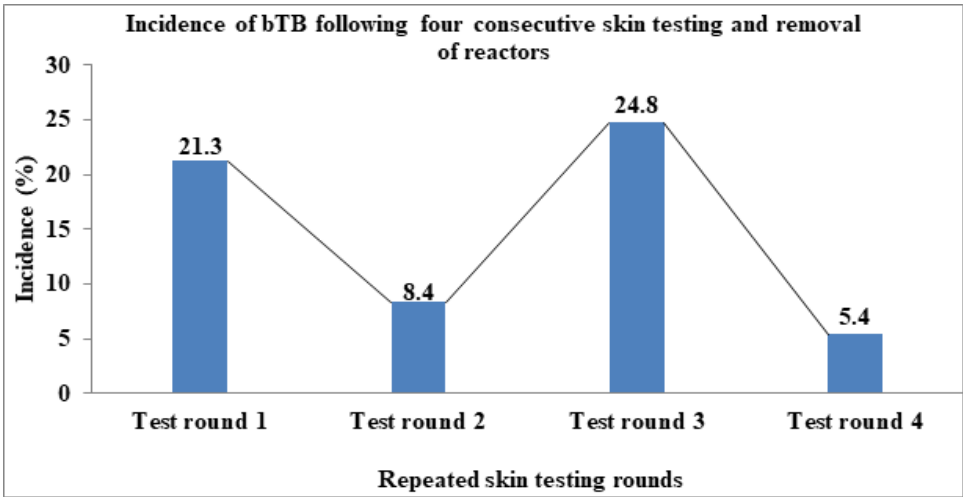


Figure 1. Trajectories showing the effect of irregular skin testing and slaughter on the prevalence of bTB in the study herd.

The prevalence, as evidenced in Figure 1, did not exhibit a direct or indirect proportionality with the repeated test and slaughter rounds**.**

The replacement heifers entering the herd were equivalent to those animals leaving the herd prior to the first-round test (Figure 2). However, following the test-and-slaughter control measures, the herd size, and the number of replacement heifers entering the herd exhibited a fluctuating trend (Figure 2). Immediately after each test round, the herd size suddenly declined due to slaughtering of a large number of reactors. On the other hand, replacement heifers started to join the herd gradually, and then the herd size experienced resurgence till the next test and slaughter rounds.

The number of animals culled from the study herd primarly affected the composition of the herd. The herd composition at a given screening test round was made of two units: new entries and retested (existing) animals. The proportion of retested animals ranged from 74% to 90% depending on the respective screening test round The rest of the animals were new entry with no tuberculin test history. During each test round, more animals (15.8%) sourced from the baseline herd (retested herd) were culled than others.

During the monitoring period, there were 810 animals involved in the screening tests, of which 342 cows were culled, and 468 were still present when monitoring ceased. Out of the 502 animals initially tested for PPD in the first-round screening test, only 392, 346, and 234 animals were subsequently passed to the second, third, and fourth round skin tests, respectively. Besides, out of 129 newly introduced animals in the second round test, only 85 passed to the fourth round test (Figure 2). The remaining animals left the herd via involuntary culling or death.

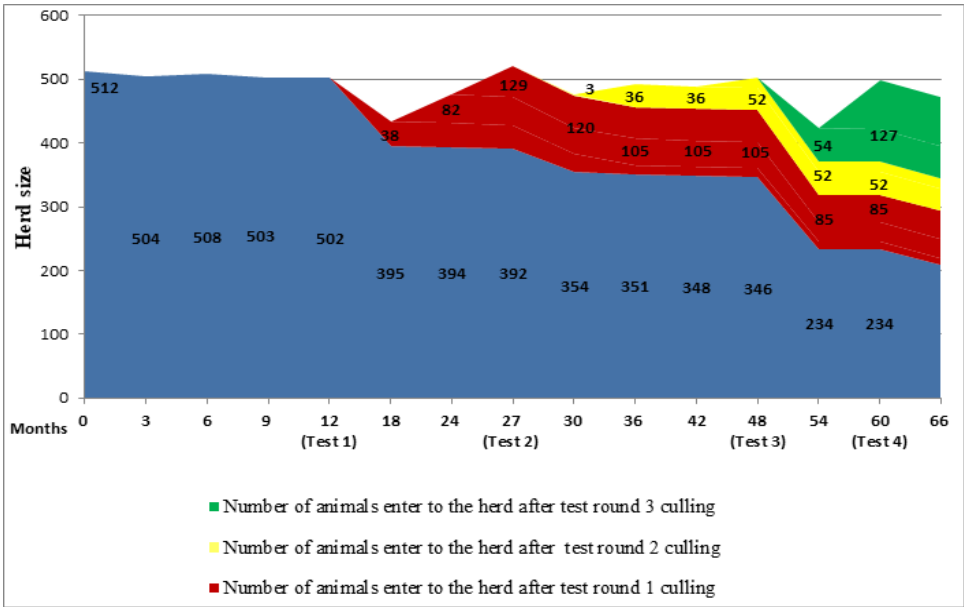


Figure 2. Chart illustrating livestock entries and exits in successive screening tests.

Influence of animal entries and exits

Animal entries and exits significantly influenced the average age and parity of the study herd. Following test and removal control measures, several cows (mean age = 8.7 years) were culled from the herd, while heifers were introduced as replacement stock. Table 2 compares the average age of culled animals and new entry animals for successive test rounds. Around 94% of culled animals in the study herd were older than 3.19 years. These entries and exits played a crucial role in shaping the average age, parity and breed composition of the

study herd. For example, 50% of the herd had ≤4.26 years of age before the first test and slaughter control intervention However, the median age decline to 3.14 years after culling of reactor animals (Figure 3). The herd included animals ranging from 6 months to 17.5 years old.

Table 2. Average herd age of new entry and culled animals at each test round.

Test round	Mean herd age (years)		Mean age of new entry	Mean age of culled animals
	Before control	After culling		
Test 1	5.75	4.60		9.99
Test 2	4.90	4.49	2.10	8.73
Test 3	5.72	5.07	2.09	7.67
Test 4	4.88	4.75	2.09	6.86

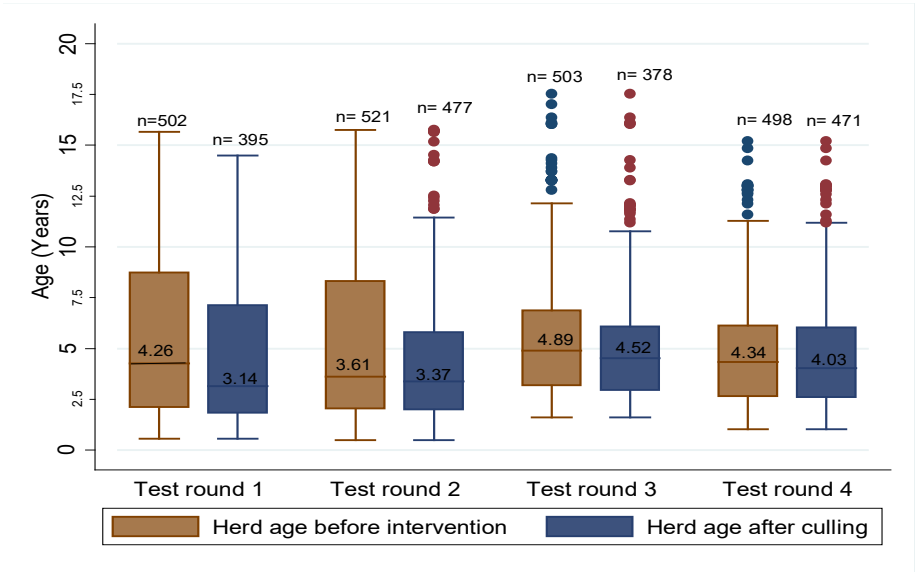


Figure 3. The median age of the study herd before and after test and slaughter control measure.

The test and slaughter interventions in the farm made the herd to have more proportion of animals with ≤2 parity. Prior to the first round test, 65% of the herd were animals with two or less parity, while the remainings 35% were productive cows with more parity. However, after test and slaughter control

intervention, the proportion of these high milk yielding multiparous cows declined to 26%. Similar trends were observed in the rest of the test rounds, as shown in Figure 4. As cows go through multiple parities, their milk production tends to increase till about the fifth or sixth parity. Older cows may produce up to 25% more milk volume than first-parity cows. In the current study, 60% of the culled animals were multiparous cows. Culling of cows at this stage highly impacts the total milk production of the herd.

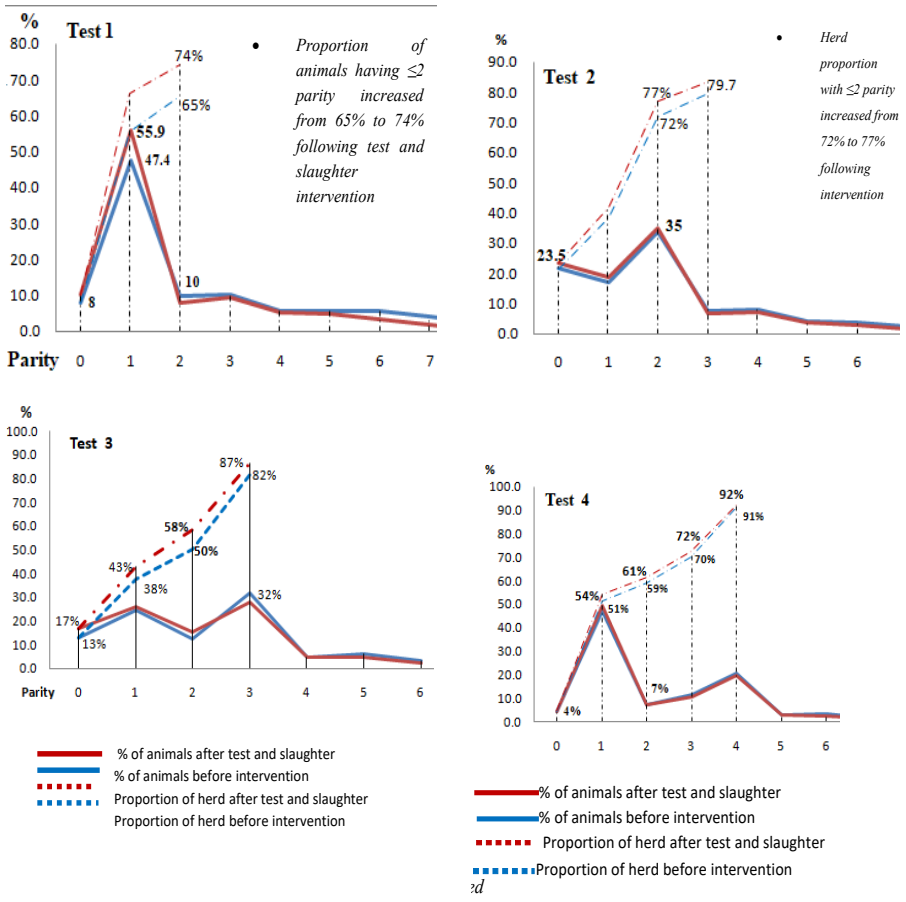


Figure 4. The proportion of herd parities before and after test and slaughter control measure.

Animal entries and exits also influenced the breed composition of the study herd. The herd consisted of a mix of pure Boran and different crossbred animals. The 50% crossbred animals consistently formed over 63% of the herd population, of which 50%F1 (Boran X Friesian) took the largest proportion in all screening test rounds. However, the proportion of purebred Boran animals declined to 5%, while high-grade animals (75% Friesian blood) increased almost five-fold between the first and the fourth-round tests. The proportion of 75% of crossbred animals was inversely proportional to that of purebred Boran animals across subsequent herd test rounds (Figure 5). The prevalence of bTB by breed for the respective test rounds is presented in Figure 5. The prevalence was higher in purebred Boran and 50% Boran-Friesian crosses than high-grade animals (75% Friesian-Boran).

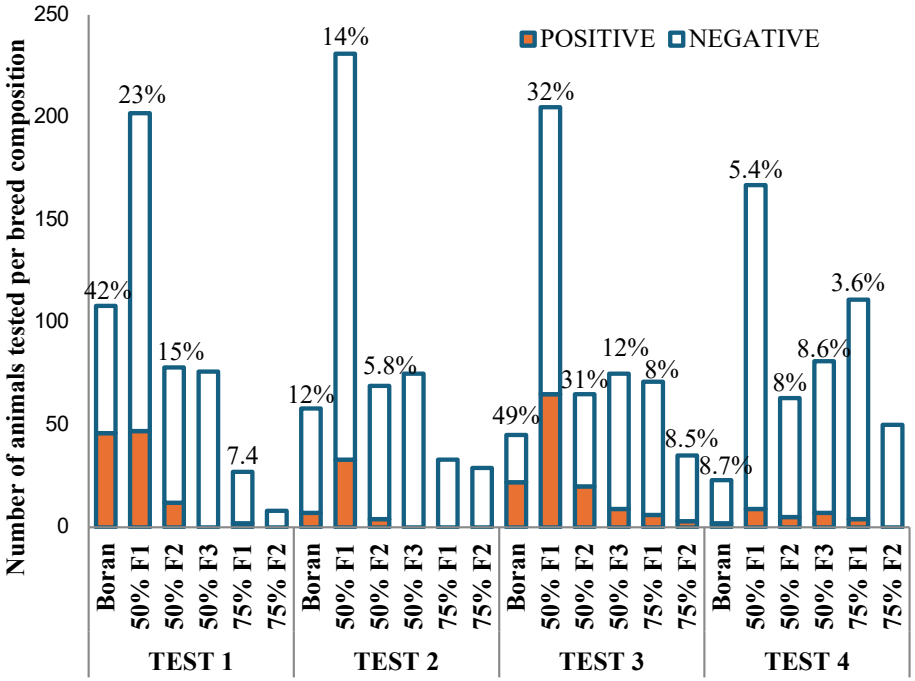


Figure 5. bTB prevalence over breed of the herd along successive screening test rounds.

Discussion

Conventionally, SICCTT is recommended as a diagnostic tool for reactor detection. A typical strategy for bTB control in domestic animals involves regular field tests and quarantine of infected herds. This prevents bTB from spreading beyond the herd. Only test-and-slaughter techniques have proven capable of eradicating bTB from domestic animal populations. Previous trials in Ethiopia found that test and slaughter, as practiced by a few dairy farms, showed an apparent improvement in prevalence (Ameni et al., 2007; Shitaye et al., 2007). The same authors also reported that test and slaughter resulted in a pronounced prevalence reduction. Moreover, the study by Proud (2006) showed a trend toward meaningful reduction of cattle-to-cattle transmission as soon as reactors and non-reactors were physically separated. In this study, despite repeated testing and removal measures, the prevalence of bTB did not exhibit a substantial reduction trend along the successive test and slaughter rounds. These failures might be due to excessively prolonged and inconsistent tuberculin testing time intervals for repeated herd retesting. In this study, three subsequent tests were utilized within four years after the first test, and the time interval between successive SICCTT varied from 0.95 years to 1.84 years. This prolonged inter-test interval facilitated the continued transmission of bTB within the herd. However, a previous study by Ameni et al. (2007) reported that the application of three consecutive tests every four months after the first test enabled earlier infection detection and culling, reducing the prevalence from 14% to 1% within a year. According to the USDA protocol, to eliminate bTB, the entire herd must have eight consecutive negative whole herd tests (WHT). This involves performing the first four tests at intervals of at least 60 days, maintaining a gap of at least 180 days between the fourth and fifth tests, and ensuring that there are at least 12 months of three consecutive tests between the fifth and eighth tests (USDA-APHIS, 2005).

The study farm took decisive action by slaughtering a number of infected animals (n=342), aiming to eliminate the infection from the herd. Commonly, culling is practiced to increase profits or reduce costs by replacing sick or non-pregnant cows (Olechnowicz and Jaskowski, 2011). This might be voluntary, for example poor production, or involuntary, including disease, injury, infertility or death (Ansari-Lari et al., 2012). Hence, the decision to cull often depends on parity, milk production, fertility, and health of cows (Bascom and Young, 1998; Groenendaal and Galligan, 2005; Olechnowicz and Jaskowski, 2011). If the culling reason is economic, a replacement animal is expected to produce great-

er profit (Fetrow, 1987), and the farmers could maintain a balance between culling and herd longevity to ensure sustainable herd management. The test and slaughter bTB control intervention at Holeta dairy farm resulted in unintended consequences on the herd demography. These demographic changes played a crucial role in shaping the average herd age, parity and breed composition of the study herd. With an increased culling rate, the average age of the herd and the average number of lactations per cow decreased which was not favorable for herd demography maintenance (Stewart, 1995). Furthermore, even if it could be nearly possible to maintain the herd size along the study period, there was aconsiderable gap in parity between the replacing heifers and culled cows. Culling is the act of, replacing a cow with another cow, often with a first-lactation heifer (Hadley et al., 2006). In the current study, younger cows or heifers replaced older ones, leading to a shift in the age distribution. Likewise, frequent culling in the subsequent test rounds of the study herd forced cows to have fewer opportunities to complete their multiple lactation cycles. This reduction in lactations per cow is likely to impact the overall milk production and reproductive efficiency of the herd. Unfortunately, the applied control intervention for bTB was not favorable for maintaining stable herd demography.

In the current study, the proportion of infected animals was significantly higher in purebred Boran than in high-grade Friesian crosses kept under the same husbandry conditions. The most likely explanation for this could be due to the older age of Boran animals in the study herd, which would give a longer time for infection and actively respond to tuberculin test after infection. This is in line with a previous study (Islam et al., 2020), which found that the odds of bTB were 2.2 and 2.5 times higher in cattle aged >3–6 years and > 6 years, compared to cattle aged ≤1 year. On the contrary, Ameni et al. (2006) reported that diverse local B. indicus breeds had lower skin test prevalence (5.6%) compared to Holstein exotic breeds, with 13.9% prevalence. Similarly, Carmichael (1939) reported that the prevalence of bTB in relation to cattle breeds was dramatically lower in Zebu cattle compared to taurine Ankole cattle, indicating that Zebu calves showed remarkable resistance compared to Ankole calves.

Conclusions

In this study, despite repeated irregular skin testing and removal of reactors, the prevalence of bovine tuberculosis did not exhibit a significant reduction trend along the successive test rounds. These failures might be due to inconsistent and excessively prolonged test-and-slaughter schemes. Compliance to

conventional test and slaughter protocol plays an important role for effective bTB disease control.

In conclusion, the findings of this study showed the effect of prolonged time interval between consecutive tests which might result in failure of test and slaughter intervention, hence, due attention should be given on maintaining the recommended regular time interval (2-6 months) during consecutive test and slaughter procedures. The present study also identified a vital knock-on effect on herd demography due to test-and-slaughter measures, which played a crucial role in shaping the average herd age, parity and breed composition of the study herd. With an increased culling rate, the average age of the herd and the average number of lactations per cow decreased, which was not favorable for herd demography maintenance. Hence, culling should be carried out with minimal significant impact on herd demography change. Finally, the study recommends further study on assessing other alternative control strategies such as test and segregation methods to reduce the economic impact of culling of bTB reactor animals at a farm level.

List of abbreviations

BCG: Bacillus Calmette and Guérin; bTB: Bovine Tuberculosis; SICCTT: Single intradermal comparative cervical tuberculin test; EIAR: Ethiopian Institute of Agriculture research; FAO: Food and Agriculture Organization of the United Nations; OIE: Office International des Epizooties, PPDs: Purified Protein Derivatives

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Authors' contributions

The following authors considerably participated in the study. B.A., M.A., and B.E. contributed to the collection of epidemiological data and interpretation of data. B.A., R.L.S., and B.G. designed the study. B.A. performed the data analyses and wrote the manuscript. R.L.S., B.G., and G.M. made substantial contributions to reviewing it critically.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Seroprevalence and associated risk factors of contagious bovine pleuropneumonia in three districts of Ilu Ababor Zone, Oromia, Ethiopia

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Abstract

Contagious bovine pleuropneumonia is caused by *Mycoplasma mycoides* subspecies *mycoides* small colony *(Mmm SC),* which causes significant economic loss in Sub-Saharan Africa, including Ethiopia. A cross-sectional study using systematic random sampling technique was conducted in Bacho, Bure, and Dorani districts of Ilu Ababor Zone to estimate seroprevalence and identify associated risk factors of contagious bovine pleuropneumonia (CBPP) in apparently healthy cattle with no history of CBPP vaccination. Thus a total of 371 blood samples were collected from the jugular vein of each cattle and tested using competitive Enzyme Linked Immunosorbent Assay (c-ELISA) to detect specific antibodies to *Mmm SC*. Data on potential risk factors for the occurrence of CBPP were collected using a questionnaire survey. Data obtained from both serological and questionnaire surveys were analyzed with SPSS version 20 software. Multivariable logistic regression was used to analyze the association of the exposure variables with *Mmm SC* serostatus, and the strength of the association was assessed using the odds ratio. The overall animal and herd-level seroprevalence of CBPP was 9.7% and 30.7%, respectively. The seroprevalence of CBPP in the Bacho, Bure, and Dorani districts was 0%, 10.8%, and 33.3%, respectively. There was a statistically significant association between Mmm SC antibody and district, age, and body condition score (p <0.03) at the individual animal-level. At the herd-level herd size was significantly associated (p <0.00) with *Mmm* SC antibody. This study indicated that CBPP was prevalent in the Dorani and Bure districts. So, methods should be devised to control the disease in the districts and prevent its spread, because CBPP is a contagious disease.

Keywords: CBPP; c-ELISA; Ilu Ababor; Risk factors; Seroprevalence.

Introduction

Contagious bovine pleuropneumonia (CBPP) is a transboundary animal disease primarily affecting cattle. It is highly contagious and has a high impact on animal production and health in Sub-Saharan Africa. In Ethiopia, field studies have shown that CBPP poses a significant threat to cattle production in different parts of the country (Ebisa *et al.,* 2015; Teklue *et al.,* 2015; Daniel *et al.,* 2016; Fulasa *et al.,* 2020).

Moreover, reports from various researchers in the country indicate considerable threat to the livestock export markets (Kassaye and Molla, 2013; Dele *et al.,* 2014; Atnafie *et al.,* 2015). The disease has a significant economic impact on cattle owners and livestock investments through direct losses (in terms of mortality, reduced milk, traction, loss of weight and workability, delayed marketing, and decreased fertility). Indirect losses are attributed to the cost of control measures and the resulting trade ban, as well as compromising food security through protein loss and the painful suffering of animals. Also, the disease retards genetic improvement (Abera *et al.,* 2016; Constable *et al.,* 2017; Demil, 2017).

There are possible challenges that increase the risk of disease in Ethiopia, which include lack of knowledge of the disease by farmers, vaccine shortage, poor diagnostic assays, management system; limitation of epidemiological information about the disease; concentration of livestock at watering points and grazing areas; and difficulty in controlling cattle movements. As a result, it is one of the most severe threats to cattle health in the study area. This disease continues to pose a threat to the livestock export market and reduce investment in livestock production in Ethiopia (Ebisa *et al.,* 2015).

Various animal diseases with unknown causative agents have been reported in western Oromia, with the primary signs being a respiratory problem, affecting livestock production and productivity and threatening the livelihoods of small-scale farmers. Ilu Ababor zone is one of the zones in western Oromia where there was no study, particularly in the current study districts (Bacho, Bure and Dorani), on the sero-prevalence and risk factors of CBPP, except for the study conducted in 1998, in a neighboring district with similar agro-ecology to the present study areas (Desta, 1998). Also, there was a report of cattle deaths in Bacho, Bure and Dorani districts. Lack of sufficient epidemiological information on livestock diseases in the area, contact of animals at grazing

and watering points which make the ease for disease spread, the difficulty of detecting carrier animals, limited resources to apply slaughter and compensation mechanisms, and the inability to restrict animal movement, all present a challenge for disease control. As a result, this study was planned to assess the magnitude of CBPP in non-vaccinated animals in three purposively selected districts in the southwestern region of Ethiopia.

Materials and methods

Description of the study area

The study was conducted in the Bacho, Bure, and Dorani districts of Ilu Ababor Zone, Oromia regional state, Ethiopia.

Bacho is located at a distance of 620 km from Addis Ababa at southwest direction. The district has 17 peasant associations, and the district is located at 8°16' N latitude and 35°48' E longitude, with an altitude of about 1875 meters above sea level. The minimum and maximum temperature of the area is 23°C and 27°C, respectively. The district has 115, 626 cattle, 18661 sheep, 28981 goats, 8861 equines, 111,220 poultry, 1152 dogs and 115 cats (BaADO, 2021).

Bure district is located in the southwest direction at about 680 km from Addis Ababa at 8°59' N latitude and 37°19' E longitude with altitudes ranging from 1450-1770 meters above sea level with temperatures ranging from 22 to 29°C. The district has 21 peasant associations. The animal population of the district includes 91,101 cattle, 28,298 sheep, 28,370 goats, 163 horses, 159 mules, 4,282 donkeys, 154, 364 Poultry, 1,519 dogs and 152 cats (BuADO, 2021).

Doreni district is located from Addis Ababa at a distance of 584 km in southwest direction at 8°26' N latitude and 35°51' E longitude, with an altitude range from 1845 to 1950 meters above sea level and rainfall of 700 mm. The temperatures of the area range from 23 to 29 °C. The animal population of the district comprises 41,792 cattle, 27,904 sheep, 15,397 goats, 14,846 equines, 76,996 poultry 967, dogs and 97 cats (DADO, 2021).

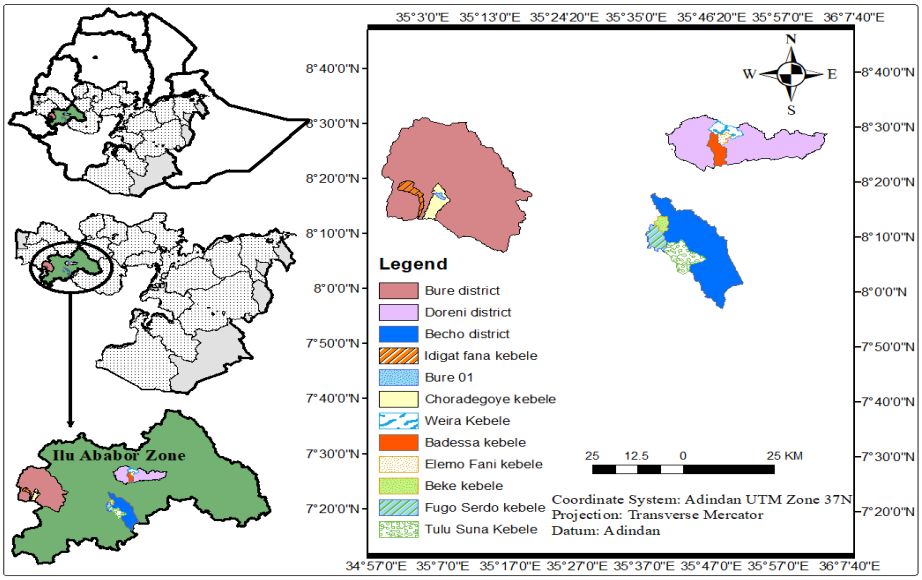


Figure 1. Map of Ethiopia and Oromia National Regional State depicting the study area (Arch GIS 10.4).

Study population and study animals

The study population consisted of all indigenous zebu cattle above six months of age, managed under extensive and semi-intensive management systems, and without a history of CBPP vaccination (information on previous CBPP vaccinations was obtained from the district veterinary office).

Study design and sample size determination

Study design

A cross-sectional study was conducted using a systematic random sampling technique to select the study animals. List of herd distribution and household size were obtained from the peasant associations (PA), and then both blood sample collection and questionnaire surveys were conducted. Pre-tested semi-structured questionnaire was used to collect information on factors influencing the occurrence of CBPP within or between herds using face-to-face interview. The age of animals was determined based on the owners' information and dental eruption,according to De Lahunta and Habel (1986).

Sample size determination

The sample size required for the study was determined using the formula given by Thrusfield (2007), with a 5% desired absolute precision, a 95% confidence interval, and an expected prevalence of 32.5% (Desta, 1998).

$$N = \frac{1.96^2 \text{ X Pexp X } (1\text{-Pexp})}{d^2}$$

Where N= is the required sample size of the study population, d= is the desired absolute precision, and Pexp= p is the previous/expected prevalence.

Accordingly, the total sample size was computed to be 337 cattle. However, to increase the precision of the outcome, the total number of study animals sampled from all three districts was increased to 371 by 0.1% precision.

Sampling technique

Both purposive sampling and systematic random sampling techniques were employed for the selection of study area and individual animals, respectively. Nine PAs (Bure 01, Core Dagoye and Idigat Fana from Bure district, Elemo Fani, Weira and Badessa, from Dorani district and Fugo Sardo, Tulu Suna, and Bake from Bacho district) were selected based on cattle population, recommendation from zonal and district experts, previous disease history and access to road facility. Accordingly, 45 cattle from Bake, 74 cattle from Fugo Sardo, 50 cattle from Tullu Suna, 53 cattle from Bure 01, 50 cattle from Idigat Fana, 36 from Chore Dagoye, 22 from Baddessa, 18 cattle from Elemo Fani, and 23 from Weira peasant association were selected.

Data collection

Questionnaire survey

A pretested semi-structured questionnaire was developed, and conducted through face-to-face interview using the local language of the community (Afan Oromo). A total of 75 cattle owners were involved in the interview during the sampling of animals to collect relevant data on the presumed risk factors of CBPP.

The questionnaires comprised of questions on factors influencing the occurrence of CBPP, such as animal origin, disease history, herd size, management

system, contact of herds with another herd, the introduction of a new animal, history of commonly observed symptoms and major clinical sign of CBPP; and age and sex of animal were collected. The body condition of the sampled animals was determined following the body condition score (BCS) assessment method set by Nicholson and Butterworth (1986), and the animals were categorized into three categories, namely poor body condition (1-3 BCS), medium body condition (4-6 BCS), and good body condition (7-9 BCS).

Blood sample collection

Blood samples were collected from the jugular vein of each animal using sterile vacutainer tubes following an aseptic procedure, and each sample was appropriately labeled. The blood samples were kept in an inclined position for 3 to 5 hours at room temperature to allow clotting of blood and then centrifuged to separate serum from blood.

The serum was transferred gently into serum storage vials (cryogenic vials) stored in an icebox and transported to the Bedelle Regional Veterinary Laboratory. Upon reaching the laboratory, the serum samples were stored at -20°C until tested. Finally, serum samples were examined for antibodies against CBPP using a c-ELISA, which is based on a monoclonal anti-*Mmm SC* antibody (Mab 117/5) test at Bedelle Regional Veterinary Laboratory.

Laboratory examination

The c-ELISA technique, as recommended by the OIE for CBPP testing, was used to examine the serum samples at Bedele Regional Veterinary Laboratory in accordance with the manufacturer's instructions (CIRAD-EMVT, France) (Amanfu *et al.,* 2000). The c-ELISA test is based on a monoclonal anti-*Mmm SC* antibody known as Mab 117/5 (OIE, 2014). The microplates were coated with *Mmm SC* purified lysate. The sera to be tested were premixed with Mab117/5 specific monoclonal antibodies in a prelate, and the content of the prelate was transferred into the coated microplate.

The immune complex reaction between any *Mmm SC* antibodies found in the serum sample forms an immune complex with Mmm SC antigen coated on the microplate, competing with Mab117/5 for the specific epitope. Following the wash of unbounded material, an anti-mouse antibody-enzyme conjugate was added. In the presence of an immune complex between *Mmm SC* antigen and antibodies in the sample, Mab117/5 cannot bind to its specific epitopes, and the

conjugate is prevented from binding to Mab117/5. On the other hand, in the absence of Mmm SC antibodies in the test sample, Mab117/5 can bind to its specific epitope, and the conjugate is free to bind to Mab117/5.

The unbound conjugate was washed away, and the enzyme-substrate Tetramethyl Benzedrine (TMB) was added. In the presence of the enzyme, the substrate is oxidized and develops a blue color but changes to a yellow color after the addition of the stop solution. The amount of anti-*Mmm SC* antibodies in the test sample was inversely proportional to the subsequent color development. Finally, the underneath of the plate was wiped, and the optical density (OD) of individual reactions was measured at 450 nm using a plate reader.

The result is expressed in percentage of inhibition by comparing the optical density in the test well with the optical densities in the Mab control wells. The percentage inhibition (PI) value for each sample was calculated by the following formula.

$$PI = \frac{(OD\ Mab - OD\ test\ serum) \times 100\%}{(OD\ Mab - OD\ Conjugated)}$$

Where OD Mab denotes the optical density of a monoclonal antibody, OD Test serum denotes the optical density of a test serum, and OD conjugate denotes the optical density of a conjugate.

Samples with a percentage inhibition of ≤ 40% were considered negative for the presence of Mmm SC antibodies. In comparison, samples with a percentage inhibition of 40% >, less than 50% were considered doubtful, and those with a percentage inhibition of ≥ 50% were considered positive (OIE, 2014).

Data management and analysis

All collected data was entered and coded into a Microsoft Excel spreadsheet and imported to the SPSS version 20 software for analysis. The total seroprevalence of individual animals was calculated by dividing the number of c-ELISA positive samples by the total number of animals tested. Herd level seroprevalence was calculated by dividing the number of positive herds by the total number of herds tested. If at least one animal in a herd was seropositive, the herd was considered seropositive.

Risk factors which, showed *p* values less than 0.25 in the univariable logistic regression were chosen for the final multivariable logistic regression analysis. The Odds Ratio (OR) was used to assess the strength of the association between the risk factors and the occurrence of the disease. Pearson correlation coefficients were used to test for co-linearity among the variables. Then, the final multivariable model was created using backward elimination method.

Ethical clearance

Ethical clearance for the study was obtained from the ethical review committee of Mamo Mezemir Campus School of Veterinary Medicine, Ambo University. The aim of the study was explained, and permission was obtained from animal owners prior to sample and data collection. Blood samples were collected by the researcher following good animal handling practices to minimize animal suffering.

Results

Seroprevalence of contagious bovine pleuropneumonia (CBPP)

Of the total of 371 sampled animals, 36 animals were seropositive for CBPP. The overall animal-level seroprevalence of CBPP was 9.7% (95% CI: 6.7-12.7). Out of the total 75 sampled herds, 23 herds were found to be infected, and the overall herd-level seroprevalence of CBPP was 30.7% (95%CI: 20.5–42.4).

Risk factors associated to seroprevalence of CBPP

Animal-level risk factors

From individual animal-level risk factors across the study district, significantly higher seroprevalence was observed in the Dorani district (33.3%) than in Bure (10.8%) and Bacho district (0%). This difference might be due to number of study animals, climate condition, socio-economic factors, and trade-routes. The study also showed that seropositivity was higher in females (10.9%) than in males (6.9%). Lower seroprevalence was observed in the young age (6 months to less than equal to 3 years) group (2.5%) than the adult (greater than three years) age group (14.9%). Poor body condition animals (14.9%) had a higher prevalence as compared to good (2.1%) body condition score animals. The seroprevalence of CBPP was highest in animals bought from a local market (11.6%) as compared to their source origin (9.3%) (Table1).

Table 1. Univariable logistic regression analysis of animal-level Seroprevalence.

Risk factors	Categories	Animals tested	№ positive (%)	95%CI	OR (95%CI)	*p* value
District	Bacho	169	0 (0.0)	0-2		
	Bure	139	15 (10.8)	6.0-17		
	Dorani	63	21 (33.3)	22-46	3.7 (1.9- 8.7)	0.002
Sex	Male	116	8 (6.9)	3.0-13		
	Female	255	28 (10.9)	7.0-15	1.6 (0.7-3.7)	0.220
Age	Young	157	4 (2.5)	1.0-6.0		
	Adult	214	32 (14.9)	10-20	6.7 (2.3-19.4)	0.000
BCS	Good	47	1 (2.1)	0.0-11		
	Medium	117	4 (3.4)	1.0-9.0	1.6 (0.7-14.9)	0.666
	Poor	208	31 (14.9)	10-20	4.9 (1.7-14.4)	0.003
Animal Origin	Born	302	28 (9.3)	6.0-13		
	Bought	69	8 (11.6)	5.0-22	1.3 (0.56-2.9)	0.557

Herd-level risk factors

The risk factors associated with the seroprevalence of CBPP at the herd level were herd size, management, introduction of new animals from the market, herd contact site, and season. Results of the study indicated that animals from large herd sizes showed a higher seroprevalence (50%) than medium herd sizes (19%) and small herd sizes (10%). From the management system, a prevalence of 35.2 % was obtained in herds reared under an extensive management system compared to a prevalence of 19% in herds reared under a semi-extensive management system. The seroprevalence of CBPP in herds with a history of new animal introduction, 35.3%, was higher than the prevalence of 20.8% in herds without the introduction of a new animal. Herds that had contact with other herds at the grazing site had shown lower seroprevalence, 28.5%, than herds that had contact with other herds at the watering and grazing site, with prevalence of 32.5%.

Table 2. Univariable logistic regression analysis of herd level risk factors.

Risk factors	Categories	Animals tested	№ positive (%)	95% CI	OR (95% CI)	*p* value
Herd size	Small	20	2 (10.0)	1-32		
	Medium	21	4 (19.0)	5-42	2.1 (0.34-13.1)	0.419
Management	Semi extensive	21	4 (19.0)	5-42		
	Extensive	54	19 (35.2)	23-49	2.3 (0.7-7.8)	0.181
New animal introduction	No	24	5 (20.8)	7-42		
	Yes	51	18 (35.3)	22-50	2.1 (0.66-6.5)	0.210
Herd contact area	Grazing place	35	10 (28.5)	15-46		
	Grazing and watering	40	13 (32.5)	19-49	1.2 (0.45-3.2)	0.713
Season	Rainy season	27	6 (22.2)	9-42		
	Dry season	48	17 (35.4)	22-51	1.9 (0.65-5.6)	0.240

Multivariable logistic regression analysis indicated that cattle from Dorani district were about four times (OR: 4.1, 95% CI:1.9-8.7, $p = 0.00$) more likely to have CBPP seropositivity of Mmm SC antibody circulation than cattle from Bacho districts. Adult cattle were five times (OR: 5.2, 95% CI: 1.2-19.2, $p = 0.01$) more likely to have CBPP than young. Poor body condition animals were two times (OR: 2.1, 95% CI: 1.5-8.6, $p = 0.03$) more likely to have CBPP seropositivity than good body condition score animals (Table 3).

Table 3. Multivariable analysis of potential risk factors for contagious bovine pleuropneumonia at individual animal level.

Risk factors	Categories	Animals tested	№ positive (%)	OR (95% CI)	*p* value
Districts	Bacho	169	0 (0)		
	Bure	139	15 (10.8)		
	Dorani	63	21 (33.3)	4.1(1.9-8.7)	0.00**
Age	Young	157	4 (2.5)		
	Adult	214	32 (14.9)	5.2(1.2-19.2)	0.01*
BCS	Good	47	1 (2.1%)		
	Medium	117	4 (3.4)		
	Poor	208	31 (14.9)	2.1(1.5-8.6)	0.03*

PA: peasant association; OR: odd ratio; CI: confidence interval; *: statistically significant.

From herd-level risk factors, a large herd size was two times (OR: 2.2, 95% CI: 1.1-15.6, p = 0.00) more likely to have CBPP seropositive than a small herd size.

Discussion

In this study, the overall animal-level seroprevalence of CBPP was 9.7%. A relatively similar result was reported by different investigators in different parts of Ethiopia and other countries: 8.6% in the Gudeya Bila and Boneya Boshe districts of the Eastern Wollega zone (Neggasa *et al.,* 2020), 8.7% in Bishoftu (Teklue *et al.,* 2015), 9.4% in Borena (Ahmed 2004), 9.5% in export quarantine center of Adama (Kassaye and Molla, 2012), 10% in Dassenech district of South Omo zone (Molla and Delil, 2014), 8.1% in the Gimbo district of southwest Ethiopia (Mamo *et al.,* 2018), 9.7% in Southwestern Kenya (Schnier *et al.,* 2006), 8.1% in Mali (Sery *et al.,* 2014), 8% in northeast states of Peninsular Malaysia Pertanika (Zarina *et al.,* 2016) and 10.65% in the Kwara state of Nigeria (Olabode *et al.,* 2013).

However, the overall seroprevalence of the present study was lower than previous research reports which include 96% in Western Gojam and 74% in Borena Zone (Yigezu and Roger, 1997), 66.3% in Banja of western Gojam (Gashaw, 1998), 46% in Konso of SNNP (Roger and Yigezu, 1995), 39% in Somali Regional State (Gedlu, 2004), 37.6% in the selected districts Afar region (Negash and Dubie, 2021), 32.5% in Ilu ababor of western Ethiopia (Desta, 1998), 31.8% in Amaro district of SNNP region (Ebisa *et al.,* 2015), 28.5% in western Oromia (Daniel *et al.,* 2016; Mersha, 2016), and 25.3% in Sidama Zone (Malicha *et al.,* 2017).

On the other hand, the present result was higher than several earlier reports from various parts of the country, including a 0.4% prevalence from the export quarantine center in Adama (Erimiyas *et al.,* 2014), 4% from Adama and its surroundings (Kassaye and Molla, 2012), 6.14% from Southern Ethiopia (Asmamaw, 2003), 1.39% from the Bale zone (Lemu and Worku, 2017), and 3.4% from South Wollo zone (Juhar, 2020). The variation of seroprevalence reported might be due to differences in the degree of cattle movement, population density (degree of confinement or crowding), sample size, and the types of tests used to determine the seroprevalence.

District had statistical association with *Mmm SC* antibody and this result agreed with previous studies conducted in different parts of Ethiopia like in western Oromia by Mersha (2016), in the Bako Tibe and Ilu Galan districts of West Shewa Zone by Fulasa *et al. (*2020), in Bench-Maji Zone of southwest Ethiopia by Kebede *et al.* (2022), in East Wollega Zone by Wakgari *et al.* (2018) and a study in the Afar region by Negash and Dubie (2021), where district/location affected seroprevalence. The statistically significant association between district and *Mmm SC* antibody circulation might be due to the presence of large herd sizes, and contact of animals at grazing and watering points in the study area.

In this study adult age group had significantly (p<0.05) higher occurrence of CBPP which agreed with several earlier reports from different corners of Ethiopia (Ebisa *et al.,* 2015; Teklue *et al.,* 2015; Mersha, 2016; Wakgari *et al.,* 2018; Juhar, 2020; Mersha *et al*., 2020; Negash and Dubie, 2021) several African countries (Thomson, 2005; Matua-Alumira *et al.,* 2006; Schnier *et al.,* 2006); Mtui-Malamsha, 2009; Elhassan and Elsadig, 2012; Swai *et al.,* 2013; Suleiman *et al.,* 2015; Alhaji and Babalobi, 2016)), where all noted the significant association of adult age (p<0.05) with occurrence of CBPP disease. This might be due to the fact that adult animals move long-distances in search of pasture and water, and they contact with herds from other households.

Animals with poor body condition had significantly higher seropositivity to Mmm SC antibodies which agreed with observations of several previous studies from various parts of Ethiopia (Biruhtesfa *et al.,* 2015; Ebisa *et al.,* 2015; Juhar, 2020; Neggasa *et al.,* 2020; Kebede *et al.,* 2022), and elsewhere in Africa (Mtui-Malamsha, 2009; Suleiman *et al.,* 2015), where animals with lower BCS had significantly higher seroprevalence. This might be due to the fact that animals with poor body condition are likely to have poor immunological response (resistance) to the infectious agent compared to animals with good body condition (Radostits *et al.,* 2007).

Conclusions

The findings showed an overall seroprevalence of 9.7% at the individual animal and 30.6% at the herd-level. Seropositivity was recorded in the Dorani and Bure districts, with a prevalence of 33.3% and 10.8%, respectively. Dorani district, adult animals and poor body condition score were the risk factors identified as having a considerable association with the prevalence of CBPP in the

study area. Therefore, regular vaccination, in seropositive areas, especially for young animals, should be given, and cattle in poor body condition should be fed well in order to help them develop resistance to diseases, including CBPP.

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Salmonella prevalence in cattle slaughtered in selected abattoirs of Amhara National Regional State, Ethiopia

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Abstract

Salmonella can spread more readily since they are present in humans, animals, and the environment. Some *Salmonella* serotypes exert huge health and economic impacts due to their virulence or antibiotic-resistance traits. This study aimed to detect *Salmonella* and determine its prevalence in slaughtered cattle of selected towns` abattoirs in the Amhara region. The study was cross-sectional, and a systematic random sampling technique was used to select the study animals. A total of 480 animals` feces, carcass swabs, liver and intestinal lymph node samples were collected for this study. Bacteriological and polymerase chain reaction (PCR) tests were used. The *Salmonella* prevalence at the animal level was 8.33% (10 out of 120) (95% CI: 4.07% - 14.79%). At sample level, 11 isolates out of 480 samples were found positive for *Salmonella*, with a prevalence of 2.29% (95% CI: 1.15% - 4.06%). The PCR amplifications gave products of 496 bp for 11 isolates by gel electrophoresis and were consequently confirmed as *Salmonella.* To know the possible source of contamination, water used for carcass washing and sanitation, as well as swab samples from butchers' hands, coats/aprons, boots, and all slaughtering materials, were tested for *Salmonella*. All of these samples were found to be negative for *Salmonella*. A significant number of *Salmonella* was identified in slaughtered cattle in this study. Emphasizing the significance of enhancing hygienic practices prior to, during, and after the slaughtering process is of utmost importance. Sources of threats for *Salmonella* contamination, including abattoirs, should be given more attention to help them establish and maintain strict hygienic measurements.

Keywords: Abattoir; Cattle; Prevalence; *Salmonella*.

Introduction

Salmonella is one of numerous foodborne pathogens that could pose a significant threat to global food safety. About 2700 serotypes (serovars) have been identified so far (Dnog *et al*., 2020); the majority of them can produce disease in animals and humans. The genus is responsible for an array of diseases, such as typhoid fever and salmonellosis (a variety of illnesses, including gastroenteritis), which cause public health issues globally (Ryan *et al*., 2017).

Salmonella contamination of meat constitutes a significant risk to the public's health as it can result in fatal illnesses. The bacteria are among the common pathogens in food animals, establishing an asymptomatic reservoir that makes it challenging to control and eradicate (Salman and Steneroden, 2014).

Animal meat is the primary reservoir for many foodborne pathogens. Meat products, mainly fresh meat, have a small number of microorganisms. However, when exposed to favorable growth conditions, they can make the meat unusable for human consumption. The presence of pathogenic bacteria like *Salmonella*, *E. coli* O157:H7, and *Listeria* or their toxic products make meat unsafe for human consumption, and lead to gastrointestinal diseases if ingested (WHO, 2015).

Salmonellosis is a significant cause of economic loss in farm animals because of the cost of diagnosis, treatment of clinical cases, cleaning and disinfection, control and prevention costs, and animal death (Pal *et al*., 2020). The globalization of the food supply, expansion of food businesses, changes in food eating habits, and the increased movements of people, animals, and goods within and between countries are bringing new food safety hazards and threats. Food safety is also facing a severe issue with the rising prevalence of foodborne pathogens that are resistant to antimicrobial drugs (Jaffee *et al*., 2018).

The issue of animal-origin foodborne diseases is still a significant threat in developing countries like Ethiopia due to inadequate hygienic standards, a habit of consuming raw meat, and undeveloped infrastructures. Salmonellosis is one of the most significant and common zoonotic illnesses. Since no practical steps have been taken to control it, the problem is highly present on the ground. The distribution of *Salmonella* has not been thoroughly studied and defined at the regional or national level. There is insufficient information regarding *Salmonella* contamination of carcasses during cattle slaughter. Thus, the present

study was conducted to estimate the prevalence and assess the distribution of *Salmonella* in slaughtered cattle of selected abattoirs of the Amhara region, Ethiopia.

Materials and methods

Study area

The study was carried out in abattoirs from selected towns in the Amhara region, namely Gondar, Bahir Dar, Debre Markos, Debre Tabor, Kombolcha, and Debre Birhan. The Amhara region is located in the northwestern part of Ethiopia between 9°20′ and 14°20′ North latitude and 36°20′ and 40°20′ East longitude. The region covers approximately 161,828.4 km^2 area. Based on the 2007 census of the Central Statistical Agency of Ethiopia (CSA), the Amhara region has a population of 17,221,976 (8,641,580 men and 8,580,396 women) (CSA, 2007). The 2016/17 CSA agricultural sample survey estimated the region's ruminant livestock population at 15.98 million cattle (representing 26.8% of Ethiopia's total cattle population), 9.79 million sheep (representing 31.8% of Ethiopia's total sheep population) and 6.08 million goats (representing 20.1% of Ethiopia's total goat population) (CSA, 2017).

Study animals

The study animals were apparently healthy slaughtered cattle from selected abattoirs in the Amhara region. The animals were mostly bought from nearby small towns and rural marketplaces and transported to the abattoirs on foot or open trucks made for the transportation of other goods. Both intensive and semi-intensive cattle husbandry systems are practiced in the areas. Those under semi-intensive management graze pasture, and they are also fed hay throughout the long dry season when the pasture is in short supply. Animals under intensive management are usually fed with hay and concentrate. Most of the cattle slaughtered in the abattoirs were adult males of the local zebu breed. All cattle in the study abattoirs ready for slaughtering during the study period were the primary sources of the test samples. Water sources that were utilized for carcass washing and sanitation in the abattoir, all materials that were utilized for slaughtering and inspection, and butchers' hand and safety dressings were also sampled to determine whether they could be the sources of contamination or not.

Study design and sampling technique

A cross-sectional study was conducted from February 2022 to January 2023. Study animals were selected using a systematic random sampling method using a three-animal sampling interval, with the first animal to be sampled on a particular sampling day selected randomly among the first three animals on the slaughter line.

Sample size determination

The sample size required for this study was determined according to Thrusfield (2018). The expected prevalence of 7.6% (Muluneh and Kibret, 2015) for *Salmonella* in the study area, a 95% confidence interval (95% CI), and a 5% level of precision were considered to calculate the sample size.

$$n=Z^2 \times P_{exp} (1-P_{exp})/d^2$$

$$n=(1.96)^2 \times 0.076(1-0.076))/0.05^2$$

$$n = 108$$

where n is the required sample size, Z is 1.96, P_{exp} is the expected prevalence, and d is the desired absolute precision of 0.05. Therefore, the calculated sample size was 108. However, twelve animals were purposefully added in order to improve the study's precision and preserve the distribution balance during sampling. So, a total of 120 animals were selected for this study. The entire sample size was distributed proportionally among the study abattoirs. Accordingly, 20 animals from each abattoir were selected and examined for *Salmonella*. The number of sampled cattle on a particular day could be from 2 to 10, depending on the number of cattle presented for slaughter.

Sample collection and transportation

Four types of samples were collected from each animal: feces (before slaughter), carcass swabs (CS), mesenteric lymph nodes (MLN), and liver. As a result, 480 samples were collected from the 120 study animals, 80 samples each from the six abattoirs.

About 25 g of fecal samples were collected directly from the rectum of each selected cattle before the animal was slaughtered using disposable sterile plastic gloves. Carcass swab samples from the flank, brisket, and rib area were col-

lected using a sterile cotton-tipped swab rubbed on the carcasses firmly. The swab was then inserted into a sampling container containing 10 ml buffered peptone water (BPW) (Oxoid, England) after cutting off the part of the stick that was in contact with the hand.

Small parts of the liver, about 25 g, adjacent to the gallbladder, were collected from each study animal. Mesenteric lymph nodes were also collected by dissecting the mesenteric fat using a sterile blade and forceps.

A total of 60 water samples (each 10 ml) from the sources of water, mainly pipe and tanks, which were used for washing hands and cleaning equipment (knives, hooks, and carts), and swab samples from butchers' hands (n = 65), coats/aprons (n = 65), boots (n = 65) and equipment used in the slaughtering processes (84 knives, 50 hooks, 26 carts) were aseptically collected for the test. All samples were collected purposely during each sampling day.

All sample types were collected aseptically and transferred immediately to sterile labeled universal bottles containing BPW. A cold chain transport method was used, keeping the collected samples in ice boxes containing ice packs until arrival at the laboratory. Samples were transported to the Veterinary Microbiology Laboratory, College of Veterinary Medicine and Animal Health Sciences, University of Gondar, within 30 minutes to 24 hours, depending on the distance of the area of sample collection. Upon arrival at the laboratory, the samples were processed immediately.

Isolation and identification of *Salmonella*

The technique recommended by the International Organization for Standardization, ISO 6579 (ISO, 2013), was employed to isolate and identify *Salmonella* organisms. Carcass swab materials and fecal samples were homogenized in 225 ml BPW. A 25 g tissue samples (liver and MLN) that had been weighed by digital balance (Heraus, Germany) were placed in a sterile Stomacher bag and homogenized in 225 ml of BPW at a ratio of 1:9. The sample suspension was prepared in a peristaltic homogenizer (Stomacher 400, UK) for 2 minutes. The samples were incubated for 18 to 24 hrs at 37 ˚C. Following this, 1 ml and 0.1 ml of the pre-enrichment broths were transferred aseptically into 10 ml of Muller Kauffmann Tetrathionate (MKTT) (Merck, Germany) and 10 ml of Rappaport-Vassiliadis (RV) broth (HiMedia, India), mixed, and then were incubated for 18 to 24 hrs at 37 ˚C and 42 ˚C, respectively. Following incuba-

tion, a loop-full of each culture was streaked onto the surface of xylose lysine deoxycholate (XLD) (HiMedia, India) and brilliant green agar (BGA) (Oxoid, England) medium and incubated at 37 ˚C for 24 to 48 hrs. The XLD and BGA plates were examined for the presence of suspect *Salmonella* colonies. Then, plates with colonies with slight growth and plates with no typical *Salmonella* colonies were re-incubated for an additional 18 to 24 hrs and re-examined. The formation of red colonies with black centers and of pink colonies with a red zone was inspected on XLD and BGA plates, respectively. Colonies showing typical morphological characteristics of *Salmonella* were sub-cultured to get pure colonies for further biochemical and molecular identification (Mooijman *et al*., 2019). A similar procedure was applied for water and equipment swab samples.

The following biochemical tests were used for further identification of all suspected *Salmonella* colonies: lysine decarboxylase (LDC), indole, citrate, triple sugar iron (TSI) agar, and urease tests. All cultural media were products of HiMedia, an Indian company. The inoculated test tubes were incubated for 24 or 48 hrs at 37 °C to see changes in their biochemical properties (ISO,2002). Colonies of the biochemically confirmed isolates were sub-cultured in a nutrient broth to refresh them. They were delivered to the National Veterinary Institute, Molecular Biology Laboratory, Bishoftu, Ethiopia, for confirmation by PCR.

Molecular analysis for the detection of *Salmonella*

DNA was extracted from an overnight pure culture of each bacterial isolate during its log phase of growth. DNA extraction was carried out by using a commercially available DNA extraction and purification MINI kit (QIAGEN™, Germany) according to the manufacturer's instructions.

Conventional PCR was conducted at the National Veterinary Institute (NVI) to confirm identification made by phenotypic tests. *Salmonella* spp isolates were detected by amplifying the 496-bp segment of the histidine transport operon gene according to the methods described by Cohen *et al*. (1993). Two different primer pairs were used for this purpose (Table 1). The amplification reaction was carried out in a final volume of 22 µl comprising 3 µl nuclease-free water, 2 µl from each primer pair, 10 µl IQTM super mix (Thermo Scientific, USA), and 5 µl template. The PCR reaction conditions were 5 minutes at 94 °C for the initial denaturation, 30 seconds at 94 °C for subsequent denaturation, 30

seconds at 56 °C for the annealing, and 45 seconds at 72 °C for the elongation, and 5 minutes at 72 °C for final elongation test which is followed by holding at 4 °C until the machine is off. The total number of cycles was 32. PCR products were subjected to gel (2% agarose, USA) electrophoresis, run in TAE Buffer by loading it with ten µl of the PCR product mixed with 4µl gel red nucleic acid stain loading dye (Gelred 10000x lot 15G1214; Biotium, USA) in water and 10 µl markers (Ladder). A parallel lane was loaded with a 100 bp DNA-marker ladder (Fermantas, USA) along with known positive control *Salmonella* typhimurium (ATCC 14028) and negative control, and the products were separated at 120 V for 1 hr, then visualized under UV light and recorded using a gel documentation system (UVtec 08100554, France).

Table 1. Forward (F) and Reverse (R) primers sequences for Histidine transport operon in *Salmonella* detection.

Primer	Sequence	Target	Amplicon Size	Reference
F	5-ACTGGCGTTATCCCTTTCTCTGGTG-3'	Histidine Transport Operon	496 bp	Cohen *et al.* (1993)
R	5'ATGTTGTCCTGCCCCTGGTAAGAGA-3'			

Data management and analysis

A Microsoft Excel spreadsheet was used to enter the data. Data consistency and completeness were achieved during data collection, data entry, and analysis. The data were analyzed using a statistical package for the social sciences (SPSS), version 25. During the calculation of the animal-level prevalence of *Salmonella*, an individual animal was considered positive for *Salmonella* if at least one fecal, swab, or tissue sample was found positive for *Salmonella* as confirmed by PCR test. The proportion of *Salmonella*-positive samples was calculated by dividing the number of positive (confirmed) samples by the total number of samples investigated (processed).

Ethical consideration

Ethical approval was obtained from the University of Gondar, College of Natural and Computational Sciences, ref CNCS/02/03/189/01/2024.

Results

Cultural and biochemical detection rates of *Salmonella*

Cultural, biochemical, and molecular testing methods were used in the evaluation procedures. A PCR test was used to confirm 24 isolates that were initially suspected of being positive for *Salmonella* based on biochemical tests. However, only 11 of these isolates were confirmed to be *Salmonella* by PCR. Overall, 10 out of 120 animals tested positive for *Salmonella*, representing 8.33% prevalence (Table 2), with a 95% confidence interval ranging from 4.07% to 14.79%.

Depending on the towns where the samples and data were collected, the majority of *Salmonella*-positive isolates were found in the Kombolcha town abattoir, followed by the Gondar town abattoir (Table 2).

Table 2. Occurrence of *Salmonella* (confirmed by PCR) in cattle slaughtered in selected town abattoirs in Amhara Region (no. of animals = 120).

Towns	No. of animals sampled	No positive for *Salmonella*	Prevalence (%)
Gondar	20	3	2.5
Bahir Dar	20	0	-
Debre Markos	20	1	0.83
Debre Tabor	20	1	0.83
Kombolcha	20	4	3.33
Debre Birhan	20	1	0.83
Total	120	10	8.33

At the sample level, 11 of 480 (2.29%) samples collected from slaughter cattle tested positive, with a 95% confidence interval (CI) of 1.15% to 4.06%. Table 3 indicates the sample type and number of samples identified as *Salmonella* positive.

Table 3. Proportions of *Salmonella*-positive samples (confirmed by PCR) collected from slaughtered cattle in selected town abattoirs of Amhara Region (no. of animals = 120).

Sample type	No. examined	No. positive	% positive
Liver	120	0	-
Feces	120	4	0.83
Carcass swabs	120	2	0.42
Mesenteric lymph nodes	120	5	1.04
Total	480	11	2.29

The culture and biochemical test results of the water samples and swab samples taken from butchers' hands, coats/aprons, boots, and materials used in the slaughtering processes were all negative for *Salmonella*.

Molecular identification of *Salmonella*

The PCR amplifications gave products of 496 bp for 11 isolates by gel electrophoresis (Figure 1), which consequently confirmed them as *Salmonella*.

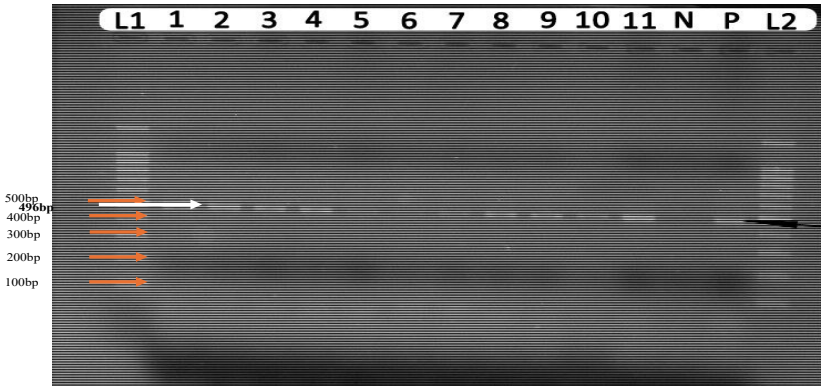


Figure 1. PCR assay to amplify Histidine Transport Operon gene of *Salmonella* isolates recovered from carcasses and feces of cattle from selected abattoirs.

Lanes 1 and 2 are ladders or molecular markers.
Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 are positive isolates for *Salmonella*.
Lane P is positive control and
Lane N is the negative control

Discussion

Our study revealed an 8.33% (10 out of 120) prevalence of *Salmonella* at the animal level. The result is comparable with previous studies in the region. Studies by Alemu and Zewde (2012), and Muluneh and Kibret (2015) reported prevalence of 7% and 7.6%, respectively, in Bahir Dar.

Our study showed a comparatively low level of contamination at the sample level. Of the 480 carcass swabs, liver, intestinal lymph nodes, and fecal samples collected, only 11 (2.29%) were found to contain *Salmonella*. *Salmonella* was found in all types of cattle samples, except the liver.

Our findings are comparable with studies that were conducted in abattoirs, such as in South Africa (2.75%) (Madoroba *et al.,* 2016), Haramaya (2.75%) (Mengistu *et al.,* 2017), Nigeria (2.6%) (Shaibu *et al.,* 2021), Iraq (2.75%) (Yousif, 2013), Addis Ababa (3.7%) (Ketema *et al.,* 2018), and Hawasa (2.0%) (Kore *et al.,* 2017). However, other researchers, including Kebede et al. (2016) (Addis Ababa), Gebremedhin *et al.* (2021) (Holeta), Wabeto *et al.* (2017) (Sodo), Beyene and Yibeltie (2015) (Asella), Alemu and Zewde (2012) (Bahir Dar), Alemayehu *et al.* (2003) (Addis Ababa), Sibhat *et al.* (2011) (Addis Ababa), Muluneh and Kibret (2015) (Bahir Dar), and Takele *et al.* (2018) (Jimma), reported prevalences significantly higher than that of the current finding. These studies reported prevalence of Salmonella from slaughtered cattle, ranging from 5.7% to 12.5%.

Although there have been several investigations on Salmonella contamination in apparently healthy slaughtered cattle, the results have not always been uniform. In this regard, the authors evaluated many papers and found that the results vary from 2.0% in Hawasa (Kore *et al.,* 2017) to 12.5% in Sodo (Wabeto *et al.,* 2017), Ethiopia. The differences in findings may be attributed to differences in the number of abattoirs included in the study, abattoir facilities, methodological differences such as study design, study period, sample size, type of sample taken, demography, area covered, diagnostic, sampling and culturing techniques, and the level of hygienic standards maintained by the abattoirs, as well as related factors that may affect the results.

In this study, compared with the other samples (liver 0 %, feces 0.83%, carcass 0.42%), mesenteric lymph nodes were identified as the most contaminated organ (1.04%). Mesenteric lymph nodes (MLN) drain the small and large in-

testine parts (jejunum, ileum, and ceacum) (Houston *et al*., 2016). The gastrointestinal tract (GIT) remains the main route of transmission for foodborne pathogens. *Salmonella* isolation from mesenteric lymph nodes indicates that the animal is naturally infected and is a carrier of the organism. Long traveling, overcrowding, and holding animals at a specific place for an extended period without feed and water can stress animals, promote *Salmonella* infection, and cause it to spread quickly around the jejuno-ileco-ceacal area, which causes swelling of the mesenteric lymph nodes (Garrido *et al*., 2014). Stress makes the animal shed *Salmonella* through feces, leading to the contamination of carcass, equipment, and abattoir environment, especially in conditions of poor hygiene.

The occurrence of *Salmonella* in MLNs in this study is comparable with the report of 1.3% from Hawassa (Kore *et al*., 2017), and 2.4% from Addis Ababa (Molla *et al*., 2003), but slightly lower than other studies, such as 3% in Iraq (Yousif, 2013), 3.2% in Bahir Dar (Alemu and Zewde, 2012), and 4.6% in Addis Ababa (Alemayehu *et al*., 2003), and still much lower than 8% (Sibhat *et al*., 2011) reported in Addis Ababa.

Salmonella isolation from feces (0.83%) reveals the pathogen's presence in the animals brought for slaughter to the abattoir. Fecal excretion is not necessarily indicative of an actual infection of the animal. After being ingested, *Salmonella* can circulate passively through the animal's gut lumen without invading the enterocytes. *Salmonella* must cross the enterocyte barrier and reach the local lymphoid system in order to cause an active infection. Carrier animals can become active fecal shedders of *Salmonella* due to the stress associated with transport and lairage, which may contribute to the spread of the infection to other animals (EFSA, 2021). The ingestion of contaminated feed, water, and pasture, as well as contaminated soil, drainages, toilet sewage, and other wastes, are some potential sources of infection. Despite the variations in the prevalence, *Salmonella* is frequently isolated from feces in various studies.

The proportion of animals found shedding *Salmonella* through their feces is different from what was recorded in other studies in Ethiopia: 4.1% (Ketema *et al*., 2018), 3.1% (Alemayehu *et al*., 2003), 6% (Sibhat *et al*., 2011), and 1.9% (Molla *et al*., 2003), all from Addis Ababa.

Two positive isolates were found from carcass swab samples. Freshly slaughtered animals' muscles are usually sterile (Kadim, 2007). Only two carcass

swab samples were found positive for *Salmonella*. Though we observed a very low prevalence of *Salmonella* (0.42%) in this study, the presence of even small numbers of *Salmonella* in carcasses and edible organs could lead to contamination of red meat and other meat products. The positive result might be due to cross-contamination from other organs due to insufficient sanitary conditions when slaughtering. Meat can be contaminated in the process of evisceration, whereby feces of contaminated animals have been transferred to the hands of the slaughtermen, which in turn contaminates other healthy carcasses. Our result is different and lower when compared with other studies, which reported a 2% (Sibhat *et al*., 2011), 2.8% (Alemayehu *et al*., 2003), and 2.5% (Ketema *et al*., 2018) carcass contamination from Addis Ababa. Other studies reported even higher values: 4.8% in Bahir Dar (Alemu and Zewde, 2012), 7.6% in Ireland (Mcevoy *et al*., 2003), and 9.8% in Addis Ababa (Nyeleti *et al*., 2000).

No *Salmonella* was isolated from the liver in this study. The finding is in accordance with the results of Alemayehu *et al*. (2003). The negative result may partly be due to the organ being far and deep situated from contamination.

In general, the level of contamination of cattle meat with *Salmonella* varies in different nations of the world. There is variation in some factors and similarity in others. Nowadays, most abattoirs have improved their hygienic conditions. The improvement is apparent in the slaughterhouses we chose for this study. We observed that they use clean and high-pressure tap water to clean the floor, tables, walls, carts, hanging chains, and hooks in the slaughterhouses. They often wash knives, axes, and other tools used for slaughtering purposes in hot water.

Isolation of *Salmonella* from various animal tissues or feces shows the spread of the organism in food animals. This emphasizes the need for regular surveillance and monitoring programs for zoonotic salmonellosis and other foodborne illnesses. It is essential to stop the organism from infecting people and spreading through tainted meat and animal products.

Contamination is likely to be worsening as meat goes through multiple chains from the time of the animal's slaughter until it reaches the customers. Meat in Ethiopia is traditionally sold in open-air retail shops without the correct hygienic display, temperature, and storage conditions. The widespread consumption of raw meat, particularly in the forms of kitfo (minced meat) and kurt (pieces of cut meat) in the study locations and across the nation, makes the

finding of *Salmonella* on beef carcasses even more significant and is believed to be another potential contributing factor to the disease's persistent presence in the community (Geresu and Desta, 2021).

Only the genus level of the bacteria (*Salmonella*) was determined in this investigation. The specific *Salmonella* serotypes circulating in the selected study abattoirs were not identified. It was also not known exactly where the contamination of the carcasses might have originated. The antimicrobial resistance pattern of isolated *Salmonella* species was not also determined. These are among some of the gaps and limitations of this study that need to be considered in future research efforts.

Conclusions

Bovine salmonellosis is a disease of significant importance to the cattle industry and public health. Consequently, improving communities' knowledge about the cause of infection and its primary sources may help lower the risk of infections in humans and cattle. The study indicates that more attention should be given to implementing and maintaining strict hygienic measurements in abattoirs before, during, and after slaughtering. As the consumption of raw and undercooked meat is a common habit in most parts of Ethiopia, public education and continuous awareness are needed to discourage the habit and minimize the occurrence of *Salmonella* infection.

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Evaluation of single shot prostaglandin F2α analog (dinoprost tromethamine) for estrus synchronization in cattle with corpus luteum

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Abstract

An experiment was conducted from June 2022 to April 2023 at Debrezeit Agricultural Research Center to evaluate the effectiveness of single shot prostaglandin based estrus synchronization protocol in Boran, Boran*Holstein-Friesian crosses, and Holstein-Friesian cattle upon manual detection of mature corpus luteum (CL). A total of 120 animals (80 cows and 40 heifers) with detected CL from different age, body condition (BC), parity, days in milk, and lactation status were included in the experiment. A single injection of 5 ml LUTALYSE® Injection (dinoprost tromethamine, a prostaglandin F2α analog) was used to synchronize the animals. The observed estrus response and conception rate were 83.3% and 73.0%, respectively. Among the predictor factors considered, BC score significantly influenced both estrus response and conception rates, while days in milk (DIM) had significant effect on conception (p <0.05). Estrus response and pregnancy rates were higher in animals with better BC, and cattle with >60 DIM had significantly higher conception rate compared to those with less DIM. Both estrus response and pregnancy rates were not significantly different between cows and heifers, as parity had no significant association (p >0.05) with both outcomes. The result showed that manual per rectal detection of CL and injection of a prostaglandin analog were effective in inducing heat. Therefore, synchronization with a single injection of LUTALYSE in heifers and cows after manual detection of mature CL could minimize drug cost and can achieve optimal results.

Keywords: Boran*Holstein crosses; Conception rate; Corpus luteum; Prostaglandin; Synchronization.

Introduction

Ethiopia owns enormous livestock in Africa, having 70 million cattle, 42.9 million sheep, 52.5 million goats, 8.1 million camels and 57 million chickens in 2020. Ethiopia's livestock consists almost entirely of indigenous breeds. Recent estimates indicate that 97.4%, 2.3%, and 0.3% of cattle are native, hybrid, and exotic, respectively (CSA, 2020). Despite their large numbers, indigenous cows are not selected for milk production, so their productivity is very low, with an average milk production of about 1-2 liters per day and a short lactation period of about 7 months (Tegegne *et al*., 2010).

Annual milk production in the Ethiopia is very low, estimated at about 4 billion liters (CSA, 2020). On the other hand, the current demand for milk is increasing rapidly due to various factors such as increasing urbanization, population growth and improving milk consumption habits. However, per capita annual consumption of milk is still lower than 20 liters (Tegegne, 2018). Based on FAO recommendations, Ethiopia's human population now needs about 22 billion liters of milk per year, indicating a supply gap of about 18 billion liters per year (Tegegne, 2018). This huge gap between demand and supply has to be met by increasing milk production largely from cows, and this should be accompanied by an increase in the number of crossbred cows (Tadesse, 2002). Cattle reproductive biotechnologies such as estrus synchronization (ES) and artificial insemination (AI) could be used to fast produce crossbred cows (Tegegne *et al*., 2016).

The inability to timely and accurately detect heat in dairy herds is a major factor limiting reproductive efficiency (Mekonnin *et al*., 2016). Fertility is an important factor in dairy herd production and profitability (Dogruer *et al*., 2010). A calving interval of 12-13 months is generally considered to be economically optimal (Rajamahendran *et al*., 2001), but is often difficult to achieve in Ethiopia. To reach this goal, cows must begin their cycle and become pregnant within an average of 85 days postpartum. In addition, efficient and accurate heat detection, proper semen handling and timely insemination in relation to ovulation are also important factors (Million *et al*., 2011).

Estrus synchronization is one of the reproductive management tools that induce estrus in groups of females to breed relatively in a confined period of time. This program improves reproductive efficiency by shortening the length of breeding, and calving intervals and increasing calf uniformity (Gupta *et al*.,

2009). Products such as prostaglandin (PG) F2α, PGF2α analog (dinoprost tromethamine), melengesterone acetate (MGA), controlled internal drug release (CIDR), and gonadotropin-releasing hormone (GnRH) are used to synchronize estrus and ovulation. Prostaglandin-based protocols depend on the presence of a functional CL aged a minimum of 5 days, especially during the diestrus phase of the estrous cycle (day 7 to 17 of the cycle) (Oaxaca *et al*., 2009).

The natural prostaglandins (PG) produced in endometrium of uterus induce functional or structural regression of the corpus luteum (CL). Regression of the CL results in the removal of progesterone's negative feedback control on the secretion of GnRH, follicle stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2), leading to the growth and development of follicles. Luteolysis leads to increased E2 concentrations (Galina and Orihuela, 2007; Sprott and Carpenter, 2007; Stocco, 2007; Araujo *et al*., 2009; Ginther *et al*., 2011).

Ethiopia has undertaken mass synchronization of cattle in various regions in recent years to improve planned breeding programs. Still various factors have led to a decline in estrus and conception rates of synchronized cattle. It was therefore hypothesized that effective detection of CL and identification of factors affecting estrus response and conception would help to minimize drug cost and improve the efficiency of single-shot PGF2α based synchronization protocol. Therefore, the current study was designed to evaluate the effectiveness of single injection of PGF2α analog (dinoprost tromethamine), following manual CL detection, in estrus synchronization and pregnancy in dairy cows and to identify cow factors associated with estrus response.

Materials and methods

Study area

The experiment was conducted from June 2022 to April 2023 at Debrezeit Agricultural Research Center (DZARC) of Ethiopian Institute of Agricultural Research (EIAR) in Bishofu town. Bishoftu is located about 45 km east of Addis Ababa, the capital city of Ethiopia. The town is located at a geographic coordinate of 8°46′ latitude N, and 38°59′ longitude E at an altitude of 1920 masl (Fig. 1) (Addis *et al*., 2015).

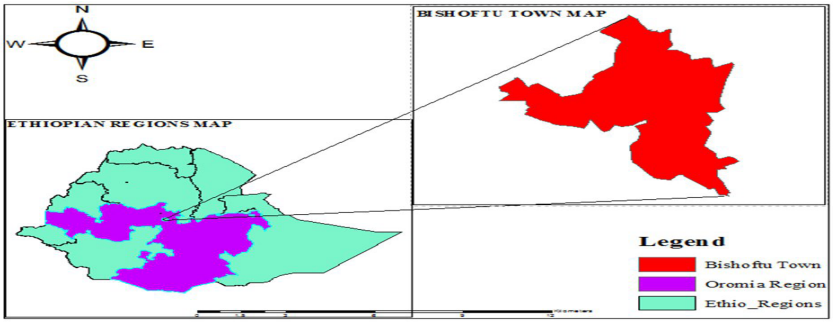


Figure 1. Map of the study location (Arc GIS).

Study animals

The study was conducted on 120 dairy cattle (80 cows and 40 heifers) at the Debre zeit agricultural research center (DZARC). Prior to the start of the experiment, reproductive organs of animals were palpated per rectum to confirm the reproductive stage, presence of CL and whether they were free from any obvious reproductive tract abnormalities. Animals having CL on palpation were selected for the experiment.

The experimental animals aged between 2 and 10 years with a parity of 0 to 5. The body conditions (BC) of the animals ranged between 2.5 to 3.5, on a 5-point scale assessment (Wildman *et al.*, 1982). On a scale of 1 through 5, condition score 1 indicates severe under condition, 3 optimal and 5 indicates severe over condition. Boran, Boran*Holstein-Friesian crosses, and Holstein-Friesian cattle were included in the experiment. Regarding parity, the experimental animals were grouped into heifers, parity 1 and 2, and ≥3. The minimum days in milk of the experimental animals was 54 days. The animals were managed intensively and fed on straw, hay, concentrates and alfalfa.

Study design

The experiment was carried out on 120 dairy cattle to evaluate estrus response and subsequent pregnancy rate for a single injection of LUTALYSE® Injection. The reproductive organs of the animals were examined manually per rectum for any gross abnormalities and for presence of mature CL. Animals with apparently normal reproductive organs and mature CL at the time of examination were included in the study and received a single shot of dinoprost tromethamine (PGF2α analog) on the day of examination and monitored for 5 days

thereafter for signs of heat. Animals that showed heat during this period were inseminated artificially.

PGF2α treatment, estrus detection and insemination

Animals which were found to have normal reproductive organs and mature CL received single injection of 5ml LUTALYSE® Injection (dinoprost tromethamine injection, Zoetis Inc., Spain) intramuscularly (IM). Estrus was detected with visual observation and the help of ESTROTECT breeding indicator. ESTROTECT were applied halfway between the hip and tail head perpendicular to the spine. In the event of estrus, because the animal allows itself to be mounted, the surface of the indicator, with each mount, will gradually change its color (to deep green in our case) indicating a true standing heat. Visual observations were made twice daily, once in the early morning (6am) and once in the afternoon (5pm). All observations made lasted approximately for 1 hour for up to 5 days. The onset of behavioral signs of estrus such as standing to be mounted, mounting others, ruffled hair on tail head or rump, pink vulvar mucosa, vaginal mucus discharge, frequent urination, anogenital sniffing, restlessness, walking in circles, and bellowing were recorded along with color change in the breeding indicator. Animals that showed behavioral estrus, within 5 days of the injection, were inseminated in the AM-PM principle (Fig. 2) i.e., animals that showed estrus in the morning were inseminated in the afternoon and those which showed estrus in the afternoon were inseminated in the next morning. Holstein-Friesian semen purchased from the National Artificial Insemination Center (NAIC), Kality, Ethiopia, was used for insemination.

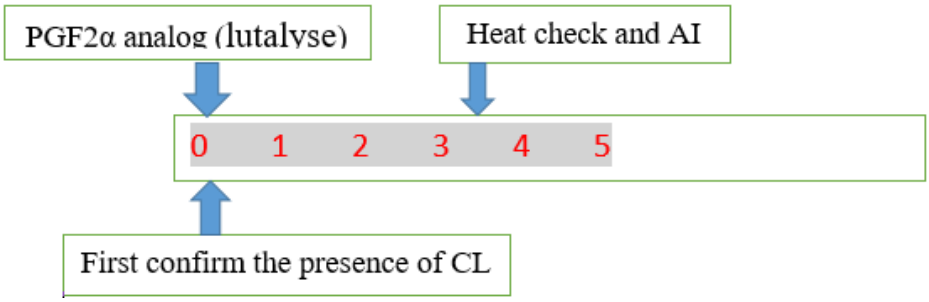


Figure 2. Synchronization and artificial insemination protocol.

Pregnancy diagnosis

History of return to estrus of inseminated animals within the normal period of interestrus was obtained from the farm attendants. The experimental animals

that did not return to estrus were checked manually for pregnancy at 60th day of insemination. Pregnancy was confirmed when there was asymmetry of uterine horn, manual detection of CL, and fluid filled uterus was grasped in the palm (Purohit, 2010).

Age of the animal, breed, parity, body condition score (BCS), days postpartum, lactation status, the estrus response of the animal to hormonal treatment, date and time of estrus detection, insemination, and pregnancy were recorded.

Data analysis

All data collected from study animals were coded, entered and filtered in Microsoft excel spreadsheet and analyzed in STATA (version 17). Descriptive statistics were employed to calculate the percentage of estrus response and pregnancy rate. The effect of age, breed, BCS, parity, days postpartum, and lactation status on the rate of estrus and conception was analyzed using Chi-square test. Factors that were significant in the X^2 were considered for further logistic regression to estimate the strength of the association. The variation between groups was considered significant when the p value was less than 0.05.

Results

Estrus response and conception rate

Out of the 120 cattle injected with a single dose of 5ml PGF2α analog (dinoprost tromethamine) during the experiment 100 (83.33%) animals showed estrus (Table 1), and of these 100 animals, which showed estrus and were inseminated in AM-PM principle 73 (73%) became pregnant (Table 3).

Association of estrus response with factors

The multivariable logistic regression demonstrated that estrus response was significantly influenced by BCS (p<0.05), whereas all the other factors considered in the study, namely days in milk, parity, and lactation status of the animals did not significantly affect estrus response (p >0.05) (Table 2).

Table 1. Chi-square analysis for estrus response to lutalyse (PGF2α analog) injection in the experiment.

Factors	Category	No. injected	No. in Estrus	Response rate (%)	X^2	*p* value
Days in milk	≤ 60 days	21	12	57.14%	12.68	0.000
>60 days	59	54	91.52%			
Lactation status	Non lactating	73	65	89.04%	4.37	0.0037
	Lactating	47	35	74.46%		
BCS	2.5	30	12	40%	54.27	0.000
	3	57	55	96.49%		
	3.5	33	33	100%		
Parity	Zero (Heifer)	40	34	85%	8.51	0.014
	One & two	39	37	94.87%		
	≥ 3	41	29	70.73%		
Breed	Boran	36	29	80.55%	1.85	0.396
	Boran*HF	58	51	87.93%		
	HF	26	20	76.92%		
Total		120	100	83.33%		

HF: Holstein-Friesian

The odds of estrus response in animals having BCS of 3 was 40.7 times more likely than cattle with 2.5 BCS, keeping other variables constant. The detail multivariable output is presented in Table 2.

Table 2. Multivariable binary logistic regression for association of lutalyse injection with estrus response.

Factor	Category	Odds ratio	95% CI	*p* value
BCS	2.5	Ref		
	3	40.68	3.77, 439.55	0.002
	3.5	1		
Lactation status	Lactating	Ref		
	Non lactating	0.66	0.077, 5.63	0.704
Days in milk	≤60 days	Ref		
	>60 days	1.599	0.257, 9.97	0.615
Parity	Heifers	Ref		
	One & two	1.43	0.146, 13.92	0.761
	≥3	1		
Cons		.691	0.082, 5.812	0.734

BCS: Body condition score; Ref: Reference; SE: Standard error; CI: Confidence interval

Association of pregnancy with factors

Pregnancy was significantly different among animals with different body conditions i.e. animals with 3.5, 3, and 2.5 BCS had 93.94%, 72.73%, and 16.67% pregnancy rate, respectively (p <0.05) (Table 3). Days in milk as a predictor factor also statistically significantly influenced pregnancy (p <0.05). Pregnancy rate, however, was not affected by lactation status, parity, and breed of the animals (p >0.05) (Table 4).

Table 3. Univariable factor wise effects Chi-square on conception rate of lutalyse.

Factors	Category	No. inseminated	No. conceived	Conception rate (%)	X^2	p value
Days in milk	≤60 days	12	4	33.33%	16.12	0.000
	> 60 days	59	47	79.66%		
Lactation status	Non lactating	65	50	76.92%	1.45	0.229
	Lactating	35	23	65.71%		
BCS	2.5	12	2	16.67%	26.66	0.000
	3	55	40	72.73%		
	3.5	33	31	93.94%		
Parity	0 (Heifers)	34	22	64.71%	2.42	0.299
	One and two	37	30	81.08%		
	≥3	29	21	72.41%		
Breed	Boran	29	18	62.07%	2.56	0.278
	Cross (Boran*HF)	51	40	78.43%		
	HF	20	15	75%		
Total		100	73	73%		

BCS: Body condition score; HF: Holstein-Friesian

The odds of conception rate in cows with greater than 60 days postpartum was 9.4 times more likely than cows with less than 60 days in milk, keeping BCS, lactation status, and parity constant. Cattle with 3.5 BCS were 32.3 times more likely to conceive than cattle with 2.5 BCS, keeping other predictors constant. Results of multivariable analysis of association between conception rate and predictors are presented in Table 4.

Table 4. Multivariable factor logistic regression on conception rate.

Factor	Category	Odds ratio	95% CI	*p* value
BCS	2.5	Ref		
	3	9.43	1.33, 66.99	0.025
	3.5	32.30	2.13, 488.73	0.012
Days in milk	≤60 days	Ref		
	>60 days	9.4	1.92, 46.26	0.006
Cons		.079	0.01, 0.68	0.021

BCS: Body condition score; Ref: Reference; SE: Standard error; CI: Confidence interval

Discussion

The experiment revealed that estrus was induced in 83.3% of the animals with a single injection of PGF2α analog (dinoprost tromethamine). Using the same protocol with the current study, a similar (84.2%) estrus response rate was reported in Tigray region, Ethiopia (Gugssa, 2015). Our result was also within the range of 75 to 90% estrus response rate recorded within 2 to 5 days of hormonal injection by Murugavel *et al.* (2003). However, the estrus response rate observed in our study was lower compared to results reported by Tegegne *et al.* (2012) from Hawassa-Dale (97.7%) and Adigrat-Mekelle (100%) milksheds, Gebrehiwot *et al.* (2015) from Wukro area (91.67%) in Tigray, and slightly lower compared to 89.3% response rate reported from Bahir Dar milkshed (Kebede *et al.*, 2013). The variations might be due to differences in the animals involved in the studies (body condition, parity, age, breed, days postpartum) and their management, and even differences in agro-ecological conditions of the study areas.

Estrus response in the present study was influenced by BCS of the animals. Cows and heifers with optimal BCS (3) showed greater response than those with sub-optimal body condition (2.5 BCS). The negative effect of low BCS on estrus response may be related to reduced functional competence of follicles produced during a period of negative energy balance (Britt, 1991; Beam and Butler, 1999). Acute nutritional deprivation leading to a negative energy balance (poor conditions) in heifers and cows has been noted to have immediate adverse effect on follicular growth and ovulation (Roche *et al.*, 2000). Energy was also reported as the most important limiting nutrient in establishing estrous cycles in the postpartum cow (Hill *et al.*, 2014). Negative energy balance

in dairy cattle influenced follicular growth and size of ovulating follicles (Armstrong *et al*., 2001; Wiltbank *et al*., 2002; Diskin *et al*., 2003).

The conception rate of 73% recorded in this study was higher than the national rate of pregnancy (27%) reported by Tegegne *et al.* (2012), and the preliminary results of mass synchronization in SNNPR (63%) and Tigray (62%) regions (Tegegne *et al*., 2010). Gebrehiwot *et al*. (2015) also reported a lower conception rate of 32.17% in Wukro area from Tigray. This difference might be due to careful selection of the animals on the basis of presence of CL in the present study, close follow-up of the animals, proper heat detection,and insemination at the right time.

Conception rate in our study was affected by body condition of the animals. Animals with better body condition had higher conception rate. This finding follows previous works of several researchers (Barros *et al*., 2000; Pancarci *et al*., 2002; Patton *et al*., 2007; Ciptadi *et al*., 2012; Tazangi and Mirzaei, 2015) who reported influence of body condition on conception rate where animals with higher body conditions had higher conception rates than animals with lower BCS. The low pregnancy rate in animals with low BCS may reflect reduced functional competence of the ovulated follicle because of its development during negative energy balance, or it may be a result of subtle changes in steroid hormone secretions that regulate gene expression and the secretion of proteins by the endometrium, thereby affecting implantation or pregnancy recognition (Beam and Butler, 1999; Roche *et al*., 2009).

Cows with longer days in milk (>60 days) during hormonal treatment had higher pregnancy rate than cows with less DIM. This is supported by different scholars who concluded that conception increased as the voluntary waiting period increased, peaking at about 80 days postpartum (Tenhagen *et al*., 2003; Stevenson, 2004).

Conclusions

Based on the finding, manual detection of CL and injection of prostaglandin analog is effective in inducing estrus in different breeds of bovine species. The present study revealed that estrus response to a single injection of lutalyse (dinoprost tromethamine*)* was significantly affected by body condition of the animals. The pregnancy rate was also significantly affected by body condition and days postpartum of the animals at the time of hormonal injection. Our

study suggested that a single injection of lutalyse (dinoprost tromethamine), following manual detection of CL, could be used for a cost-effective synchronization of estrus in dairy cows and heifers in Ethiopia. More controlled studies are recommended to support the findings of our study.

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Multivariate characterization of Wollo sheep populations in the cool highlands of Ethiopia

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Abstract

The objective of this study was to assess variation among populations of Wollo sheep managed in different locations. Three locations were selected purposely based on their sheep population size, sheep production potential and experiences of farmers in sheep breeding. Body weight, linear body measurements and qualitative data were recorded from a total 405 sheep (322 females and 83 males) in 68 flocks. Body weight and most of the linear body measurements of the indigenous sheep populations were significantly affected by location ($p<0.05$). The stepwise discriminant analysis procedure identified eight most significant discriminating traits: rump height (RH), wither height (WH), body weight (BW), tail circumference (TC), body condition score (BCS), ear length (EL), chest girth (CG) and body length (BL) from females; and five: RH, BW, WH, BL and horn length (HL) from males. The overall average error count estimate of sheep was 30%, accounting for 44%, 33% and 14% for Wogide, Borena and Legambo sheep populations, respectively. This means that 70% of sheep for all populations were correctly classified in their source population. In conclusion, the lowest misclassification of Legambo sheep population could be an indication of more uniformity within the population, whereas the highest misclassification of Wogide and Borena might be due to low geographical distance between their habitats. The largest distance was found between the Wogide and Legambo sheep populations and between the Borena and Legambo sheep populations, whilst the sheep populations from the Wogide and Borena were poorly differentiated. More genetic analysis research is recommended to assess variations and similarities of sheep and to identify the potential of genetic resources.

Keywords: Indigenous sheep; Linear-measurements; Morphological traits; Multivariate analysis.

Introduction

Ethiopia has 31.3 million sheep, and sheep play a significant role in the livelihood of rural communities of Ethiopia (CSA, 2018). Ethiopia is home for at least 9 breeds and 14 traditional sheep populations (Gizaw *et al*., 2007). The country has about 14 varied traditional sheep populations in four major groups i.e., sub-alpine short fat-tailed, highland long fat-tailed, lowland fat-rumped/tailed, and lowland thin-tailed (Gizaw *et al.,* 2008). Wollo sheep population is among the sub-alpine short fat-tailed group predominantly found in the central highlands at an altitude of ≥2500 m above sea level. Sub-alpine short fat-tailed sheep are mainly reared for income generation from the sale of lambs at market age, although they are also important as a source of food, hair, manure and socio-cultural benefits (Gizaw *et al.,* 2008; Getachew *et al.,* 2010).

Breed diversity is high in peripheral and remote areas of the country (Gizaw *et al.,* 2008) due to less human intervention for crossing. Similarly, sheep are adapted to various ecological niches and the differing needs and preferences of their breeders who belong to different ethnic communities. High phenotypic diversity for morphological characteristics, significant within and between breeds, was observed in sheep found in the country (Gizaw *et al.,* 2007, Getachew *et al*., 2009; Michael *et al*., 2016).

Body measurements have been used to predict body weight by several authors in many breeds of sheep (Sowande and Sobola, 2007; Cankaya, 2008; Iqbal, 2010; Michael *et al*., 2016). The use of multivariate analysis to classify different goat (Zaitoun *et al*., 2005; Dossa *et al*., 2007) and sheep (Riva *et al*., 2004; Traore *et al.,* 2008) populations, based on various qualitative and quantitative measurements, has also been reported. When all measured morphological characteristics are taken into account at the same time, this can be thought of as an appropriate instrument for evaluating population variance and discriminating between distinct population types.

In general, there is information gap regarding the phenotypic characteristics of Wollo sheep populations since, the overall local sheep morphological characteristics were not studied at the district level. Accordingly, there hasn't been any research on morphological characterization of sheep, including body weight, in Wogide, Borena and Legambo districts, and no research has been conducted to identify the genetic resources of sheep in the districts. It is important to identify the sheep genetic resources potential of the areas, as the information may be

used to establish breeding programs and to conserve the genetic resources. It is also necessary to understand the breeding practices and breeding objectives of farmers. Hence the aim of this study was to assess the variation of Wollo sheep populations using multivariate analysis to understand the potential of sheep in the specific places.

Materials and methods

Description of the study area

The study was conducted in three selected districts of South Wollo zone of the Amhara Regional State, namely Wogide, Borena, and Legambo districts. Wogide is bordered by Woleka River to the south, which separates it from the Oromia Region; by the Abay River to the west which separates it from East Gojjam zone; by Borena district to the north; by Legambo to the northeast and by Kelala district to the east. Borena is bordered by Wogide to the south, by the Abay River to the west which separates it from the East Gojjam zone, by Mehal-Sayint to the north, by Sayint to the northeast, and by Legambo to the east. Legambo is bordered by Legehida and Kelala to the south, by Wogide to the southwest, by Borena to the west, by Sayint-Ajibar to the northwest, by Tenta to the north, by Dessie Zuria to the east, and by Woreilu to the southeast. The capital town of Legambo is Akesta (CSA, 2008). The geographic coordinate points, rainfall, and temperature of the study districts are presented in Table 1.

Table 1. Description of the districts.

Characteristics of the study area	Districts		
	Wogide	Borena	Legambo
Latitude and longitude	10°40′N, 11°38′E	10°55′N, 38°30′E	11°00′N, 39°00′E
Rainfall (mm)	600 to 1100	1500 to 3660	700 to 1200
Temperature	23 °C	16.5 °C	13 °C
Area (km^2)	1,110.69	1,027.61	1,017.35
Sheep population	15,442	68,642	146,954

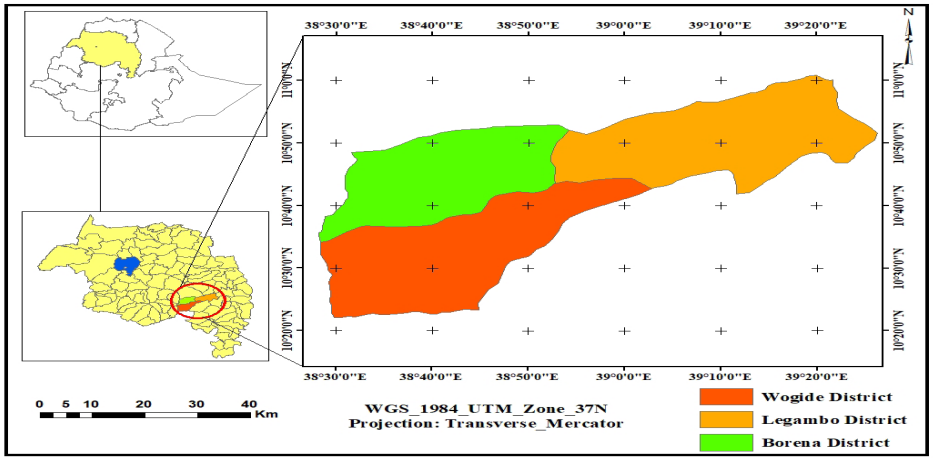


Figure 1. Map of the study area.

Animal sampling and body measurements

For qualitative and quantitative data collection purpose, animals were restrained and held in a natural position. The body weight and linear measurements were taken using a measuring tape and a measuring stick early in the morning before the animals were fed to avoid biases on certain traits due to feed intake. A total of 405 sheep (322 female and 83 male) were sampled from the study areas. The number of animals sampled per site is presented in Table 2. Five to 7 animals in each flock were used for body measurements and observation in order to avoid sampling of related animals.

Sampling techniques

Three districts were selected purposely from South Wollo zone based on their sheep population size, sheep production potential, and farmers' experience in sheep rearing. Three kebeles from each Wogide and Borena districts and two Kebeles from Legambo district (a total of 8 Kebeles) were selected based on their sheep production potential and road accessibility. *Kebele is* the lowest administrative hierarchy in Ethiopia.

Physical measurements were taken according to FAO (2012) guidelines, from a representative set of adult animals (as judged by dentition of 0PPI to ≥2PPI): 100-300 for females and 10-30 for males. Body weight and linear body mea-

surements were obtained from a total of 405 sheep (322 female and 83 male). Within each Kebele, measurements were made on individual indigenous sheep from randomly selected flocks.

Table 2. Distribution of samples across district and kebele.

Locations	*Kebele*	Samples	Total per district
Wogide	Seka-Shimbira	45	135
	Abado	45	
	Makefta	45	
Borena	Deramie	50	150
	Hulagosh	50	
	Chefe Belo	50	
Legambo	Dembesh	60	120
	Temu	60	

Methods of data collection

Qualitative data collection

Visual observations for qualitative traits were made and morphological features were recorded based on breed morphological characteristics descriptor list of phenotypic characterization of sheep. Generally six qualitative traits were observed like coat color pattern (plain, patch, spotted), coat hair type (short and smooth, long and coarse and short and coarse), coat color type (white, red, black, red and white, black and white, black with white head, ark grey /Jibma/, black with red/white belly and brown), head profile (straight/flat, concave and convex), ear formation (rudimentary, short/inclined downward and dropping/semi pendulous), and horn (present and absent) (FAO, 2012).

Quantitative data collection

Quantitative traits cover the size and dimensions of animals' bodies or body parts, which are more directly correlated to production traits than are qualitative traits. The body measurements collected were body weight (BW), chest girth (CG), body length (BL), wither height (WH), tail length (TL), tail circumference (TC), rump height (RH), ear length (EL), horn length (HL), scrotal circumference (SC) and body condition score (BCS). Linear body measurements

were made using measuring tape while live body weight was taken using suspended spring balance having 50 kg capacity with 0.2 kg precision.

Each animal was identified by its sex, dentition (0PPI, 1PPI and ≥2PPI) and sampling site. A dentition record was included, as this was the only reliable means to estimate the approximate age of an animal. The ages of the animals were estimated from dentition class following the procedure described by ESGPIP (2009). Adult sheep were classified into three age groups: no pair of permanent incisors (0PPI), 1 pair of permanent incisors (1PPI), 2 pairs of permanent incisors and above (≥ 2PPI), to represent age of sheep below 12 months, 12 to 18 months, 18 to 24 months and more than two years, respectively based on ESGPIP (2009).

Multivariate analysis

In order to ascertain the assignment (%) of each individual animal, identify significant discriminative traits, obtain distances between sample populations, and observe the spatial distribution of sample populations, multivariate discriminant analysis was carried out using quantitative traits for locations of sheep (Aziz and Al-Hur, 2012; FAO, 2012). Stepwise discriminant analysis, percent classified into each location for populations using discriminant analysis squared mahalanobis distances between sampled populations based on pooled covariance were analyzed by using quantitative traits of female and male sheep.

Data management and statistical analysis

Data collected through body measurement were entered into Microsoft EXCEL 2007. The data collected from each study area were checked for any errors, coded, and entered into computer for further analysis. Preliminary data analysis like homogeneity test, normality test and screening of outliers were carried out using PROC UNIVARIATE in Statistical Analysis System (SAS) before conducting the main data analysis. Observations on morphological characters were analyzed for male and female sheep using frequency procedure of SAS (SAS version 9.1, 2003). Quantitative characters (body weight and linear body measurements) were analyzed using the Generalized Linear Model (GLM) procedures of the SAS (SAS 9.1, 2003). Scrotum circumference was analyzed for each population by fitting age group as fixed factor. When analysis of variance declares significance, least square means were separated using adjusted Tukey-Kramer test.

The model to analyze adult body weight and other linear body measurements for indigenous sheep except scrotum circumference was**:**

$$Yijk = \mu + Ai + Lj + Sk + (AxS)\,ik + eijk$$

Where: Y*ijkl*= the body weight and linear body measurements except scrotum circumference in the i^{th}age group, j^{th} location and k^{th} sex

μ = overall mean
Ai = the effect of i^{th} age group ($i = 0, 1and \geq 2$ dentition)
Lj = the effect of j^{th} location (j = Wogide, Borena and Legambo)
Sk = the effects of k^{th} sex (k= male, female)
(AxS) ik = the effect of the interaction of *i* of age group with *k* of sex
Eijk = random residual

Results

Qualitative traits

Summary of qualitative traits observed in indigenous male and female sheep by district is presented in Table 3. In the study areas, sheep were short fat tailed (100%), and the tail was curved upward at the tip (96.79%), the remaining small proportion was straight and tip downward.

Table 3. Frequency and percentage of each class level of the qualitative traits scored in Wollo sheep.

Qualitative traits	Locations												Overall	
	Wogide (N = 135)				Borena (N = 150)				Legambo (N = 120)					
	Male		Female		Male		Female		Male		Female			
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Coat color pattern														
Plain	17	56.7	79	75.2	22	73.3	75	62.5	18	78.3	71	73.2	282	69.63
Patchy	8	26.7	16	15.2	7	23.3	41	34.2	4	17.4	23	23.7	99	24.44
Spotted	5	16.7	10	9.5	1	3.3	4	3.3	1	4.3	3	3.1	24	5.93
Coat hair type														
Short and smooth	2	6.7	1	1.0	-	-	-	-	-	-	-	-	3	0.74
Long and coarse	25	83.3	99	94.3	28	93.3	120	100.0	23	100.0	93	95.9	388	95.80
Short and coarse	3	10.0	5	4.7	2	6.7	-	-	-	-	4	4.1	14	3.46
Coat color type														
White	8	26.7	48	45.7	13	43.3	47	39.2	11	48.7	38	39.2	165	40.74
Red	2	6.7	9	8.6	2	6.7	14	11.7	4	17.4	15	15.5	46	11.36
Black	5	16.7	7	6.7	5	16.7	12	10.0	3	13.0	18	18.6	50	12.35
Red and white	2	6.7	3	2.9	2	6.7	2	1.7			3	3.1	12	2.96
Black and white	3	10.0	5	4.8	-	-	8	6.7	2	8.7	5	5.2	23	5.68
Black with white head	-	-	3	2.9	-	-	1	0.8			1	1.0	5	1.23
Dark grey/ Jibma/	8	26.7	14	13.3	2	6.7	22	18.0	2	8.7	10	10.3	58	14.32
Black with red/ white belly	-	-	1	1.0	1	3.3	-	-	-	-	-	-	2	0.49
Brown	2	6.7	15	14.3	5	16.7	14	11.7	1	4.3	7	7.2	44	10.86
Head profile														
Straight/flat	23	76.7	71	67.6	21	70.0	94	78.3	19	82.6	68	70.1	296	73.09
Concave	2	6.7	4	3.8	1	3.3	6	5.0	2	8.7	5	5.2	20	4.94
Convex	5	16.7	30	28.6	8	26.7	20	16.7	2	8.7	24	24.7	89	21.98
Ear formation														
Rudimentary	2	6.7	11	10.5	2	6.7	6	5.0	1	4.3	3	3.1	25	6.17
Short/inclined downward	13	43.3	27	25.7	9	30.0	15	12.5	1	4.3	4	4.1	69	17.04
Dropping/ semi pendulous	15	50.0	67	63.8	19	63.3	99	82.5	21	91.3	90	92.8	311	76.79
Horn														
Present	30	100.0	10	9.5	30	100.0	17	14.2	23	100.0	4	4.1	114	28.15
Absent	-	-	95	90.5	-	-	103	85.8	-	-	93	95.9	291	71.85

N: numbers of observations

Quantitative traits

Body weight, body length, chest girth, tail length, ear length, and rump height in Wogide and Borena sheep populations were not statistically different (p >0.05), but statistically different (p<0.05) from Legambo sheep population. The overall body weight of Wogide, Borena and Legambo sheep populations were 24.90±0.38, 25.71±0.39 and 22.19±0.41 whereas the chest girth were 70.18±0.46, 70.67±0.47 and 64.16±0.50, respectively.

Association of quantitative traits with fixed factors

The effect of location, sex, age group and interaction of age group and sex on body weight and other linear body measurements are presented in Table 4. In this study, body weight and most of the linear body measurements (body length, chest girth, wither height, tail length, tail circumference, rump height and ear length) were significantly (p <0.01) affected by location. Body condition score was also affected (p <0.05) by location, whereas horn length and scrotum circumference were not influenced by location (p >0.05).

Table 4. Least squares means ± standard errors of body weight (kg), linear body measurements (cm) for the effects of locations, sex, age group and sex by age group for indigenous sheep in the study areas.

Effects & levels		BCS	BW	BL	CG	WH	TL	TC	EL	RH	HL	SC
	N	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE
Overall	405	2.71±0.04	24.27±0.28	54.04±0.28	68.34±0.34	62.16±0.28	21.49±0.22	20.88±0.23	8.70±0.13	66.52±0.26	13.87±0.84	24.38±0.35
Locations		*	**	**	**	**	**	**	**	**	NS	NS
Wogide	135	2.69±0.06^{ab}	24.90±0.38^{a}	55.69±0.38^{a}	70.18±0.46^{a}	63.42±0.38^{b}	22.09±0.29^{a}	21.45±0.31^{a}	9.03±0.18^{a}	69.45±0.36^{a}	13.42±1.12	24.74±0.52
Borena	150	2.82±0.06^{a}	25.71±0.39^{a}	55.40±0.39^{a}	70.67±0.47^{a}	65.45±0.38^{a}	21.67±0.30^{a}	21.02±0.32^{ab}	9.33±0.18^{a}	69.44±0.36^{a}	13.61±0.99	23.83±0.54
Legambo	120	2.61±0.06^{b}	22.19±0.41^{b}	51.03±0.41^{b}	64.16±0.50^{b}	57.60±0.40^{c}	20.74±0.32^{b}	20.19±0.34^{b}	7.72±0.19^{b}	60.67±0.38^{b}	14.59±1.42	24.57±0.64
Sex		**	**	NS	**	**	**	**	NS	**	**	NA
Male	83	2.88±0.07^{a}	26.09±0.47^{a}	54.46±0.47	69.50±0.57^{a}	63.66±0.46^{a}	22.63±0.36^{a}	23.12±0.38^{a}	8.62±0.22	68.19±0.44^{a}	21.47±0.73^{a}	24.38±0.35
Female	322	2.54±0.05^{b}	22.46±0.31^{b}	53.62±0.30	67.17±0.37^{b}	60.64±0.30^{b}	20.37±0.24^{b}	18.65±0.25^{b}	8.78±0.14	64.85±0.29^{b}	6.23±1.48^{b}	-
Age group		*	**	**	**	**	**	**	**	**	**	**
0PPI	137	2.71±0.05^{ab}	19..65±0.35^{c}	49.65±0.35^{c}	63.68±0.43^{c}	58.77±0.35^{c}	20.00±0.27^{b}	19.74±0.29^{b}	8.30±0.16^{b}	63.05±0.33^{c}	9.32±1.19^{b}	20.50±0.42^{c}
1PPI	46	2.55±0.09^{b}	25.00±0.58^{b}	55.10±0.57^{b}	69.00±0.70^{b}	62.77±0.57^{b}	21.58±0.44^{a}	20.77±0.47^{ab}	8.56±0.27^{ab}	67.23±0.54^{b}	15.77±1.86^{a}	24.54±0.63^{b}
≥2PPI	222	2.86±0.08^{a}	28.17±0.51^{a}	57.36±0.50^{a}	72.33±0.61^{a}	64.93±0.50^{a}	22.92±0.39^{a}	22.15±0.41^{a}	9.22±0.23^{a}	69.29±0.47^{a}	16.46±1.03^{a}	28.05±0.72^{a}
Sex by age		**	**	NS	**	**	**	**	NS	**	**	NA
Male 0PPI	46	2.67±0.09	20.24±0.57^{cd}	49.73±0.57	63.31±0.69^{c}	59.16±0.56$_{de}$	20.47±0.44^{bc}	20.69±0.47^{b}	8.31±0.26	63.78±0.54^{de}	13.22±0.88^{b}	-
Male 1PPI	21	3.03±0.13	27.72±0.85^{ab}	55.35±0.84	71.26±1.03^{a}	64.41±0.84^{ab}	22.62±0.65^{ab}	23.36±0.69^{a}	8.18±0.39	68.89±0.79^{ab}	25.21±1.31^{a}	-
Male ≥2PPI	16	2.93±0.15	30.31±0.98^{a}	58.31±0.97	73.94±1.18^{a}	67.41±0.96^{a}	24.79±0.75^{a}	25.31±0.79^{a}	9.38±0.45	71.91±0.91^{a}	25.99±1.50^{a}	-
Female 0PPI	91	2.75±0.06	19.04±0.41^{d}	49.58±0.41	64.04±0.49^{bc}	58.38±0.40^{c}	19.53±0.31^{c}	18.78±0.33c	8.30±0.19	62.32±0.38^{c}	5.53±2.20^{c}	-
Female 1PPI	25	2.07±0.12	22.28±0.78^{c}	54.86±0.77	66.75±0.94^{b}	61.13±0.77^{cd}	20.53±0.60^{bc}	18.18±0.64^{c}	8.98±0.36	65.57±0.73^{cd}	6.34±3.46^{bc}	-
Female≥2PPI	206	2.79±0.04	26.04±0.27^{b}	56.41±0.27	70.72±0.33^{a}	62.42±0.27^{bc}	21.04±0.21^{b}	18.99±0.22^{c}	9.07±0.13	66.67±0.26^{bc}	6.93±1.38^{c}	-

Means with different superscripts within the same column and class are statistically different. Ns: Non-significant; *significant at 0.05; **significant at 0.01; NA: none applicable; N: number of sheep; BCS: body condition score; BW: body weight; BL: body length; CG: chest girth; WH: wither height; TL: tail length; TC: tail circumference; EL: ear length; RH: rump height; HL: horn length; SC: scrotum circumference; 0PPI: 0 pair of permanent incisor; 1PPI: 1 pair of permanent incisor; ≥2PPI: 2 or more pairs of permanent incisors; LSM: least square mean; SE: standard error.

Multivariate analysis

The stepwise discriminant analysis procedure identified eight (RH, WH, BW, TC, BCS, EL, CG, and BL) most significant discriminating traits between females while five discriminating traits (RH, HL, BW, WH and BL) were identified in males. Table 5 illustrates how each predictor for both sexes increased the discriminant function's predictive power between sample populations. Each predictor's association with Wilk's Lambda and Average Squared Canonical Correlation was statistically significant (p <0.05) for each variable. Percent of classification into each location for populations using discriminant analysis is presented in Table 6. Squared Mahalanobis distances between sampled populations based on pooled covariance are shown in Table 7. Every sheep pairwise distance was statistically significant (p<0.05).

Table 5. Quantitative characters selected by stepwise discriminant analysis.

Sex	Step	Variable	Partial R^2	F value	Pr>r	Wilks' lambda	Pr< lambda	ASCC	Pr> ASCC
Female	1	Rump height	0.545	191.38	<.0001	0.455	<.0001	0.273	<.0001
	2	Wither height	0.102	18.06	<.0001	0.408	<.0001	0.324	<.0001
	3	Body weight	0.063	10.65	<.0001	0.383	<.0001	0.338	<.0001
	4	Tail circumference	0.046	7.68	0.0006	0.365	<.0001	0.351	<.0001
	5	Body condition score	0.034	5.53	0.0044	0.352	<.0001	0.361	<.0001
	6	Ear length	0.042	6.87	0.0012	0.338	<.0001	0.374	<.0001
	7	Chest girth	0.019	2.98	0.0522	0.331	<.0001	0.377	<.0001
	8	Body length	0.014	2.20	0.1121	0.327	<.0001	0.383	<.0001
Male	1	Rump height	0.425	29.52	<.0001	0.575	<.0001	0.212	<.0001
	2	Horn length	0.359	22.15	<.0001	0.369	<.0001	0.316	<.0001
	3	Body weight	0.111	4.89	0.0101	0.328	<.0001	0.349	<.0001
	4	Wither height	0.191	9.10	0.0003	0.265	<.0001	0.419	<.0001
	5	Body length	0.089	3.71	0.0290	0.241	<.0001	0.446	<.0001

ASCC: Average Squared Canonical Correlation

Table 6. Percent classified into each location for populations using discriminant analysis.

Locations	Wogide	Borena	Legambo	Total
Wogide	56.30	33.33	10.37	100
Borena	28.00	66.67	5.33	100
Legambo	5.00	9.17	85.83	100
Total	30.62	38.52	30.86	100
Error rate	0.44	0.33	0.14	0.30

Table 7. Squared Mahalanobis distances between sampled populations based on pooled covariance.

Locations	Wogide	Borena	Legambo
Wogide	**		
Borena	0.83	**	
Legambo	6.84	6.76	**

Discussion

Qualitative traits

In the study areas, sheep were short fat tailed (100%), and the tail was curved upward at the tip (96.79%), the remaining small proportion was straight and tip downward. This finding was in agreement with observations of that of Gizaw *et al.* (2008) for Wollo sheep, Getachew *et al.* (2009) for Menz sheep, Mohammed *et al.* (2014) for sheep in Habru and Gubalafto districts of North Wollo zone and Bimerow *et al.* (2011) for Farta sheep, all of whom reported short fat tail type for the respective sheep populations they studied.

About 69.63% of the sheep had plain coat colour pattern, 24.44% patchy and 5.93% spotted. The current result was in line with Bireda *et al.* (2016) who reported that majority of the sheep in Gode and Adadile districts also had plain coat colour pattern. About 95.80% of indigenous sheep in the study areas had long and coarse hair, while only 3.46% and 0.74% had short and coarse, and short and smooth (0.74) hair, respectively. The present result is in agreement with Getachew *et al.* (2009), who reported a 98.80% long and coarse hair for Menz sheep. As indicated in coat colour preferences ranking, black coat colour had the least preference among the wide ranges of coat colours and this was

confirmed by small proportions of animals exhibiting black coat colour in the sampled population. This finding was similar to the findings of Edea *et al.* (2009) and Bireda *et al.* (2016) who reported least preference to black coat color for Bonga and Horro sheep and for Gode and Adadile sheep rearing areas, respectively.

Quantitative traits

Body weight and most of the linear body measurements (body length, chest girth, wither height, tail length, tail circumference, rump height and ear length) were significantly (p <0.05) affected by location. Body condition score was also affected (p <0.05) by location, whereas horn length and scrotum circumference were not influenced by locations. This result was in line with the findings of Amelmal (2011) and Michael *et al.* (2016), who reported significant differences (p <0.05) in most of the linear body measurements and live body weight in the study areas. But the present result contradicts with a report by Shibabaw (2012) which indicated that location had no effect (p >0.05) on body measurements, except on body weight which was higher in Metta (29.4±0.2) than Deder (28.8±0.2) district in Hararge highland sheep.

In this study, most of the linear body measurements of the Wogide and Borena sheep population were almost similar but higher than sheep in Legambo areas. For example the rump height of Wogide and Borena sheep were 69.45±0.36 and 69.44±0.36, respectively, but the rump height of indigenous sheep in Legambo was 60.67±0.38. The body weight of indigenous sheep in this study was similar to the observation by Bimerow *et al.* (2011) who reported 25.8±0.26 kg for Farta sheep. Still, it was lower than that of Taye *et al.* (2016) who reported 31.64±0.43 kg for Doyogena sheep, and Michael *et al.* (2016), who reported 29.0±0.2 for East Gojam (Gozamen, Sinan and Huleteju) sheep.

For indigenous sheep, sex of the sheep had significant (p <0.01) effect on body condition score, body weight, chest girth, wither height, tail length, tail circumference, rump height and horn length. Whereas body length and ear length of indigenous sheep were not affected (p >0.05) by sex of the sheep. This finding was in agreement with Getachew *et al.* (2009). who reported a significant association of sex with body condition score, body weight, chest girth, wither height, tail length, tail circumference and rump height in Menz and Afar sheep. Similar to our finding body length and ear length were not affected by sex of the sheep in the same study. Gebreyowhens and Tesfay (2016)

reported significant (p <0.05) effects of sex on body weight and other linear body measurements except ear length from Atsbiwonberta district of Tigray Region. Mengistie *et al.* (2010) obtained a slightly different result in which they reported a significant effect of sex on body weight, heart girth and height at wither, but also a significant association of body length with sex - different from our study, in Washera sheep. In contrast to our result Amelmal (2011) reported no significant association of sex of sheep with body weight and other linear measurements (p >0.05), except BCS. Haylom *et al.* (2014) also reported that sex had no significant (p >0.05) effect on body weight, chest girth, body length and height at wither in highland sheep in Atsbiwonberta.

In this study body weight and all other linear body measurements of indigenous sheep were significantly (p <0.05) affected by age of the sheep. Except BCS, body weight and all linear body measurements increased as the age increased from the youngest (0PPI) to the oldest (≥ 2PPI). The result of the current study was in line with a previous report by Gebreyowhens and Tesfay (2016), which stated that the average values of live body weight, chest girth, body length, and height at wither significantly (p<0.05) increased as the age of animals advanced, in a highland sheep population in Tigray Region. As the age of the male and female sheep increased, their body size also increased. At the same age, males were heavier than female sheep. Both sex and age have a synergistic effect on body size of the animal (Gebreyowhens and Tesfay, 2016). The interaction of sex and age was significant (p <0.05) for BCS, body weight, chest girth, wither height, tail length, tail circumference, rump height, and horn length but not for body length and ear length. The significant effect of interaction between sex and age on body measurements observed in the current study was in agreement with reports of Getachew *et al.* (2009) for Menz and Afar sheep, Mohammed *et al.* (2014) for Habru and Gubalafto districts, and Gebreyowhens and Tesfay (2016) for Atsbiwonberta district. Body weight, chest girth, height at wither, and height at rump were significantly affected p<0.05), while body length and ear length were not significantly affected (p >0.05) by sex by age interaction. However, in contradiction to our finding, Michael *et al.* (2016) reported significant effect of age by sex interaction only for body weight. In all age groups, indigenous male sheep were heavier (p <0.05) than female sheep.

Multivariate analysis

According to Peter *et al.* (2012), the stepwise discriminant analysis of RH and WH in this study is significant. At the 5% level of significance, the relative relevance of the found morphometric features in differentiating the three populations of sheep was evaluated. Wilk's Lambda decreased to 0.33 and 0.24 with a significant difference between the three location sheep populations ($F = 2.20$; $p<0.05$) and ($F = 3.71$; $p <0.05$), respectively, indicating the proportion of total variability not explained by the discriminator variables between populations when the most discriminating traits of both females (RH, WH, BW, TC, BCS, EL, CG, and BL) and males (RH, HL, BW, WH, and BL) respectively. This suggests that differences across populations, as opposed to variance within populations, accounted for the majority of the variability in the discriminator variables (67% for females and 76% for males).

As important discriminating variables were added chronologically, the partial R2 static decreased, indicating the proportion of each variable's variability that was explained by population differences. This pattern was similar to Wilk's Lambda value. The results indicate that RH had the strongest discriminating power in females, with BW, TC, BCS, EL, CG, and BL following in descending order, as indicated by the corresponding partial R2 and F-values. Meanwhile, RH exhibited the greatest discriminating power in males, followed by HL, BW, WH, and BL. This suggests that compared to females, who needed the measurement of eight traits, males needed slightly fewer trait measures to distinguish between the sheep populations in the three locations. When multivariate normality was checked, multi-attributes did not exhibit a combined multivariate normal distribution for the populations, according to the estimated significant ($p <.0.05$) multivariate skewness and kurtosis values. By calculating the likelihood of misclassification or error rates, one can assess how well a discriminant function performs in classifying new observations.

The three locations' combined average error count estimate for sheep was 30%, accounting for 44%, 33%, and 14% of the population of Wogide, Borena, and Legambo sheep, respectively. This indicates that 70% of sheep across all populations were identified in their original population with accuracy. The population of sheep classed as Legambo (85.83%) was found to be part of its source population, with Wogide (56.30%) and Borena (66.67%) following suit. The percentage of sheep that were incorrectly classified as Wogide was approximately 33.33%, whereas the percentage of sheep that were incorrectly classed as Borena was

28.00%. There was a misclassification of approximately 5.00% and 9.17% of the Legambo sheep population into the Wogide and Borena sheep populations. Ten point four percent of Wogide sheep and 5.3% of Borena animals were incorrectly assigned to the Legambo area. The highest misclassification of Wogide (33.3% in the Borena location) and Borena (28.0% in the Wogide location) may be related to the close proximity of their habitats, while the lowest misclassification of the Legambo sheep population may indicate greater homogeneity within the population.

To determine the Mahalanobis distances between the sample populations, the canonical discriminant analysis was used. The study was carried out via a step-wise discriminant analysis approach to find the most significant discriminant variables for females (RH, WH, BW, TC, BCS, EL, CG, and BL) and males (RH, HL, BW, WH, and BL). The squared Mahalanobis distance between the places, however, was determined to be greater. The sheep populations from the Wogide and Borena locations were minimally differentiated (0.83), whereas the greatest distance was seen between the Wogide and Legambo locations (6.84) and between the Borena and Legambo locations (6.76). Any conservation and development plan should begin with evaluating the level of diversity in the population since genetic variation is essential for populations to respond to artificial selection and adapt to changing environments (Toro *et al.,* 2011).

Conclusions

Sheep in the study locations were categorized as short fat-tailed (100%), with a primarily upward-curving tail tip. Around 96.79% of sheep had long and coarse hair, while the remaining sheep had short smooth hair. Three coat color types were noted in the research areas: red, dark grey, and plain white. In all linear body dimensions and body weight, there was a difference in these places. Genetic variations and environmental factors could be attributed to this.

The most significant distinguishing characteristics between females were found to be eight (RH, WH, BW, TC, BCS, EL, CG, and BL) in the stepwise discriminant analysis approach, and five (RH, HL, BW, WH, and BL) in the case of males. While the misclassification of Wogide and Borena sheep populations may be higher because of their close proximity to one another, the lowest misclassification of the Legambo sheep population may indicate greater homogeneity within the population. The sheep populations from the Wogide and

Borena locations were not well differentiated, whereas the greatest distance was identified between the Wogide and Legambo locations and between the Borena and Legambo locations.

Sheep has the ability to boost smallholder farmers' economies and standard of living in the study areas. It was discovered that the indigenous sheep populations of Wogide and Borena were bigger. Thus, before diluting the adapted and reasonably productive genotype, it would be imperative to conserve and enhance the native sheep in Wogide and Borena in their natural habitat. More genetic analysis research is recommended to assess variations and similarities of sheep and to identify potential of the genetic resources.

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Ethiop. Vet. J., is the Official Scientific Organ of the Ethiopian Veterinary Association

ETHIOPIAN VETERINARY JOURNAL

(*Ethiop. Vet. J.*)

Objectives

- To stand as the official scientific organ of the Ethiopian Veterinary Association and serve as medium of communication with professionals in the fields of animal health and production and other related disciplines.
- To effectively disseminate research outputs in all spheres of veterinary science, veterinary public health, animal production and other related fields of study.
- To help promote agricultural development in Ethiopia and the subregion as a whole through generation via research of technologies, methods etc and dissemination of scientific knowledge to stakeholders and ultimately to end users.

Type of papers

- Original research papers
- Review articles
- Short communications

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This includes original articles presenting outputs from basic, applied and adaptive research activities related to animal and public health and diverse aspects of animal production in Ethiopia, and from regional and international sources. The material must not have been previously submitted or published elsewhere. The research article should not contain more than 6000 words.

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These include papers covering baseline data/information as well as advances in the field of veterinary medicine, public health and related fields of research and development. Often review articles will be prepared by specialist or researchers actively engaged in particular area of study for a considerable period of time with proven success. This must be supported by a strong track record

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This is devoted to entertain results of original research, selected clinical case reports, brief scientific notes including preliminary results, scientific observation, experimental techniques, recent technological advances and news of interest in the field. Short communication must be written in a synthesis form where all necessary information are presented in a condensed manner. The material must not have been previously published elsewhere. Short communication should not contain more than 3000 words

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The title page should consist of:

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c) Name and address of the corresponding author, in case of more than one authors.

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The second page should provide an abstract of not more than 300 words summarizing the background, objective, materials and methods, major findings and their significance, and conclusions. Avoid the use of undefined abbreviations.

Keywords

Below the abstract, in a separate line, keywords, up to six in number should be given in alphabetical order separated by ";".

Introduction

Give sufficient information on the associated background zooming in to the subject in question; articulate the specific research question, and the contribution of your current research in bridging the gap, followed by statement of objective(s) is in order.

Materials and methods

Under this section, briefly state the materials used, the selection of study animals, subjects, herds or materials, and the design for observational or experimental study and the nature of controls. Briefly describe the methods and procedures followed in sufficient detail and give reference; if new or modified methods are used, give justification for using them. Precisely specify apparatus, instrument or chemicals used in terms of manufacturer's name and ad-

dress in parentheses. Similarly, drugs, biologicals, medicinal plants or others used should be sufficiently described in terms of generic name(s) or scientifically accepted identifications, dose(s), and route(s) of administration. Describe sufficiently the statistical methods used and how the magnitude or precision of analysis results reached.

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Quantify qualitative findings and present them with appropriate indicators of measurement of error or uncertainty, such as by using confidence intervals. Likewise, when reporting quantitative data, authors must indicate the extent of variability by either using standard deviation or standard error. Present the results in logical sequence in the text, tables, or illustrations. The results have to be presented in the same order as the questions raised in the objective(s) and methods sections. Results should be concise and no need of interpretation. In clinical or therapeutic trials, report complications or losses or even dropouts of such observations giving numbers. Support all findings by using appropriate statistical analysis.

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Discussion

Under this heading you should explain the findings and the associated interpretations. Relate the results with the objective of the study and with previ-

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Conclusion

This section should be separately presented with supporting evidences based on the major findings of the study. Appropriate recommendations can be made if necessary

Acknowledgements

Acknowledgments should be briefly stated after the Conclusions. Under this, technical, financial and material support can be mentioned.

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Authors should declare that they have no conflict of interest.

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Jobre, Y., Malone, J. B., McCarroll, J. C., Erko, B., Mukaratirwa, S. and Xinyu, Z., 2001. Satellite climatology and the environmental risk of Schistosoma mansoni in Ethiopia and east Africa. *Acta Trop.*, (*In press*).

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D) Books or chapters in books:

Thrusfield, M., 1995. Veterinary Epidemiology, 2nd edition, Blackwell Science Ltd, Oxford, UK.

Malone, J. B and Jobre, Y., 1999. Predicting outbreaks of fasciolosis from Ollernshaw to satellites. In: Dalton, J.P. (Ed.), Fasciolosis. CBA International Publications, Cambridge, Pp. 151-183.

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