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## Bovine tuberculosis reactor cattle in Southwest Ethiopia: Risk factors for bovine tuberculosis

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#### ABSTRACT

Bovine tuberculosis, caused by Mycobacterium bovis, is a chronic zoonotic disease that persists in Ethiopia despite global control efforts, impacting public health and the economy. However, little is known about the epidemiology of bovine tuberculosis in Southwest Ethiopia. This study aims to assess the prevalence of bovine tuberculosis and identify associated risk factors in dairy cattle. A cross-sectional study was conducted from October 2022 to October 2023 in four districts in the Sheka, Bench-Sheko, and Keffa zones. A multistage sampling approach was employed, resulting in the selection of 1.152 cattle from various herds. Tuberculin skin testing was performed to detect bovine tuberculosis, and data on potential risk factors were collected through questionnaires. Logistic regression models were used to analyze the association between bovine tuberculosis and various risk factors. The overall prevalence of bovine tuberculosis was 4.95% at the cow level and 22.19% at the herd level in the study areas. Significant herd-level risk factors for bovine tuberculosis included lowland areas, larger herd sizes, and the introducing new animals. At the cow level, risk factors included age, breed, body condition, herd size, introduction of new animals, and management system. The study reveals a high prevalence of bovine tuberculosis in dairy cattle in Southwest Ethiopia, identifying key risk factors. Effective control requires targeted interventions, such as improved management practices, enhanced farmer awareness, and a better understanding of and guidance on bovine tuberculosis strategies in Ethiopia. Future research should isolate and characterize M. bovis in the study areas.

## 1. Introduction

Bovine tuberculosis is a chronic zoonotic disease caused by *Mycobacterium bovis* (*M. bovis*), a pathogen that primarily affects cattle but can also infect other animals and humans. It poses significant public health and economic challenges, particularly in developing countries where livestock plays a crucial role in livelihoods [1–3]. Bovine tuberculosis is transmitted mainly through the inhalation of infected aerosols, direct contact, or ingestion of contaminated feed or water [4]. Globally, bovine tuberculosis remains a persistent problem despite efforts to control and eradicate it, especially in low- and middle-income countries [5]. The World Health Organization (WHO) recognizes bovine tuberculosis as a significant zoonotic disease that requires coordinated efforts for control and prevention [6]. In Ethiopia, bovine tuberculosis is endemic, particularly in areas with high cattle density and close human-

animal interactions. Studies have reported varying prevalence across the country, highlighting the need for investigations to tailor control measures effectively [7–9].

The prevalence of bovine tuberculosis in Ethiopia varies by region, with higher prevalence often observed in intensive dairy farms and urban settings where cattle are kept in close proximity. Factors such as herd size, breed, management practices, and the presence of wildlife reservoirs contribute to the disease's epidemiology. For example, wild animals such as the African buffalo (*Syncerus caffer*), bushbuck (*Tragelaphus sylvaticus*), warthogs (*Phacochoerus africanus*), and various rodents are known to harbor *M. bovis*, the causative agent of bovine tuberculosis [10]. Previous studies in different regions of Ethiopia have reported prevalence ranging from 3 % to 50 %, indicating a widespread but uneven distribution of the disease across the country [11–14]. Several risk factors have been associated with the occurrence of bovine

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tuberculosis in cattle. These include herd size, breed susceptibility, age of the cattle, and management practices such as housing and feeding [15,16]. For instance, larger herds and exotic or cross-bred cattle are often at higher risk due to increased animal contact and genetic susceptibility. Furthermore, poor management practices, such as inadequate ventilation and poor hygiene, can exacerbate the spread of the disease within and between herds [10,17,18].

The impact of bovine tuberculosis on the productivity of dairy cattle is profound, leading to significant economic losses for farmers. Infected cattle often exhibit reduced milk yield, weight loss, and increased susceptibility to other diseases, which together diminish their overall productivity [19,20]. In Ethiopia, where the dairy industry is a critical component of the agricultural economy, the prevalence of bovine tuberculosis in dairy cattle presents a major challenge to food security and livelihoods, particularly for smallholder farmers who depend on milk production as a primary source of income [5,14,21]. The control and prevention of bovine tuberculosis in Ethiopia face numerous challenges, including a lack of awareness among farmers, inadequate veterinary services, and limited access to diagnostic facilities [5,7]. Despite the availability of diagnostic tests such as the tuberculin skin test (TST) and interferon-gamma assay, their use in routine screening is limited in the country due to logistical and financial constraints [22,23]. Furthermore, the lack of effective vaccination against bovine tuberculosis in cattle further complicates efforts to control the disease, necessitating the implementation of comprehensive control programs that include both livestock and public health components [24,25].

Southwest Ethiopia, characterized by its agro-ecological diversity and a cattle population of 1.6 million, is a region where dairy farming is a major livelihood [26]. However, the prevalence and risk factors of bovine tuberculosis in this region remain underexplored. Understanding

the epidemiology of bovine tuberculosis in this specific context is crucial for designing effective control strategies tailored to the region's unique environmental and socio-economic conditions. This study aims to fill the knowledge gap by assessing the prevalence and identifying the associated risk factors of bovine tuberculosis in dairy cattle in Southwest Ethiopia [16,27]. The objective of this study is to determine the prevalence of bovine tuberculosis in dairy cattle in Southwest Ethiopia and to identify the associated risk factors contributing to its occurrence. The findings provide valuable insights into the epidemiology of bovine tuberculosis in this region, informing policymakers and stakeholders in the development of targeted intervention strategies. Furthermore, by identifying specific risk factors, the study contributes to a broader understanding of bovine tuberculosis dynamics in similar settings, ultimately aiding global efforts to control and prevent this significant zoonotic disease [28,29].

#### 2. Materials and methods

#### 2.1. Study areas

The study was conducted in four districts located in Southwest Ethiopia, specifically in the Sheka, Bench-Sheko, and Kaffa zones. These districts are Yeki, Chena, Debub Bench, and Gimbo. The Bench-Sheko Zone has elevations ranging from 1200 to 1959 m above sea level, with an average annual temperature of 20 °C to 40 °C and annual rainfall between 1500 and 1800 mm. The altitude of Sheka Zone ranges from 1200 to 3000 m above sea level, experiencing an average annual temperature of 15.1 °C to 27.5 °C and rainfall between 1201 and 1800 mm. The Kaffa Zone has a mean annual temperature ranging from 10.1 °C to 27.5 °C, with rainfall varying between 1001 and 2200 mm (Fig. 1).

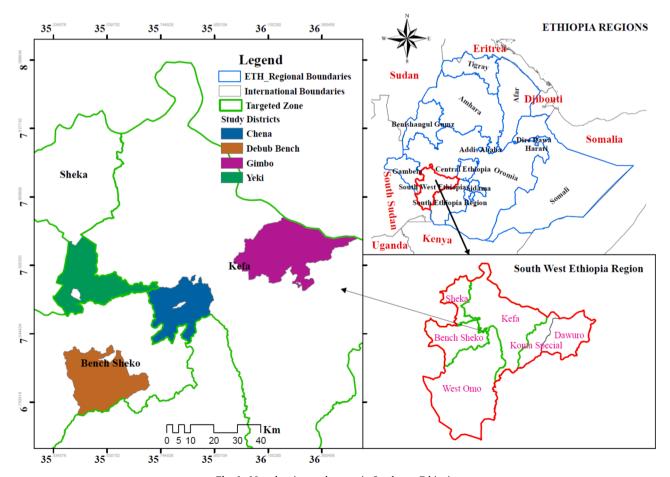


Fig. 1. Map showing study areas in Southwest Ethiopia.

According to Ethiopia's agro-ecological classification [30], these zones are categorized as lowland (below 1500 m asl), mid-altitude (1500–2300 m asl), and highland (above 2300 m asl). Accordingly, the selected districts fall within the following agro-ecological zones: highland (Chena and Gimbo), mid-altitude (Debub Bench), and lowland (Yeki). The study area is home to a livestock population of 71,047 goats, 73,384 sheep, and 1,596,803 cattle [31]. The dominant cattle breeds are Zebu and Sheko, with some crossbreeds of Holstein-Friesian and Jersey. The area supports both extensive (crop-livestock integration) and semi-intensive (urban production) management systems.

#### 2.2. Study design and population

A cross-sectional study was conducted between October 2022 and October 2023 to determine the prevalence and risk factors associated with bovine tuberculosis in dairy cattle in Southwest Ethiopia. The study focused on a population of dairy cows, including crossbred Holstein-Friesians, Jersey cows, and local breeds such as Zebu and Sheko. These cows varied in age, body condition, and were raised across different agro-ecological zones, under various management systems.

#### 2.3. Sampling methods and sample size determination

We used a multistage sampling method with random selection at each stage to conduct our study [32]. The research took place in three zones of Southwest Ethiopia: Sheka, Bench-Sheko, and Keffa. These zones were purposely chosen because they have a high incidence of bovine tuberculosis and large cattle populations (unpublished data). The sampling process involved proportional simple random sampling across several levels: districts, kebeles (local administrative units), villages, and herds. We randomly selected districts such as Yeki, Chena, Debub Bench, and Gimbo using a lottery system. From these districts, we then randomly selected 28 kebeles, followed by the selection of 84 villages within those kebeles. Subsequently, we applied proportional simple random sampling to select 383 herds. Within each herd, cattle were chosen randomly using a lottery system, with the number of animals sampled adjusted based on the herd size. Since there were no prior studies on bovine tuberculosis in these areas, we calculated the sample size needed to achieve an absolute precision of 5 % with a 95 % confidence interval (CI) for an estimated prevalence of 50 %, following the method of Thrusfield [33]. This calculation indicated a need for a sample of 384 cattle. To further improve precision, we tripled the sample size, resulting in a total of 1,152 cattle included in the study.

## 2.4. Tuberculin skin testing

A comparative intradermal tuberculin test (CIDT) was conducted using Bovine Tuberculin PPD 3000 (30,000 IU/ml) and Avian Tuberculin PPD 2500 (25,000 IU/ml) (Animal Science Group, Wageningen UR, Netherlands) on the neck skin. Two injection sites, spaced 12 cm apart, were marked and shaved on the left side of the mid-neck region. For calves, due to limited space, the opposite sides of the neck were used. The initial skin thickness at each site was measured with an electronic caliper (Preco Machine Tool Co., Ltd, Qingdao/Shandong, China). One site received an injection of 0.1 ml of bovine PPD, while the other was injected with the same volume of avian PPD, using an insulin syringe (Shanghai Care Life International Trading Co., Ltd). After 72 h, the thickness of the skinfold at both sites was re-measured and recorded. The difference in skin thickness at each site before and 72 h after injection was calculated and used for result interpretation, following Office International des Epizooties [34] guidelines. An animal was deemed positive if the difference between PPD-B and PPD-A was 4 mm or greater; if the difference was less than 4 mm, the animal was considered negative. A positive result at the PPD-A site indicated exposure to Mycobacterial avian species other than M. bovis. A herd was classified as positive if at least one animal tested positive [35,36].

#### 2.5. Data collection

Data on herd-level risk factors were collected using a pretested questionnaire administered by the researcher during farm visits coinciding with tuberculin testing. The questionnaire, which included both open-ended and closed questions, was translated into the local language to ensure clear understanding and comfort for respondents. Animal-level risk factors recorded during skin testing included age, breed, origin, body condition score, pregnancy status, and lactation. Other factors such as agro-ecology, livestock management practices, new animal introductions, herd size, cattle contact with other animals, and wildlife contact with cattle were documented. Body condition scores were categorized into poor (1-2), medium (3), and good (4-5), based on the visibility of ribs and vertebral spinous processes [37]. Herd sizes were classified as small (up to 15 cattle), medium (15-30 cattle), and large (more than 30 cattle). Cattle management systems were categorized as extensive, semi-intensive, or intensive [38]. Cattle age was grouped into less than 3 years, 3–6 years, and more than 6 years, reflecting the typical age at first calving in tropical conditions [39]. Herds were classified based on hygiene practices into "good hygiene," with daily manure removal and effective drainage, and "poor hygiene," without these practices. Information on cattle-wildlife interactions, including the presence of wildlife in grazing and watering areas, was gathered through the questionnaires.

#### 2.6. Data analysis

The collected data were imported into Microsoft® Excel for Windows 2010, and subsequently recorded, saved, and analyzed using STATA version 14.0 (Stata Corp., College Station, TX, USA). The prevalence of bovine tuberculosis at the animal level was calculated by dividing the number of positive animals by the total number of animals tested. At the herd level, prevalence was determined by dividing the number of herds with at least one positive animal (based on the CIDT test result) by the total number of herds sampled. Associations between bovine tuberculosis and explanatory variables were examined using logistic regression models for both positive animals and herds. Variables with a P-value  $\leq$ 0.25 in univariable analyses were included in the multivariable analyses. Multicollinearity in the final multivariable models was evaluated using generalized variable inflation factors (GVIF), and variables with a GVIF^(1/2\*Df) exceeding 2 were excluded. Furthermore, biologically significant two-way interactions among variables in the final model were tested. Multivariable logistic regression was utilized to assess the effects of potential risk factors for bovine tuberculosis at both the herd and individual animal levels. Non-significant components of the model with a p-value greater than 0.05 were removed through backward elimination. Model fit was evaluated using the Hosmer-Lemeshow test, and variables with P < 0.05 were retained. Covariates that altered the estimated odds ratio (OR) by more than 25 % were considered confounders and included in the model [32]. A significance level of 0.05 was applied to all statistical tests.

#### 3. Results

#### 3.1. Prevalence of bovine tuberculosis at cow and herd levels

A total of 1,152 dairy cattle were examined, revealing an overall cow-level prevalence of 4.95 %. The highest prevalence was observed in the Gimbo district at 6.0 %, followed by Debub Bench at 5.0 %, Yeki at 4.98 %, and Chena at 4.0 %. A chi-square test indicated no significant difference in cow-level prevalence across the study areas (P=0.813). At the herd level, 383 herds were examined, with an overall prevalence of 22.19 %. Yeki had the highest herd-level prevalence at 32.43 %, followed by Gimbo at 20.34 %, Chena at 15.0 %, and Debub Bench at 13.54 %. The chi-square test revealed a significant difference in herd-level prevalence across the study areas (P=0.001) (Table 1).

**Table 1**Prevalence of bovine tuberculosis in dairy cattle at both the animal and herd levels in Southwest Ethiopia.

	Cow-level		Herd-level		
Study areas	Total animal examined	Prevalence (%) (95 %CI)	Total herd examined	Prevalence (%) (95 %CI)	
Chena	250	4.0 (1.57–6.43)	80	15.0 (7.18–22.82)	
Debub Bench	300	5.0 (2.53–7.47)	96	13.54 (6.70–20.39)	
Gimbo	200	6.0	59	20.34	
Yeki	402	(2.71–9.29) 4.98	148	(10.07–30.61) 32.43	
. "		(2.85–7.10)		(24.89–39.97)	
Overall	1152	4.95 (3.70–6.20)	383	22.19 (18.03–26.35)	
Chi square value (χ2)		0.951		15.662	
P-value		0.813		0.001	

CI: Confidence Interval.

#### 3.2. Potential risk factors of bovine tuberculosis at herd level

The prevalence of bovine tuberculosis was significantly higher in herds located in lowland areas, with a rate of 32.43 %. Univariable analysis showed that herds in lowland areas had a crude odds ratio (OR) of 2.3 compared to those in highland areas, indicating a higher risk of bovine tuberculosis in lowland areas. This association remained significant in the multivariable analysis, with an adjusted OR of 3.0. Larger herds also exhibited a higher prevalence of bovine tuberculosis, with 27.84 % of small herds testing positive. The univariable analysis indicated that medium and large herds had significantly higher odds of bovine tuberculosis, with crude ORs of 2.1 and 2.2, respectively, compared to smaller herds. The multivariable analysis supported this finding, showing adjusted ORs of 1.9 for medium herds and 2.2 for large herds. Herds managed under an intensive system had a higher prevalence of bovine tuberculosis (30.97 %), with the association being significant in the univariable analysis. However, no significant association was observed in the multivariable analysis. The introduction of new animals into herds was a significant risk factor for bovine tuberculosis, with a prevalence of 24.36 % in herds that introduced new animals.

Univariable analysis yielded a crude OR of 3.4, which remained significant in the multivariable analysis, with an adjusted OR of 4. Hygiene, accessibility to wild animals, and contact with other animals were not significantly associated with bovine tuberculosis prevalence in either the univariable or multivariable analyses (Table 2).

#### 3.3. Potential risk factor of bovine tuberculosis at cow-level

In the univariable logistic regression analysis, age was found to be a significant risk factor. Cattle older than 6 years had a higher prevalence of bovine tuberculosis (6.51 %) compared to those aged 3-6 years (2.29 %) and those younger than 3 years (1.81 %). The crude odds ratio (OR) for cattle older than 6 years was 3.8, while for those aged 3-6 years, it was 1.4. Crossbred cattle had a higher prevalence of bovine tuberculosis (9.40 %) compared to local breeds (3.51 %), with a crude OR of 2.1. Jersey cattle had a prevalence of 6.54 %, with a crude OR of 1.7 compared to local breeds. Cattle in poor body condition had a prevalence of 4.95 % and a crude OR of 4.8, while those in medium body condition had a prevalence of 6.08 %. Cattle in good body condition had the lowest prevalence (1.35 %). Large herds had a prevalence of 6.21 %, and medium-sized herds had a prevalence of 5.81 %, compared to small herds (1.95 %). Cattle in intensive systems had the highest prevalence (10.37%), with a crude OR of 3.9. Cattle in semi-intensive systems had a prevalence of 5.81 %, while those in extensive systems had a prevalence of 2.87 %. Cattle with new animal introductions had a higher prevalence of 7.03 % compared to those without new introductions (3.72 %). Pregnancy status, lactation status, body condition score, hygiene, accessibility to wild animals, and contact with other animals did not show significant associations with the prevalence of bovine tuberculosis (p > 0.05) (Table 3).

The multivariable logistic regression analysis revealed that age was a significant risk factor for bTB, with cattle older than six years having significantly higher odds of testing positive for bovine tuberculosis. Cattle aged between 3–6 years also showed a higher risk, with an adjusted OR of 1.6, while those younger than three years had the lowest prevalence of the disease (1.81 %). The breed was another significant factor, with crossbred cattle having higher odds of testing positive. Jersey cattle also exhibited a relatively higher risk, with an adjusted OR of 1.5. Cattle with a poor body condition score were significantly more

 Table 2

 Analysis of potential herd-level risk factors for bovine tuberculosis in Southwest Ethiopia using univariate and multivariate methods.

Variable	Category	Total herd examined	Total herd positive (%)	Univariable		Multivariable	
				Crude OR (CI 95 %)	P-value	Adjusted OR (CI 95 %)	P-value
Agroecology					0.001		0.001
	Highland	139	24 (17.27)				
	Mid-land	96	13 (13.54)	0.8 (0.36-1.56)	0.442	1.4 (0.65-2.87)	0.404
	Lowland	148	48 (32.43)	2.3 (1.32-4.02)	0.003	3.0 (1.51-6.00)	0.002
Herd size					0.016		0.028
	Large	161	24 (14.91)	2.2 (1.18-4.60)	0.013	2.2 (1.18-4.19)	0.014
	Medium	125	34 (27.20)	2.1 (1.18-4.10)	0.011	1.9 (1.06-3.49)	0.032
	Small	97	27 (27.84)				
Hygiene	Good	184	34 (18.48)				
	Poor	199	51 (25.63)	1.5 (0.93-2.48)	0.094		
Management system					0.030		
	Intensive	113	35 (30.97)	2.0 (1.14-3.46)	0.016		
	Sem-intensive	102	19 (18.63)	1.0 (0.54-1.91)	0.294		
	Extensive	168	31 (18.45)				
Introduction new animal							
	Yes	234	57 (24.36)	3.4 (1.84-6.31)	0.002	4.1 (1.76-8.54)	0.003
	No	149	28 (18.79)				
Accessibility to wild animal							
•	Yes	168	32 (19.05)	1.4 (0.85-2.28)	0.191		
	No	215	53 (24.65)				
Cattle contact with other animals							
	Yes	166	35	1.1 (0.69-1.83)	0.648		
	No	217	50				

CI: Confidence interval; OR: Odds Ratio.

**Table 3**Univariable logistic regression analysis of potential risk factors for bovine tuberculosis in cattle in Southwest Ethiopia.

Variable	Category	Total cattle examined	Total cattle positive (%)	Crude OR (95 %CI)	P- value
Age	>6 years	768	50 (6.51)	3.8	0.005 0.027
	3–6 years	218	5 (2.29)	(1.17–12.28) 1.4	0.029
Dd	<3 years	166	2 (1.81)	(1.22–4.60)	0.015
Breed	Cross	117	11 (9.40)	2.1	0.015 0.013
	Jersey	321	11 (6.54)	(1.99–4.09) 1.7	0.022
Pregnancy	Local	712	35 (3.51)	(0.35–1.37)	
status	Pregnant	852	46 (5.40)	1.5	0.237
Lactation	None pregnant	289	11 (2.83)	(0.77–2.93)	
Lactation	None lactating	444	23 (5.18)	1.1 (0.63–1.86)	0.773
Body condition	Lactating	708	34 (4.80)	(0.00 1.00)	0.033
score	Poor	222	11 (4.95)	4.8	0.010
	Medium	707	43 (6.08)	(1.46–15.46)	0.042
Agroecology	Good	223	3 (1.35)	(1.05–13.90)	0.00
	Lowland	300	15 (5.0)	1.0	0.997 0.945
	Mid-land	402	20 (4.98)	(0.52–2.01) 1.1	0.954
Herd size	Highland	450	22 (4.89)	(0.55–1.90)	0.025
neiù size	Large	354	22 (6.21)	3.4	0.025 0.008
	Medium	499	29 (5.81)	(1.37–8.57) 3.1 (1.27–7.57)	0.013
Hygiene	Small	308	6 (1.95)	(1.2/-/.3/)	
Trygiene	Poor	546	30 (5.49)	1.2 (0.73–2.13)	0.418
Management	Good	606	27 (4.46)	(2 2 2.1.0)	0.001
system	Intensive	164	17 (10.37)	3.9 (1.95–7.85)	0.0001
	Sem- intensive Extensive	396 592	23 (5.81) 17 (2.87)	(1.93–7.83) 2.1 (1.10–3.96)	0.024
Introduction new animal	Yes	427	30 (7.03)	2.0	0.014
	No	725	27 (3.72)	(1.15–3.33)	
Accessibility to wild animal					
	Yes	495	26 (5.25)	1.1 (0.66–1.91)	0.679
Cattle contact with other animals	No	657	31 (4.72)		
	Yes	655	35 (5.34)	1.2 (0.71–2.11)	0.478
	No	497	22 (4.43)	(0./ 1-2.11)	

CI: Confidence interval; OR: Odds Ratio.

likely to be bTB positive, with an adjusted OR of 4.5. Cattle with a medium body condition also had a higher risk, though not statistically significant, with an adjusted OR of 3.3. The size of the herd was associated with bovine tuberculosis prevalence, with cattle from large herds having an adjusted OR of 2.8, while those from medium-sized herds had an adjusted OR of 2.5. The management system was another significant risk factor. Cattle under intensive management had a much higher risk of bovine tuberculosis, with an adjusted OR of 11.0. Those under semiintensive management also had a significantly higher risk, with an adjusted OR of 3.8, compared to those under extensive management, which had a prevalence of 2.87 %. The introduction of new animals into a herd significantly increased the risk of bovine tuberculosis. Herds that introduced new animals had an adjusted OR of 2.1 compared to those that did not, which had a prevalence of 3.72 % (Table 4). There were no significant differences (P > 0.05) detected among the variables regarding interaction and multicollinearity. The model showed a good fit to the data, as indicated by Hosmer and Lemeshow's test ( $\chi 2$  = 12.765, P = 0.120). Furthermore, the model's area under the ROC curve (AUC = 0.747, 95 % CI: 0.745–0.943) highlighted its predictive accuracy.

#### 4. Discussion

This study identified that several factors at the individual animal level, including age, breed, body condition, herd size, management

**Table 4**Multivariable logistic regression analysis of potential risk factors for bovine tuberculosis in Southwest Ethiopia.

Variable	Category	Total cattle examined	Total cattle positive (%)	Adjusted OR (95 %CI)	P- value
Age					0.002
	>6 years	768	50 (6.51)	4.5 (1.35–14.79)	0.015
	3–6 years	218	5 (2.29)	1.6 (1.23–5.03)	0.023
	<3 years	166	2 (1.81)		
Breed	Cross	117	11 (0 40)	2.4	0.001 0.023
	GIOSS	11/	11 (9.40)	(1.92–6.49)	0.023
	Jersey	321	11 (6.54)	1.5	0.040
				(1.19–1.14)	
Body	Local	712	35 (3.51)		0.042
condition score					0.042
	Poor	222	11 (4.95)	4.5 (1.36–14.86)	0.014
	Medium	707	43 (6.08)	3.3 (0.89–12.44)	0.073
	Good	223	3 (1.35)		
Herd size		054	00 (6 01)	0.0	0.023
	Large	354	22 (6.21)	2.8 (1.08–7.11)	0.033
	Medium	499	29 (5.81)	2.5	0.042
				(1.01-6.32)	
	Small	308	6 (1.95)		0.0001
Management system					0.0001
oj otem	Intensive	164	17	11.0	0.0001
			(10.37)	(3.98-30.47)	
	Sem-	396	23 (5.81)	3.8	0.0001
	intensive Extensive	592	17 (2.87)	(1.86–7.65)	
Introduction new animal	DATCHSIVE	3,2	1/ (2.0/)		
	Yes	427	30 (7.03)	2.1 (1.15–3.65)	0.014
	No	725	27 (3.72)	•	

CI: Confidence interval; OR: Odds Ratio.

practices, and the introduction of new animals, influenced the prevalence of bovine tuberculosis in cattle. Furthermore, it was observed that herd size, agroecology, and the introduction of new animals significantly impacted the prevalence of bovine tuberculosis at the herd level. The high prevalence of bovine tuberculosis in dairy cattle in the study areas resulted in substantial economic losses and presented a serious public health risk. This research provides crucial epidemiological data on bovine tuberculosis in cattle in Southwest Ethiopia. As the first study in this region to investigate the epidemiology of bovine tuberculosis, it offers valuable insights that could guide the implementation of effective management strategies to control and prevent the disease in cattle.

The overall cow-level prevalence of 4.95 % is consistent with previous studies conducted in Ethiopia, which have reported a prevalence of 4.3 % [40], 5.2 % [7], 5.5 % [27], and 5.8 % [10]. However, the prevalence recorded in this study is lower than in other studies conducted in Ethiopia, where bovine tuberculosis prevalence in cattle has been reported to range from 11.24 % to 39.3 % [17,16,41,42]. The variation in prevalence between this study and previous reports may be attributed to differences in environmental conditions, breed composition, and husbandry practices. The highest cow-level prevalence observed in Gimbo could be attributed to differences in farming practices, herd management, and possible variations in environmental conditions that may influence the transmission of M. bovis [21].

The overall herd-level prevalence of bovine tuberculosis observed in this study (22.19 %) is consistent with previous studies that have reported varying prevalence across different regions, which reflects the impact of regional factors on disease dynamics [7,40]. However, the current result is lower than the prevalence reported in other parts of the country [16,17,27]. This discrepancy may indicate potential regional variations in bovine tuberculosis prevalence, which could be influenced by local environmental, management, and epidemiological factors. The variation in herd-level bovine tuberculosis prevalence across different study areas in Southwest Ethiopia, with the highest in Yeki (32.43 %) compared to Chena (15.0 %) and Debub Bench (13.54 %), demonstrates the heterogeneity of the disease. This disparity might be attributed to differences in management practices, environmental conditions, or cattle population density in these districts [41].

The significant association between agroecology and the risk of bovine tuberculosis underscores the impact of environmental factors on disease prevalence at the herd level. Specifically, the higher risk in lowland areas could be attributed to differences in climatic conditions, wildlife interactions, or management practices compared to highland areas [3,43]. These results align with studies that suggest environmental conditions play a crucial role in the epidemiology of bovine tuberculosis [7,29]. Similar findings have been reported in other research [35,44], indicating that agroecological factors influence the distribution of bovine tuberculosis.

The findings from this study underscore the crucial role of herd size as a risk factor for bovine tuberculosis at the cow level. Larger herds show a higher prevalence of bovine tuberculosis, which aligns with previous studies suggesting an increased transmission risk associated with greater animal density [17,27,40,41]. In larger herds, the greater number of animals may facilitate the spread of the disease due to closer contact between animals, reduced biosecurity measures, and potentially inadequate testing and control practices [21,45]. Herd size also emerged as a significant risk factor for bovine tuberculosis at the herd level, with larger herds being at increased risk. This finding is consistent with similar studies, which have reported that larger herds may face more transmission opportunities and challenges in maintaining biosecurity [7,27]. Furthermore, the significant association between herd size and bovine tuberculosis prevalence aligns with other research [16,46,47], suggesting that larger herds may have a higher risk of bovine tuberculosis due to increased animal-to-animal contact and stress factors that could compromise immunity.

The introduction of new animals was associated with a higher prevalence of bovine tuberculosis at the cow level. This finding corroborates earlier research suggesting that new introductions can increase the risk of disease spread within herds [7,27,47]. Allowing unrestricted movement of animals without adequate precautions may heighten the risk of bovine tuberculosis spreading to broader regions. Similar observations have been made in other countries [48–51]. It has been recommended to source animals from bovine tuberculosis-free herds, limit cattle trade overall, and prioritize the trade of younger animals over adults as effective measures to mitigate the spread of bovine tuberculosis [52]. The introduction of new animals was also identified as a significant risk factor at the herd level, with a substantial increase in risk associated with this practice. This finding highlights the importance of strict biosecurity measures when integrating new animals into herds to prevent the introduction and spread of bovine tuberculosis [53]. This aligns with previous studies [7,54] that have identified animal movement as a critical factor in the transmission of bovine tuberculosis.

Age was a significant predictor of bovine tuberculosis prevalence, with older cattle showing a markedly higher risk compared to younger animals. The increased risk in older cattle may be attributed to prolonged exposure and potentially less effective immune responses as cattle age [1,55]. This finding aligns with previous studies suggesting that older animals are more likely to have accumulated exposure to bovine tuberculosis [7,27,41,46,47]. The study also highlights the significant influence of breed on bovine tuberculosis prevalence. Crossbred cattle exhibited the highest risk, followed by Jersey breeds. This may be attributed to the higher genetic susceptibility of these breeds compared to local breeds, which might have developed some level of resistance to the disease due to long-term adaptation to the local environment [56,57]. Previous studies have similarly reported a higher susceptibility of exotic and crossbred cattle to bovine tuberculosis, likely due to the lack of resistance developed in these breeds [16,40,41]. The body condition score was another significant risk factor, with cattle in poor condition showing a higher likelihood of bovine tuberculosis. This finding is consistent with the notion that poor nutritional status can impair immune function, thereby increasing susceptibility to infections [16,27,42].

The findings underscore the significant role of management systems in influencing the prevalence of bovine tuberculosis. The high prevalence of bovine tuberculosis in cattle managed intensively highlights potential risk factors associated with this management style, including higher density and potentially more frequent close contact among cattle, which could facilitate the spread of the disease [10,29]. The intensive management system likely creates conditions conducive to the transmission of bovine tuberculosis due to factors such as limited ventilation, high stocking density, and shared feeding and watering points, which are less prevalent in extensive systems. This aligns with findings from previous studies that have identified intensive systems as high-risk environments for bovine tuberculosis transmission due to factors such as higher animal density and increased pathogen exposure [7,16,17,27]. Similarly, the elevated risk observed in semi-intensive systems, while lower than in intensive systems, suggests that even partial confinement and restricted movement can increase bovine tuberculosis transmission risks compared to fully extensive systems [21].

The cross-sectional nature of the study captures data at a single point in time, which limits the ability to infer causality or track changes in bovine tuberculosis prevalence over time. Longitudinal studies would offer more insight into the dynamics of disease transmission and the impact of risk factors. Furthermore, the molecular techniques used were constrained by the available resources, which prevented the use of more advanced methods that could provide deeper insights into *M. bovis* isolation and molecular characterization. Despite these limitations, the study provides valuable insights into the epidemiology of bovine tuberculosis in Southwest Ethiopia and contributes to informing interventions aimed at mitigating the impact of this disease on dairy productivity and farmer livelihoods.

#### 5. Conclusion

This study provides a comprehensive assessment of the prevalence and risk factors associated with bovine tuberculosis in dairy cattle in Southwest Ethiopia. The findings reveal a higher prevalence of bovine tuberculosis at both the cow and herd levels. At the cow level, cattle older than six years and those in poor body condition are at greater risk, with crossbred and Jersey breeds showing increased susceptibility. The study also highlights that larger herds and intensive management systems are associated with a higher prevalence of bovine tuberculosis. Moreover, the introduction of new animals into herds significantly increases the risk of infection. At the herd level, the study emphasizes a higher prevalence of bovine tuberculosis in larger herds, with the introduction of new animals identified as a notable risk factor. The higher prevalence observed in lowland areas may be attributed to factors such as increased cattle density, limited veterinary services, or specific environmental conditions conducive to disease transmission. The occurrence of bovine tuberculosis in dairy cattle poses a significant threat to human health. To effectively control bovine tuberculosis in Ethiopia, it is essential to move beyond general intervention strategies and focus on four prioritized steps: 1) improving cattle management practices, particularly in lowland areas; 2) enhancing farmer education and awareness regarding bovine tuberculosis transmission; 3) implementing strict biosecurity measures to limit the introduction of new animals into herds; and 4) establishing robust monitoring and surveillance systems for early detection and control. These steps align with the One Health approach, which emphasizes the interconnectedness of animal, human, and environmental health, particularly given the zoonotic nature of bovine tuberculosis and its potential risk to human health. Future studies should focus on isolating and characterizing M. bovis in the study areas.

#### **Ethical statement**

The research adhered to the protocols and standards approved by the Animal Welfare and Research Ethics Committee of the Ethiopian Institute of Agricultural Research (reference number EIAR-2662/2010) and followed international guidelines for animal welfare. Furthermore, verbal consent was obtained from the cattle owners, ensuring their voluntary participation and cooperation, while safeguarding the confidentiality of their involvement.

## CRediT authorship contribution statement

Dereje Tulu Robi: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. Tsegaye Teklemariam: Writing – review & editing, Methodology, Investigation, Data curation. Belay Gezahegn Gebreyes: Writing – review & editing, Methodology, Investigation, Data curation. Ararsa Bogale: Writing – review & editing, Validation, Software, Data curation. Tamirat Haile: Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. Melkam Aleme: Writing – review & editing, Investigation, Data curation. Dawit Dejene: Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. Shiferaw Temteme: Writing – review & editing, Visualization, Supervision, Resources. Beksisa Urge: Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition.

## **Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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