



Prevalence and Factors Associated of Bovine Tuberculosis in North Shawa Oromia and Addis Ababa Dairy Farms, Ethiopia

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Keywords: *Mycobacterium bovis*; Tuberculin test; CIDT Test; Bovine tuberculosis; Cattle; Ethiopia.

Abbreviations: BTB: Bovine Tuberculosis; CIDT: Comparative Intradermal Tuberculin Test; PPD: Purified Protein Derivative; M. Bovis: Mycobacterium Bovis; TST: Tuberculin Skin Tests; SICCT: Single Intradermal Comparative Cervical Tuberculin; BT: Bovine Tuberculin; AT: Avian Tuberculin.

Abstract

The infectious chronic disease known as Bovine Tuberculosis (BTB) affects cattle and can spread to humans, domestic animals, and certain wild animals. In the dairy herds located in and around Addis Ababa, Bovine Tuberculosis (BTB) has emerged as a disease of economic significance. *Mycobacterium bovis* is the bacterium that causes its primary zoonotic disease. This disease is widespread in most of Africa, as well as in several regions of Asia and the Americas. Due to a lack of effective management strategies, BTB is a highly prevalent disease in Ethiopia and Africa. It results in large financial losses in animal production, especially in the dairy sector. Test-and-slaughter techniques are used in many developed countries to decrease or eradicate disease. However, because testing and control measures in Africa are limited by a lack of infrastructure and skilled manpower, comparative intradermal tuberculin skin tests are frequently used in developing nations like Ethiopia.

A cross-sectional study was carried out from June 2024 to December 2024, in a few areas of Ethiopia, including Oromia and Addis Ababa. This study's primary goal was to use a comparative intradermal tuberculin skin test on 617 dairy cattle from four dairy farms—Muka Turi, Sale, Shararo, and Nifas Silk—to estimate the prevalence and risk factors of bovine TB in cattle. Age, breed, body condition score, and Peasant association for the occurrence of BTB were among the various risk factors that were taken into consideration.

The dairy farm's animals were tested using the random sampling method. In dairy cattle from four dairy farms, the overall prevalence of bovine tuberculosis was 12.5% (77/617). 12.5 % of the total animals were positive, (6) were doubtful, and the remaining 86.5% (534) were negative.

In terms of bovine tuberculosis reactions with the associated risk, there were statistically significant differences ($P < 0.05$) between positive reactor animals by age ($\chi^2 = 7.9745$, $P = 0.01855$), body condition score ($\chi^2 = 8.0887$, $P = 0.01752$), breed ($\chi^2 = 241.545$, $P = 9.52e-10$), region ($\chi^2 = 281.5$, $P < 2.2e-16$), and farms ($\chi^2 = 284.05$, $P < 2.2e-16$). According to the study, bovine tuberculosis is very common. The disease's transmission and prevalence in cattle were influenced by related risk factors. Therefore, in order to lower the prevalence of tuberculosis in developing countries, control measures must be put in place.



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Introduction

Ethiopia's livestock economy is still severely hampered by infectious diseases, which cause between 30 and 50 % of all yearly losses. The infectious disease known as bovine tuberculosis, which is brought on by the *Mycobacterium bovis* (*M. bovis*), can affect cattle, other domesticated animals, and certain wildlife species that are either free-range or kept in captivity. A bovine with tuberculosis loses 10–25% of its productive efficiency; the infection's direct effects are manifested by a 10%–18% drop in milk production and a 15% reduction in meat production.

Acute respiratory distress and severe emaciation may occur in the last stages of tuberculosis. In addition to having an impact on animal production, it has a major impact on public health. Undercooked meat and tainted or unpasteurized raw milk are believed to be the primary ways that humans contract *M. bovis*. Ten to fifteen % of human TB cases worldwide are thought to be caused by *M. bovis*. This suggested that TB is endemic in Ethiopia, affecting both humans and animals [1]. *Mycobacterium bovis*, an acid-fast, gram-positive bacterium that belongs to the *Mycobacterium tuberculosis* complex of the *Mycobacteriaceae* family, is the causative agent of bovine tuberculosis (bTB). As a zoonotic disease, it continues to pose a threat to human health, livestock, and wildlife. Almost all mammals are susceptible to this illness, which can lead to coughing, general illness, and death. The nodules, known as "tubercles," that develop in the lymph nodes of afflicted animals are the source of the name TB [1].

It was one of the most common diseases affecting domestic animals worldwide until the 1920s, when developed countries started to implement control measures. The disease is widespread and highly prevalent in both domestic and wild animals in Africa. Although other domestic animals like pigs, cats, dogs, horses, and sheep are thought to be spill-over hosts, cattle are the primary host for *Mycobacterium bovis*, which is genetically related to and clinically identical to TB caused by *Mycobacterium tuberculosis* [2]. The role of *M. bovis* in human TB is well established in developed world, though it is not well known in developing countries [3]. In Africa to which 82% of human and 85% of animal population live in area where BTB is either partly or not controlled at all, it was a significant zoonotic human pathogen that aggravate the 'triple trouble' of HIV/AIDS, TB infection and malnutrition [4]. The primary risk factors for bTB transmission are close contact between animals or the sharing of food and water between infected and non-infected animals. BTB is primarily aerogenic. The international trade of animals and animal products, productivity losses in milk yields, meat production, and fertility, restrictions on the animal market, extensive control and eradication programs, and increased costs for human health are all ways that bTB outbreaks can have a significant negative economic impact on society [5]. History, clinical and necropsy findings, and a tuberculin test are frequently used to diagnose *M. bovis* infection in cattle. For MALDI TOF identification, culture and biochemical properties, and confirmatory diagnosis. Polymerase Chain Reaction (PCR) and DNA genome sequencing are used to further distinguish *M. bovis* from other members of the *M. tuberculosis* complex. A number of novel laboratory techniques, such as γ -interferon assays and lymphocyte proliferation, have been developed to help diagnose mycobacterium infections [6].

Bovine Tb has significant impact on international trade of livestock and animal products. The Tuberculin Skin Tests (TST), which are based on delayed hypersensitivity reactions, are now

the best methods for worldwide field diagnosis of bovine TB in live animals and World Organization for Animal Health (WOAH) recommended difference between the increases in skin thickness for the test after 72 hours [7]. In contrast to the Single Intradermal Tuberculin (SIT) test, which employs only BT, the Single Intradermal Comparative Cervical Tuberculin (SICCT) test involves the intradermal injection of Bovine Tuberculin (BT) and Avian Tuberculin (AT) at different places in the skin of the neck [8]. Early *M. bovis* infection in cattle can be successfully detected by TST, which also enables quick evacuation of sick animals, minimal transmission, and quick eradication of bovine TB, reduce the risk of Zoonosis [9].

The tuberculin skin test may demand physical exertion in the field but, it is also simple and relatively inexpensive and offers reliable means of screening cattle populations in an entire region [10]. The effectiveness of the TST may be impacted by ambient conditions, host characteristics state of immunity, genetics, and the type of tuberculin being utilized. Additionally, the sensitivity, specificity, and prevalence of the disease in the test population [11]. BTB is regarded as a significant animal disease burden in Ethiopia and other African nations. The main causes of BTB in developing nations include illiteracy, culture and customs, eating habits, living and socioeconomic status of families, close contact with animals, HIV/AIDS, and demographic factors. Although there are no national statistics on the prevalence of BTB in Ethiopia, it is thought that the expansion of the dairy industry is contributing to the disease's rise. A few studies have been carried out in Ethiopia using skin testing for tuberculin [12]. Therefore, establishing disease control and prevention at the national level will benefit from a nationwide study. Determining the prevalence and risk factors of BTB in a dairy farm in Addis Ababa and oromia north shawa Ethiopia is the aim of the current study.

Materials and methods

Study area

The study was carried out in four dairy farms chosen from Oromia Regional State and Addis Ababa. North Shawa (Shararo, Muka Turi, and Sale) are located in the Oromia region, and Addis Ababa (Nifas Silk) respectively. North Shawa is located 91 kilometers north of Ethiopia's capital, Addis Ababa. North Shewa shares borders with Addis Ababa to the south, West Shewa to the southwest, North Shewa (Amhara) to the north, and East Shewa to the southeast. 9° 29' 59.99" N latitude and 38° 39' 59.99" E longitude [13]. Nifas Silk sub city it shares borders with the districts of Akaky Kaliti, Kirkos and Bole, Lideta, and Kolfe Keranio. Longitude: 38° 43' 39" E; latitude: 8° 57' 41.76" N. The sub-city's total land area is 5876.02 hectares, and its landscape exhibits a notable elevation difference of 2074–2885 meters above sea level [14].

Study animals

The study was conducted on Holstein, and local cross breeds of kept in the intensive dairy farms.

Study design, Sample size and Sampling method

The cross-sectional study was design by using Random sampling method in order to test animals in the farms. The comparative intra dermal tuberculin test determines the factors associated and prevalence of BTB in the study area. In this study 617(543 and 74 cattle from oromia and Addis Ababa respectively) with different breed, sex, age and origin to be included.

The comparative intra dermal tuberculin test methods

Two sites were used, one for avian PPD (upper site) and the other for bovine PPD (lower site). The upper site was 10 cm below the crest and the lower site was 12.5 cm from the upper site, on a line drawn parallel with the line of the shoulder. The selected site was shaved and skin thickness was measured using a caliper before injection of the Purified Protein Derivative (PPD) and recorded as A1 for avian PPD site and B1 for bovine. Then 0.1 ml of Avian Tuberculin PPD and 0.1ml Bovine of Tuberculin PPD was injected intradermally in the upper and lower site, respectively. A correct injection was checked by developing papula (a small pea-like swelling) at each injection site. The two injection sites were remeasured after 72 hours by the same person who measured the skin thickness before the injection and recorded as A2 for avian PPD and B2 for bovine PPD. The same person should measure the skin fold thickness before and after tuberculin injection [14].

Interpretation of the reaction was considered to be positive if the difference in skin fold thickness at the bovine site of injection was 4 mm or more higher than the reaction shown at the site of the avian injection. When the difference in the skin fold thickness at the bovine site of inoculation was greater than 1 mm but lower than 4 mm, it was considered as doubtful, but if lower than or equal to 1 mm it was taken as negative. If the increase was observed at both sites of the injection the difference was considered between the two sites of reactions [7].

Data analysis

During the study, individual animal identification number, breeds, sex, age, data were entered MS Excel sheets. Then, coded and analyzed using SPSS version 20 statistical software. The associated factors with *M. bovis* infection were calculated by using Chi-square (χ^2) [15].

Results

Demographic features

The Comparative Intradermal Tuberculin Test (CIDT) in the current study showed that from a total of 617 cattle tested, 12.5% (77) were found to be positive, 1.0% (6) doubtful and the rest 86.5% (534) animals were negative for BTB test (Table 1).

Table 1: Study animals' overall parameter.

Characteristics	Frequency	Percent (%)
Age		
Adult	418	67.7
Young	199	32.3
BCS		
Good	421	68.2
Medium	196	31.8
Breed		
Holstein	612	99.2
Local	5	0.8
Result		
Doubtful	6	1.0
Negative	534	86.5
Positive	77	12.5

Risk factors associated with bovine tuberculosis

From the total of 617 cattle 99.2% (612) Holstein, 0.8% (5) local breed are tested for the presence of bovine tuberculosis by the comparative intradermal tuberculin test (Table 2). There were statistically significant differences ($P < 0.05$) in proportions of positive reactor animals among Age

($\chi^2 = 7.9745$, $P = 0.01855$), body condition score ($\chi^2 = 8.0887$, $P = 0.01752$) breed ($\chi^2 = 241.545$, $P = 9.52e-10$), Region ($\chi^2 = 281.5$, $P < 2.2e-16$) and farms ($\chi^2 = 284.05$, $P < 2.2e-16$) with the associated of bovine tuberculosis reactions.

Table 2: Summary of associated risk factors for Bovine tuberculosis analysis (n=617).

Variable	Category	No of Positive Animals	% of Positive	X-squared	P-value
Age	Young	14	7.03	7.9745	0.01855
	Adult	63	15.07		
BCS	Good	42	9.97	8.0887	0.01752
	Medium	35	17.85		
Region	Addis Ababa	53	71.62	281.5	<2.2e-16
	Oromia	24	4.41		
Farms	Muka Turi	12	4.95	284.05	<2.2e-16
	Nifas silk	53	71.62		
	Sale	7	5.03		
	Shararo	5	3.08		
Breed	Holstein	73	11.92	41.545	9.52e-10
	Local	4	80		

Discussion

Bovine TB caused by *Mycobacterium bovis* is considered one of the most important diseases facing farming industry, cattle owners, government, abattoir workers and veterinary profession in Ethiopia. In urban intensive dairy farms, BTB transmission high with prevalence [16]. In this study, we assessed cattle bTB prevalence in four intensive dairy farms and identified risk factors for bTB in cattle. The study was carried out in Muka Turi, Sale, Shararo and Nifas silk to diagnosis Bovine tuberculosis and its risk factors using Comparative Intradermal Tuberculin Test (CIDT). The overall prevalence of BTB in all four towns were 12.5% (77) in dairy farms. This finding is moderately in line with [17], who found 8.2% animal BTB prevalence from smallholder farms of Arba Minch Zuria and Chenchu districts of southern, Ethiopia. Another important finding was the reported of [16,18] who found 20.3% and 30% high Prevalence of Bovine Tuberculosis in Dairy Cattle in eastern and Central Ethiopia respectively. This variation may be brought by the various research designs, methods and production systems. Dairy cattle kept under intensive management are reportedly more likely to contract the disease than other cattle because of their closer confinement, longer life spans, and higher levels of productivity stress [19]. All bovine tuberculosis positive animals' sex were female due to large proportion female cattle in the study. Animal body conditions score analyses also 9.97% (42) good and 17.85% (35) medium respectively. The highest prevalence of tuberculin positive age reactivity was adult age animals 15.07% (63) and 7.03% (14) young. Also, this finding showed the age, body condition scores, breed and dairy farms as significant associated with bTB infection. The results of this study reveals in consisted with previous report of [5], bTB prevalence increased with the age of the

animals, probably because of the longer exposure to the agent over time of older animals[20]. In the present study, the most dominant positive prevalence of the region was 71.62% (53) in Addis Ababa and 4.41% (24) in north shao oromia region. In the present study, the most dominant positive prevalence dairy farms were record in 71.62% (53) Nifas silk, when compared with 4.95% (12) Muka Turi, 5.03% (7) Sale, 3.08% (5) Shararo respectively. Due to not test for long time from its establishments. The result of the current study revealed that, there is statistically significant associations ($P < 0.05$), between the tuberculin test positivity and all associated risk factors.

Conclusions

BTB causes impact on animal and human health particularly in developing countries. The absence of a gold standard single test to detect all cases of bTB, the absence of a realistic vaccine against the disease, and the zoonotic impact are the main challenges. The prevalence reported in the current study only using CIDT test revealed that BTB is present and is well-established in dairy farms especially in Addis Ababa very alarming results. The farm owners and community have the habit of consuming raw meat and milk and share the same microenvironment with their livestock. This further disseminates the causative agent, both through inhalation and ingestion resulting in high economic loss and public health effect.

Bovine tuberculosis can be controlled by test-and-slaughter or test-and-segregation methods. Affected herds are re-tested periodically to eliminate cattle that may shed the organism; the tuberculin test is generally used. Infected herds are usually quarantined, and animals that have been in contact with reactors are traced. Only test-and-slaughter techniques are guaranteed to eradicate tuberculosis from domesticated animals. However, some countries use test- and-segregation programs during the early stages of eradication, and switch to test-and-slaughter methods in the final stage. Once eradication is nearly complete, slaughter surveillance, with tracing of infected animals, may be a more efficient use of resources.

Recommendation

Introduction of new animal should be tested before mixing to the herd. After test isolation of animals in to category of positive, doubt full and negative in separate room. Hygiene and avoidance of contamination should be implemented to prevent herd infection. Awareness creation on zoonotic importance of BTB to should be given on safety precaution for animal's attendant and milker, Pasteurized or Boiling milk before drinking. The relevance of screening test for BTB before purchasing the animals and after. Moreover, further detailed agent characterization and public awareness should be done to investigate bovine tuberculosis in the nationwide in order to design appropriate strategic prevention and control measures.

Author declarations

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Conflict of interest: There is no conflict of interest regarding the publication of this manuscript.

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