Original Research Article

The incidence of bovine tuberculosis in a dairy herd practicing irregular skin test and slaughter control program

Abstract

The combination of intensified animal husbandry and development of peri-urban systems have corresponded with increased bovine tuberculosis incidence. Its economic impact is primarily driven by direct effects, particularly due to test and culling of affected animals. A retrospective study was conducted to assess the incidence of bTB in a dairy herd practicing repeated irregular skin testing and slaughter control program. The incidence at the subsequent test rounds ranged from 5.4% to 24.8%. These incidences exhibited an oscillating pattern: it initially decreased from 21.3% to 8.4%, then resurged to 24.8% in the third round, and ultimately declined back to 5.4%. Penultimate test result, breed of the animal, and herd composition were significantly associated with the odds of them becoming a reactor to the SICCTT at a subsequent test (P<0.05). The study findings indicated that animals undergoing two consecutive repeated skin tests had an approximately 11 times higher risk of bovine tuberculosis (bTB) infection compared to newly introduced animals. Similarly, animals with an inconclusive penultimate test result were 2.64 times more likely to be infected with bTB than those with a negative penultimate test result. Likewise, reactors that had been embedded by inconclusive penultimate SICCTT result were more likely to have visible lesions at slaughter than those with a negative penultimate SICCTT result. The study herd consisted of a mix of purebred Boran and different crossbred animals. The prevalence of bTB was high in purebred Boran than crossbred animals. In conclusion, the study confirmed the necessity of considering inconclusive SICCTT test results

and the retested herd (with inconsistent and extremely prolonged retesting schemes) contribution, which all were likely responsible for the chance to increase the number of new bTB cases.

Key word: Bovine tuberculosis; Herd, Incidence; Inconclusive; Reactor

Introduction

Ethiopia has one of the largest livestock inventories in Africa (Tadesse and Yilman, 2018). The prevailing approach of relying on indigenous cattle and extensive rural production may not adequately satisfy the increasing domestic demand for animal products. As a result, there is a growing trend toward intensification of livestock farming (Marcotty et al., 2009; Vordermeier et al., 2012). An intensive dairy sector is emerging in Ethiopia to respond to an increasing demand for milk and milk products under rapid population growth and high rate of urbanization (Demissie *et al.*, 2014). Unfortunately, these combinations of intensified animal husbandry and the development of peri-urban systems for livestock production have corresponded with increased incidence of bovine tuberculosis (bTB) (Vordermeier et al., 2012). In low-income countries where resources are scarce, the consequences of bovine tuberculosis (bTB) are especially severe. These nations often lack the financial means to effectively execute expensive test-and-slaughter approaches (Bemrew et al., 2015; Moore, 2018).

Bovine tuberculosis (bTB) is a serious animal health problem in Ethiopia, ranking among the top three livestock diseases (Lakew *et al.*, 2022). Its economic impact is primarily driven by direct effects, particularly due to test and culling of affected animals. The existence of potential associated factors conducive to the spreading and persistence of bTB would make the situation worst (Girmay *et al.*, 2012; Regassa *et al.*, 2008).

According to the World Health Organization (WHO), there is no single intervention that can effectively control bTB on its own, unless it is able to block all routes of transmission of the disease (WHO, 2023). Conventionally, a common approach for bovine tuberculosis control in domesticated animals includes conducting regular field tests and culling of infected herds. This prevents disease spreading beyond the herd, while slaughter of diseased animals removes the infection from the herd. However, this method is difficult to enforce in most low-income countries because the value of cattle is deeply interwoven into the fabric of socio-cultural systems and plays a crucial role in the financial well-being of rural communities (Adeyemo and Silas, 2020). Valuable breeding stock may easily be lost through slaughter, as well. This can be of extensive socio-economic significance in non-industrial nations where replacement of equivalent breeding stock might be excessively unaffordable (McCrindle and Michel, 2007). The cost associated with these values go well beyond the economic losses resulting from testing and culling (Caminiti *et al.*, 2016). For these reasons, there has been no concerted effort to control the disease at the national level to date in Ethiopia, and the disease remains endemic. Despite largely remain uncontrolled, a few attempts have been undertaken to control bovine tuberculosis at the farm level in Ethiopia.

The single intradermal comparative cervical tuberculin test (SICCTT) is the most common ante-mortem bTB diagnostic test and has been implemented for decades. Another test which targets quantifying the amount of the most stable cytokine (INF γ) produced in response to bTB antigen exposure has more recently come into use. Unluckily, they have limitations in terms of sensitivity and specificity, which means some infected animals are missed from culling or some non-infected animals are culled needlessly (De la Rua-Domenech *et al.*, 2006; Lahuerta-Marin *et al.*, 2018, 2016; Nunez-Garcia *et al.*, 2018). The missed infected animals may be infectious, and thus play great role in the transmission of the disease; the available ante mortem tests are not able to distinguish these animals.

Post-slaughter diagnosis of bTB mainly targets the protection of the community from the disease through carcass condemnation. However, slaughterhouse surveillance may be able to predicting the extent of the disease if not estimate the true prevalence (Biffa *et al.*, 2010). The diagnostic methods that are used to investigate the disease further are dependent on several factors. For instance, some infected animals may have tiny lesions that remain undetected during postmortem examination. Subsequent laboratory examination is highly dependent on the tissue selected for further examination; if lesions are not easily visible, tissue that is diseased may not be selected, significantly reducing the chances of finding the bacterium. The *M. bovis* also grows very slowly, which makes culture difficult and slow to provide results. Given all of these difficulties, receiving a negative result at post mortem or the laboratory tests does not mean that the animal did not have bTB.

Holetta dairy farm had been practicing a test-and-slaughter measure for the previous years to control bovine tuberculosis infection from its herd. The effectiveness of the applied control measure is evaluated by trajectories of subsequent incidences. Estimate of bTB incidence is essential to attain a comprehensive and accurate insight into the subject (bTB incidence reduction trend in subsequent skin testing) which could help the decision makers and researchers to identify any barriers or limitations for the incompetence of the strategy. With this understanding, therefore, the study was conducted to assess the incidence of bTB, and identify some determinants associated with the odds of becoming a reactor in a dairy herd practicing repeated irregular skin testing and slaughter control program.

Materials and methods

Study area

The study was conducted at Holetta Agricultural Research Center (HARC) with altitude: 2400masl; annual rainfall: 1100mm; average temperature minimum: 6°C, maximum: 24°C) located at 35 km west of Addis Ababa, Ethiopia (3°24'N to 14°53'N and 33°00'E to 48°00'E). The study area experiences the wet (June to September) and the dry (October to May) seasons. HARC's dairy farm was established in 1977 and contributes significantly to research and development efforts aimed at improving dairy cattle productivity in Ethiopia. The farm contains a breeding unit, an animal health unit, a feed production unit, and an animal husbandry unit with two sub-units: herd management and milk production.

Animals and management

Boran cows were inseminated using WWS (worldwide sire) Friesian semen to produce 50% F1 crossbred calves, which (50% F1) were further crossed with pure Friesian semen to produce the 75%F1. Besides herdsmen, a teaser bull was reared with cows for heat detection. Pregnancy diagnosis was conducted 60 days after service (Artificial insemination). Animals are left to grazing from early morning 8.00 AM to 4.00 PM in the afternoon and are fed with natural pasture hay as required at night. Concentrate feed composed of wheat bran (32%), wheat middling (32%), noug cake (34%), and salt (2%) was supplemented based on their physiological states. Milking cows received the concentrate feed at a rate of 0.250 kg for every kg of milk they produced daily during milking periods. All cows had unrestricted access to fresh drinking water. Calves were allowed to suckle their dam immediately after birth for about four days to receive colostrum. Weighing and ear tagging were completed within 24 hours after birth. After 4 days, calves were moved to calf rearing pens and fed whole milk for 98 days through an artificial rearing system (bucket feeding) except the 50% F1 calves, which suckled their dams until weaning. Weaned calves were then transferred to a group pen and kept indoors until 6 months of age. Milking was conducted by milking machine twice daily (early morning and evening).All animals were vaccinated against Anthrax, Bovine Contagious pleura pneumonia (BCPP), foot and mouth disease (FMD), and lumpy skin disease (LSD) as outlined by the manufacturer, National veterinary institute (NVI), vaccination programs. The herd was de-wormed orally prior to vaccination as prescribed by the manufacturer of the de-worming product. Multi-vitamins were also administered in drinking water as supplements after veterinary interventions.

Study design

A retrospective study was conducted to assess the incidence of bovine tuberculosis across different skin test rounds. In each testing round, all animals in the herd (except thesuckler/rearing calves and replacement heifer calves $\leq$ 6 months of age) were tested using SICCTT. Cows were classified as reactors, doubtful/inconclusive, and negative. Reactors were slaughtered immediately and not involved in the subsequent retests. There were a total of four testing rounds on the herd in which all reactors were removed. The time interval between consecutive test rounds varied from 0.95 years to 1.84 years. In this study, an animal entry is defined as the number of new animals entering into the study herd and receiving its first SICCTT test at a given screening test round. Self sourced replacement heifers and outsource Boran heifers as damline are the main entry animals. However, retested animals were engaged in two or more previous skin testing.

Single intradermal comparative cervical tuberculin test

The SICCTT was used as described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals(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 (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 reactor is defined as an animal in which the relative increase in skin thickness at the injection site for PPD-B is at least 4 mm greater than the increase in skin thickness at the injection site for PDD-A. A negative result is defined as a difference of the skin thickness at the injection sites that does not exceed 2 mm. An inconclusive result is defined as a difference in skin thickness between 2 mm and 4 mm.

Post mortem examination

Reactors were slaughtered and subjected to post mortem examination in order to inspect whether visible lesions was detected or not. Each lobe of the lung was sliced into about 2cm-thick slices so as to facilitate the detection of lesions. Similarly, five lymph nodes namely, mandibular (right and left), medial retropharyngeal, bronchial (left and right), mediastinal (caudal and cranial), and mesenteric from each of the cows were sliced into thin sections and inspected for the presence of visible lesions.

Data analysis

Data were entered into an excel worksheet. Data management and analyses were carried out using MS Excel and Stata 14 (STATA Corp. Ltd). Descriptive statistics were generated for each variable of interest. The percentages and their 95% confidence intervals (95% CI) were calculated to determine the prevalence and incidence of the disease. Differences in proportions were evaluated by Pearson's Chi-square test. Unconditional associations between two binary or categorical variables (nominal or ordinal) were assessed by the odd ratio. For multivariable analysis, logistic regression model was used. In the final model, only the significant variables ($p < 0.05$) were included.

Results

Herd composition based on previous skin testing

The study had completed four screening tests rounds to control bovine tuberculosis infection. During the respective skin test round, the herd was subdivided into two groups: new entries and retested animals. 74 – 90 % of animals at a specific test round were retested, of which large percentage (90%) of retested animals were identified at the third test round. As illustrated in the figure 1, 47**%** (234 out of 498) of the animals participated in all test rounds. Additionally, during the second, third, and fourth test rounds, 25%**,** 10%, and 26% of the herd animals, respectively, were new entry animals. These new entries had no prior history of tuberculin testing.

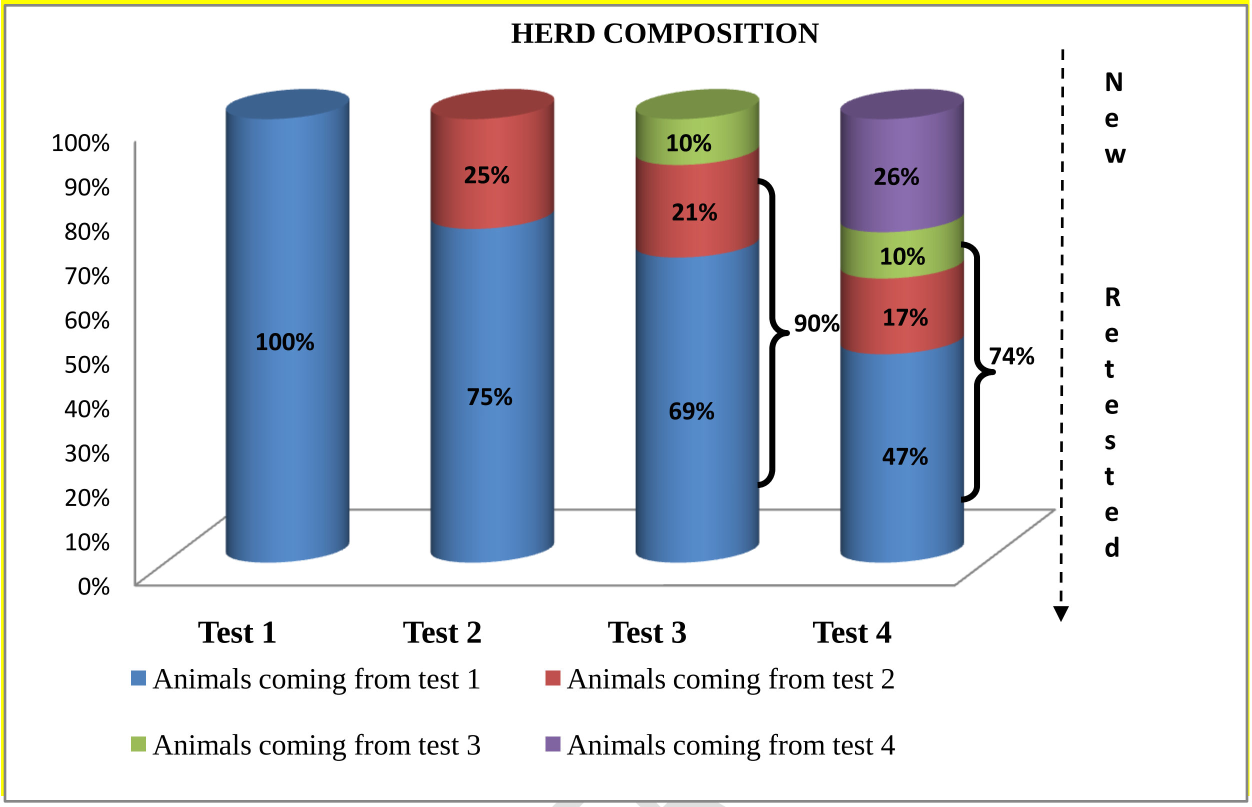

Figure 1 Profile of study herd composition at subsequent test rounds

Herd incidence

The result of the bTB screening test is summarized in Table 1. Bovine tuberculosis prevalence in the dairy farm when the study began was 21.3% (107/502). Incidence of bTB at the subsequent test rounds ranged from 5.4-24.8%. Comparing test rounds, higher incidence was recorded in test round 3, while lower incidence was obtained in test round 4. In terms of herd composition, a higher proportion of retested animals tested positive (reactors) compared to the new entry animals (Table 2).

Table 1 bTB test results in a dairy herd with repeated herd test-and-removal

Tests	**n**	**Negative %**	**Reactors%**	**Doubtful%**
Test-1	502	72.5	21.3	6.2

Test-2	521	21.9	8.4	6.9
Test-3	503	10.14	24.8	3.4
Test-4	498	24.9	5.4	4.4

Table 2 bTB test results based on herd compositions

Tests	Herd composition	n	Reactors% (n)	Doubtful% (n)
Test-1	New entry	502	21.3	6.2
	Retested	-	-	-
Test-2	New entry	129	4.65	6.98
	Retested	392	9.69	6.89
Test-3	New entry	52	0	1.92
	Retested	451	27.72	3.55
Test-4	New entry	127	0	2.36
	Retested	371	7.28	5.12

The study herd consisted of animals with negative, doubtful, and no (new entry) penultimate test results. Table 3 provides an overview of the skin test results obtained from the four test rounds based on their penultimate test results. At a specific test round, animals with doubtful penultimate test result had significantly higher incidence than those animals with negative penultimate test result. 27.6 % (8/29), 34.3% (12/35) and 40% (6/15) of animals with doubtful penultimate test results were reactors in test round 2, 3 and 4, respectively. In contrast, 8.3%, 27.2% and 5.9% of retested animals embedded by negative penultimate test result were identified as reactors in the perspective test rounds.

Table 3 Skin test results based on their penultimate test results

Tests (N)	Penultimate test result	n	Reactors% (n)	Doubtful% (n)
Test-1	No test	502	21.3	6.2
Test-2	No test	129	4.7	7.0
	Negative	363	8.3	6.9

	Doubtful	29	27.6	6.9
Test-3	No test	52	0	1.9
	Negative	416	27.2	3.6
	Doubtful	35	34.3	2.9
Test-4	No test	127	0	2.4
	Negative	356	5.9	5.1
	Doubtful	15	40%	6.7

Results of multivariate logistic regression analysis are shown in (Table 4). The analysis revealed that the chance of bTB infection in animals participating with two consecutive repeated skin testing was almost 11 times higher than new entry animals. Similarly animals with doubtful penultimate test result had 2.64 times higher chance of bovine tuberculosis infection comparing with animals having negative penultimate test result. The study herd consisted of a mix of purebred Boran and different crossbred animals. The prevalence of bTB was high in purebred Boran than crossbred animals.

Table 4 Analysis of selected determinants having association with bovine tuberculosis infections using multivariate logistic regression

Variables	P-value	OR	95% CI	
			Lower	Upper
Animals having one time repeated skin testing compare to new entry animals	0.001	4.28	1.805	10.143
Animals having two time repeated skin testing compare to new entry animals	0.000	11.31	4.847	26.407
Animals having three time repeated skin testing compare to new entry animals	0.002	4.29	1.712	10.739
Animals with doubtful penultimate test result compare to negative penultimate test	0.000	2.64	1.572	4.416
Boran animals compare to 75% crossbred animals	0.000	3.94	1.942	7.987

50% crossbred animals compare to 75% crossbred animals	0.001	2.82	1.552	5.108

Visible bovine tuberculosis lesion detection

We detected a total of 122 tuberculosis lesions from 195 reactor cattle which were presented for postmortem examination at slaughter (Table 5). The detection rate averaged one visible lesion per 1.6 reactors, although this varied by penultimate test result and herd testing round. A higher percentage of visible lesions were found in reactors with inconclusive penultimate tuberculin test results.

Table 5 Visible lesion detection rate per reactors based on their penultimate test result

Test no	Number of slaughtered animals (N)	Slaughtered animals		Visible Lesion detected	
		Penultimate test result	Frequency	n	Detection rate
Test-1	71	Baseline	71	47	0.662
Test-2	43	Negative	29	13	0.448
		Inconclusive	8	7	0.875
		New/ no retested	6	4	0.667
Test-3	54	Negative	42	28	0.667
		Inconclusive	12	10	0.833
Test-4	27	Negative	21	11	0.524
		Inconclusive	6	2	0.333

Discussion

Conventionally, SICCTT are recommended as a diagnostic tool for reactor detection. A typical strategy for disease control in domestic animals involves regular field tests and quarantine of infected herds. This prevents disease spread beyond the herd, while slaughter of diseased animals removes the infection from the herd. In this study, incidence of bTB at the subsequent test rounds ranged from 5.4-24.8%. High incidence was recorded in the third round test, although relatively low incidence was identified in the fourth round skin testing. Previous trials in Ethiopia found that test and slaughter as practiced by a few dairy farms showed an apparent improvement in incidence (Ameni *et al.*, 2007; Shitaye *et al.*, 2007). Ameni *et al*. (2007) reported a pronounced incidence reduction after three successive rounds of skin testing. 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.

Failures of incidence reduction in the current study might be due to excessively prolonged and inconsistent tuberculin test time intervals for repeated herd retesting. The previous study by Ameni *et al.* (2007) reported that application of three consecutive tests every four months after the first test enabled earlier infection detection and culling, reducing the incidence from 14% to 1% within a year. Similarly, USDA protocol requires the entire herd to have eight consecutive negative whole herd test (WHT), performing the first four tests at intervals of at least 60 days, at least 180 days between the fourth and fifth tests, and at least 12 months for 3 consecutive tests between fifth and eighth tests, to release quarantine and eliminate bTB (USDA-APHIS, 2005). In the study reported here, three subsequent tests were utilized within four years after the first test, and the time interval between successive SICCTT tests varied from 0.95 years to1.84 years. This prolonged inter-test interval would have allowed for continued transmission within the herd.

Domestic cattle are the most susceptible and represent the main animal reservoirs (Brosch *et al.*, 2002); albeit, possible differences in susceptibility between different cattle subspecies have been hypothesized (Rodríguez-Campos *et al.*, 2014). Different studies showed that Boran and Holstein cattle have differences in their relative susceptibilities to bTB.

In the current study, the prevalence was significantly higher in purebred Boren (Bos indicus) than in high-grade Holstein cattle (B. taurus taurus) kept under the same husbandry conditions. The most likely explanation for this variation among local breed Boran and high-grade Holstein cattle maintained under identical conditions could be due to 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 (95% CI: 1.0–4.5) and 2.5 times (95% CI: 1.1–5.4) higher in cattle aged >3–6 years and > 6 years, compared to cattle aged ≤1 year. In contrary, Ameni *et al.* (2006)reported that local *B. indicus* breeds had lower skin test prevalence (5.6%) compared to 86.4% in mainly Holsteins exotic breeds, with 13.9% prevalence in crosses. Similarly, Carmichael (1939) reported that the incidence of bTB was dramatically lower in Zebu cattle compared to taurine Ankole cattle, indicating that Zebu calves showed remarkable resistance compared to Ankole calves.

The study measured the association between inconclusive status of animals and the odds of them becoming a reactor to the SICCTT at a subsequent test. In our findings, the inconclusive penultimate SICCTT result was importantly associated with the odds of them becoming a reactor to the SICCTT at a subsequent test, as also had been demonstrated in England and Wales (May *et al.*, 2019). This was in agreement with previous study (May *et al.*, 2019), reporting inconclusive animals more likely to become reactor animals than animals which tested negative.

Apart from this, reactors that had a doubtful or inconclusive result in the previous SICCTT were more likely to have visible lesions at slaughter than those with a negative penultimate SICCTT test result. Similar results in Irish cattle evidenced our findings through a greater proportion of animals with inconclusive penultimate test result and tested as bTB-positive at subsequent slaughtered compared to animals with negative penultimate test results (Clegg *et al.*, 2011a, 2011b). Furthermore, Byrne *et al.* (2017) in Northern Ireland stated animals with an inconclusive penultimate skin test result having an elevated adjusted OR of 2.84–3.89 ($p < 0.001$) for the presence of bTB lesions at slaughter. These animals may actually have been false negative or doubtful result at the penultimate test, as the skin test is imperfect (Whipple *et al.*, 1996). The delay in detection would give the animal a longer time to develop visible lesions after infection. This is in line with a previous study (Rodgers et al., 2007), which found that increasing time from infection to slaughter resulted in more extensive pathology on post-mortem examination.

Conclusions

In conclusion, the current study found that incidence of bTB at the subsequent test rounds ranged from 5.4-24.8%. The incidences exhibited an oscillating pattern, initially declined from 21.3% to 8.4%, resurged to 24.8% in the third round, and finally decreased to 5.4%. In our findings, the inconclusive penultimate SICCTT result was significantly associated with the odds of them becoming a reactor to the SICCTT at a subsequent test. Likewise, these reactors that had been embedded by inconclusive penultimate SICCTT result were more likely to have visible lesions at slaughter than those with a negative penultimate SICCTT result. Besides, the prevalence was higher in purebred Boren than in high-grade Holstein cattle. The most likely explanation for this variation could be due to 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.

List of abbreviations

BCG: Bacillus Calmette and Guérin;

BO X F: Boran versus Friesian cross;

bTB: Bovine tuberculosis;

SICCTT: Single intradermal comparative cervical tuberculin test;

FAO: Food and Agriculture Organization of the United Nations;

OIE: Office International des Epizooties,

PPDs: Purified Protein Derivatives

Availability of data and materials

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

Consent for publication

Not applicable

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