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

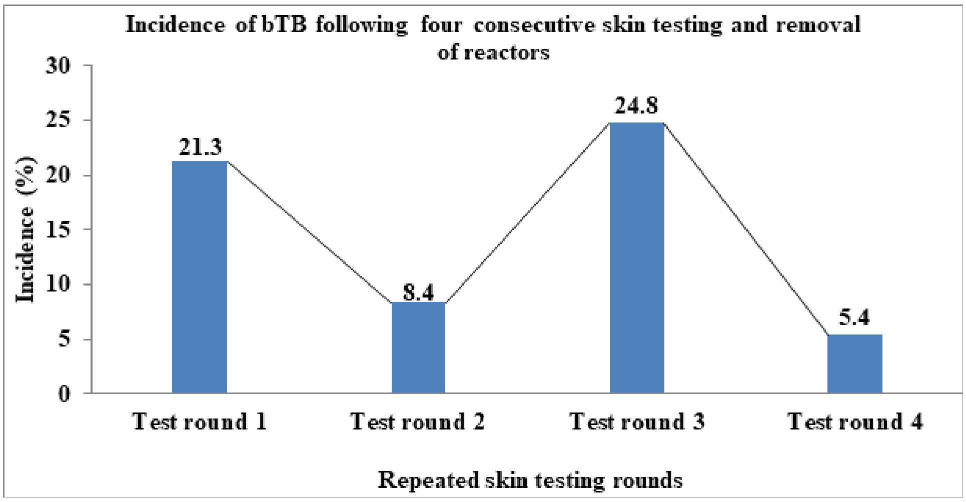

Figure 1. Trajectories showing the effect of irregular skin testing and slaughter on the prevalence of bTB in the study herd.

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 primarily 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.

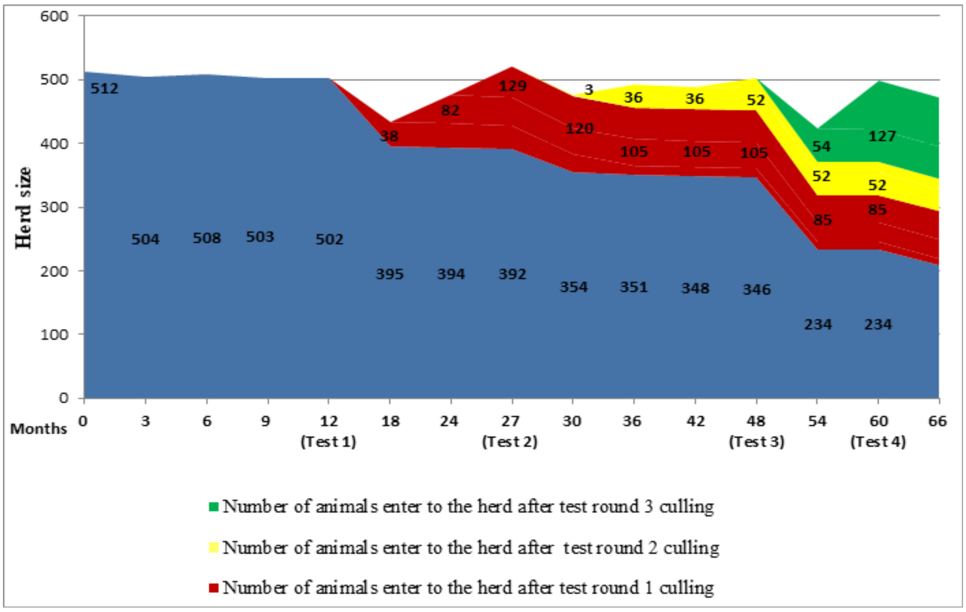

Figure 2. Chart illustrating livestock entries and exits in successive screening tests.

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

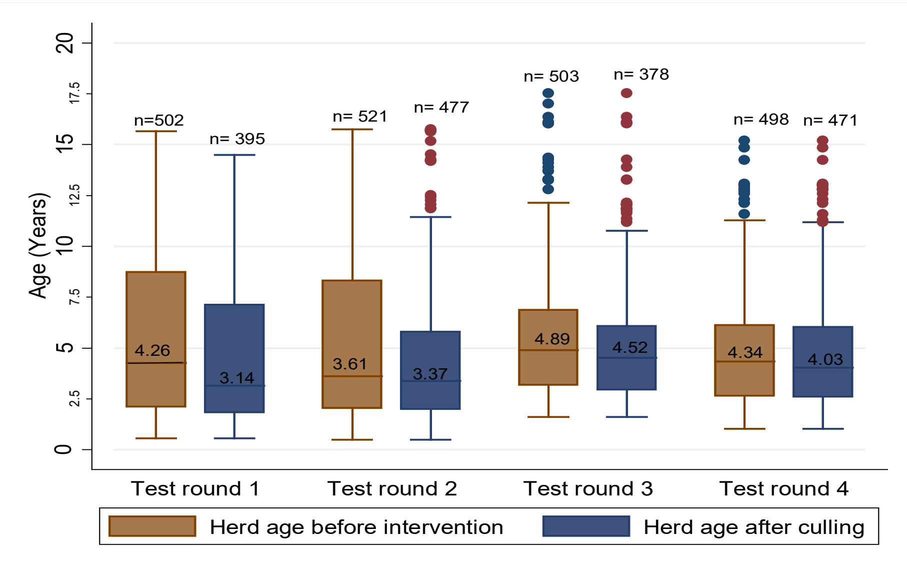

Figure 3. The median age of the study herd before and after test and slaughter control measure.

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.

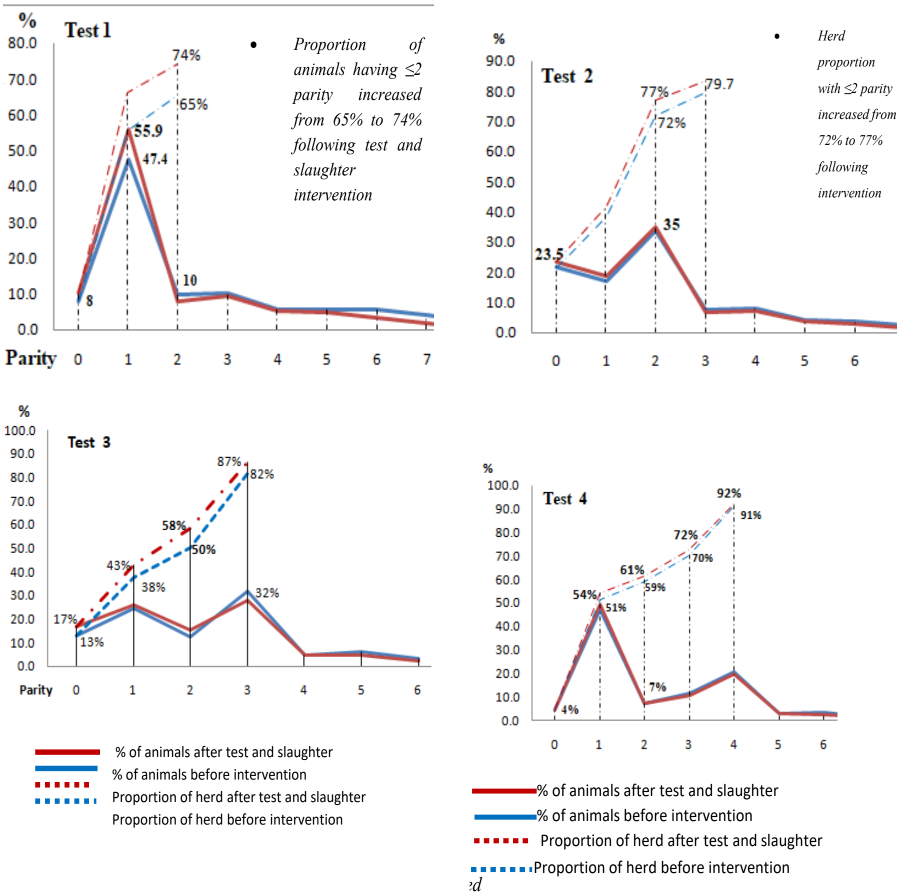

Figure 4. The proportion of herd parities before and after test and slaughter control measure.

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).

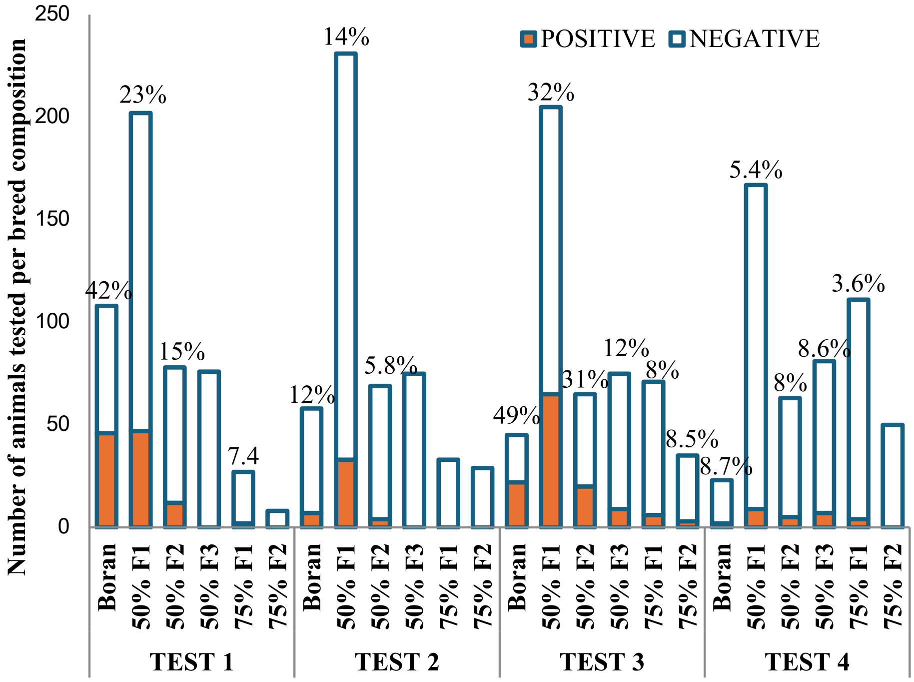

Figure 5. bTB prevalence over breed of the herd along successive screening test rounds.

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 greater 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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