Original Research Article

Herd level bovine tuberculosis incidence, and assessingtheimpact of test and slaughter measures on herd demography and structure

Abstract

Intensified dairy sectors are emerging in response to rapidly growing human population and high rate of urbanization. The combination of intensified animal husbandry and development of peri-urban systems have corresponded with increased bovine tuberculosis incidence. Bovine tuberculosis has a significant economic impact, primarily a considerable direct effect due to culling and high cost of investment for eradication. The study assessed disease incidence,persistence, and herd structures in view of bTB incidence. It also evaluated the impact of test and slaughter program on prospective incidences and herd demography. Based on subsequent SICCTT, the study animals were classified as reactors (culled and not involved in the subsequent retests), doubtful, and negative reaction. The incidence ranged from 5.4% to 24.8%. This variation associated with herd composition, breed, and penultimate test result. New-entry and retested animals with negative penultimate test result experienced lower incidence than retested with inconclusive penultimate result. Binomial logistic regression analysis revealed that animals with doubtful penultimate test result had 2.91 times higher prevalence comparing with animals having negative penultimate test result. The prevalence was significantly higher in purebred Boran than crossbred animals in a specific screening test round. Using univariable analysis, the prevalence of bTB infection in local breed (Boran) and 50% Boran-Friesian cross was about 10.5 and 4.1 times higher comparing with the prevalence of high-grade animals (75%

Friesian-Boran), respectively.Herd structure and dynamics were important, with significant differences in average herd age and breed distribution among subsequent tests rounds. These findings suggested the necessity of considering inconclusive SICCTT test results and the inconsistent and extremely prolonged retest-and-slaughter schemes, which all were likely responsible for the failures to reduce the number of new bTB cases. Generally, results in this study confirmed the ineffectiveness of the current approach, as applied, to control bTB from the study herd.

Key word: Bovine tuberculosis;Disease control;Incidence;Reactor;Test-and-slaughter

Introduction

Ethiopia has one of the largest livestock inventories in Africa(Tadesse and Yilman, 2018). The predominant system of indigenous cattle and extensive rural production are likely insufficient to meet the rising domestic demand for animal products; consequently, intensification of livestock farming is growing(Cosivi *et al.,* 1998). An intensive dairy sector is emerging in Ethiopia to respondto anincreasing demand for milk and milk products under rapid population growth and high rate of urbanization(Demissie *et al.*, 2014). Unfortunately, the combination 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).

Although still presentin some industrialized countries with a well-developed veterinary control systems, nowadays the worst impact of bTBis in low-income countries lacking the resources to apply expensive test-and-slaughter strategies (Bemrew *et al.*, 2015; Humblet *et al.,* 2009; Kaneene *et al.*, 2002). In Ethiopia, there is sufficient evidence to indicate the existence of potential associated risk factors conducive for the spreading and persistence of bTB(Ayele*et al.*, 2004; Girmay *et al.*, 2012; Regassa *et al.*, 2008).

Bovine tuberculosis is incurable in cattle. A typical strategy for bTB control in domesticated animals involves regular field tests and quarantine of infected herds. This prevents disease spreading beyond the herd, while slaughter of diseased animals removes the infection from the herd(Kao *et al.,* 1997). However, this method is difficult to enforce in most low-income countries because the value of cattle is deeply interwoven with the socio-cultural system and the savings of the rural poor (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 replacement of equivalent breeding stock might be excessively unaffordable (McCrindle and Michel, 2007). All of these costs are abovethe direct costs of testing and culling, which can be significant(Caminiti *et al.,* 2016). As a result, the disease remainsendemic, and there has been no concerted effort to control the disease at the national level to date in Ethiopia. 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 bTBdiagnostic 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 methods that are used to investigate the disease further are dependent on several factors. Some infected animals may have very small disease lesions that may not be seen on postmortem examination in the abattoir. 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*bacterium 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(Geronimo the alpaca, 2021).

Despite the limitations listed above, the study was conducted on a dairy farm,whichhad been conducting a test-and-slaughter measureto control and eliminate bTB. The study purpose was to assess disease incidence, persistence, and the impact of test and slaughter on prospective incidences and herd demography. The goal was to estimate the efficiency of test-and-slaughter measure taken and identify any barriers or limitations for successful bTB control and prevention.

Materials and methods

Farm description

The dairy farm in this study was established for genetic improvement by an 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: herd management and milk production.

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

The cattle were grouped based on breed, pregnancy, lactation stage, and age. Uniform feeding and management practices were adopted for all animals within each group. Natural grazing, hay, and concentrate supplement constituted the major feed supply. Concentrate feed was supplemented based on body weight, productivity and physiological categories. Milking cows, heifers and calves were 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 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).

In the animal health unit, there were two interrelated tasks: veterinary clinic (animal health services) and research. The unit comprises of veterinarians, assistant veterinarians, laboratory technicians, and animal health researchers, and the building was partitioned as a treatment room, laboratory, slaughtering and postmortem examination room, quarantine pens, and pharmaceutical and utility stores. Any information on livestock diseases and mortality as presented to and diagnosed at the clinic was recorded and available for analysis. Besides regular monitoring, sick animals were diagnosed and treated within the clinic. Biological specimens (fecal, blood, swabs, and tissues) were collected from both clinically sick and apparent healthy animals to perform microbiological, serological, pathological, and parasitological laboratory procedures. All animals were vaccinated against Anthrax, Bovine Contagious pleura pneumonia (BCPP), foot and mouth disease (FMD), and lump skin disease (LSD) as outlined by the manufacturer, National veterinary institute (NVI), vaccination programs. The herd was dewormed orally prior to vaccination as prescribed by the manufacturer of the deworming product. Multi-vitamins were also administered in drinking water as supplements after veterinary interventions.

Study design

A retrospective cohort study was conducted to generate comparison information on bTB incidents following four tuberculin skin-test rounds and removal of the positive reactors from the herd. 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 culled 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, the definitions of animal entries and animals exits were defined based on context to explain the herd demograghy and dynamics. An animal entry is defined as the number of new animals entering into the study herd and recieving 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. Exit is defined as animals which had been tested by SICCTT in penultimate test round but lostfrom the study herd at a given screening test round. Animals might exit from the herd either due to culling or death.

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, 2007). 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.

Results

Herd incidence

The dairy farm conducted four rounds of SICCTT tests and removal of reactors during the monitoring period, irrespective of laboratory and molecular confirmation (Figure 1). The study assessed herd structures and disease dynamics in view of bTB incidence and persistence. During the monitoring period, there were 810 animals with complete data, of which 342 cows were culled, and 468 were still present when monitoring ceased.

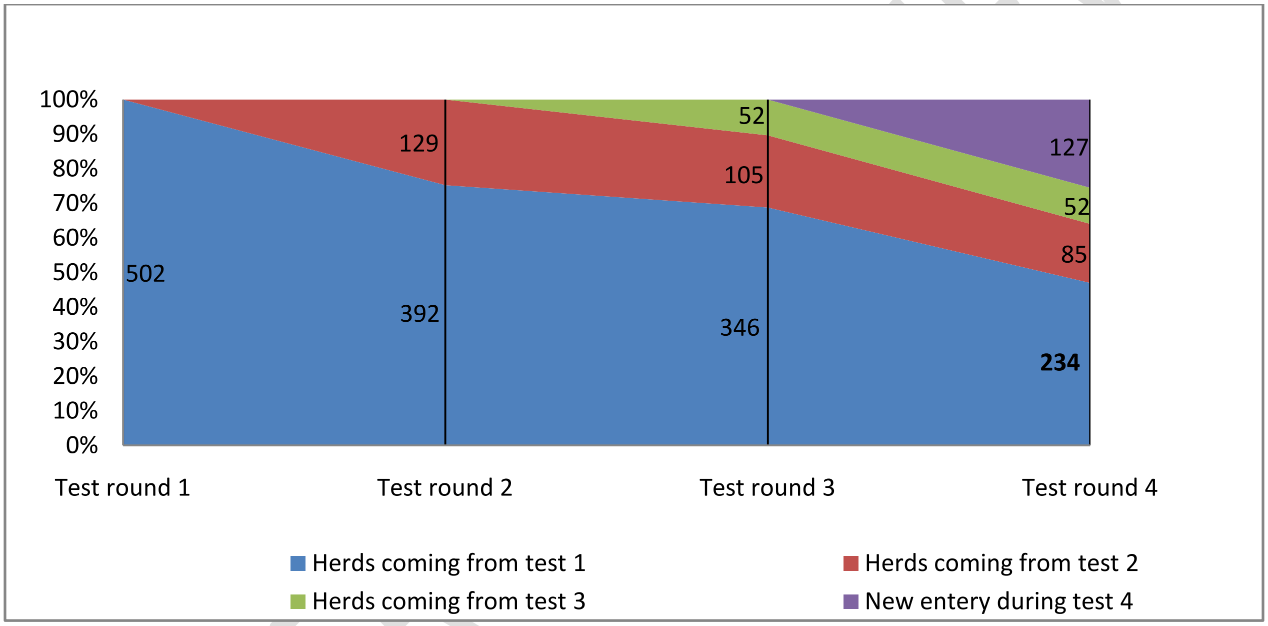

Figure 1Chart illustrating of livestock entries and exits in successive screening tests.

Overall, these 810 dairy animals were tested in one or more SICCTT test rounds, yielding a total of 2024 screening test assessments. As a result, 15% (303/2024) of tests were classified as positive. Aggregate results of the bTB screening tests are described in Table 1. Prevalence of bTB based on the screening test round ranged from 5.4-24.8%. Comparing test rounds, higher prevalence was recorded in test round 3, while lower prevalence was obtained in test round 4.

Table 1bTB test results characterized by herd composition and previous tuberculin test results in a dairy herd with repeated whole-herd test-and-removal

Tests (N)	**Herd composition**	**Penultimate test result**	**n**	**Negative % (n)**	**Reactors % (n)**	**Doubtful% (n)**
Test-1 (502)	New entry	-	502	72.5	21.3	6.2
	Retested	-		-	-	-
Test-2 (521)	New entry	-	129	21.9	1.15	1.73
	Retested	Negative	363	59.1	5.75	4.8
		Doubtful	29	3.65	1.53 (8)	0.38
Test-3 (503)	New entry	-	52	10.14	0	0.2
	Retested	Negative	416	57.26	22.46	2.98
		Doubtful	35	4.40	2.3(12)	0.2
Test-4 (498)	New entry	-	127	24.9	0	0.6
	Retested	Negative	356	63.65	4.2	3.6
		Doubtful	15	1.6	1.2 (6)	0.2

The herd composition at a given screening test round was made of two units: new entries and retested animals. The proportion of retested animals ranged from 74% to 90% depending on the respective screening test round, and the rest portions were new entry animals, with no tuberculin test history before.Concerning tuberculin test based herd composition, more retested animals (15.8%) were reactor than new entry animals (13.6%) with non-significant difference (P >0.05)(Table 2).

Table 2Univariable analysis of selected determinants having association with PPD test results

Determinants	N	X^2	P	95% CIs
Herd composition (New entry vs Retested herd)	2024	1.91	0.167	[0.93,1.54]
Breeds	2024	117	0.000	[0.50, 0.63]

Based on previous SICCTT test result, the study herd was made upof animals with negative, doubtful, and no (new entry) penultimate test results. In general, at a specific test round, animals with doubtful penultimate test result hadsignificantly higher prevalence than those animalswith negative penultimate test result. 27.6 % (8/29), 34.3%(12/35) and 40%(6/15) of animals with doubtful penultimate test resultswere 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 1).Binomial logistic regression analysis (Table 3) revealed that animals with doubtful penultimate test result had 2.91 times higher prevalencecomparing with animals having negative penultimate test result.

Table 3Univariable model for penultimate test result and breed difference having association with PPD test results

Variable	**B**	**S.E.**	**Wald**	**Sig.**	**Exp(B)**	**CI for EXP(B)**	
						Lower	**Upper**
Penultimate test result							
Negative	-	-	19.1	0.00	-	-	-
Inconclusive	1.07	0.25	17.7	0.00	2.91	1.77	4.79
Breed							
High grade breeds (75% cross)	-	-	71.7	0.00	-	-	-
50% Cross breed	1.42	0.27	26.7	0.00	4.12	2.41	7.05
Boran	2.35	0.30	62.5	0.00	10.48	5.85	18.76

The study herd consisted of a mix of purebred Boran and different crossbred animals irrespective of their proportional affiliation.Prevalence of bTB regarding breed composition of the herdfor the respective test rounds are presentedin Figure 2, in which prevalence was significantly higher in purebred Boran than crossbred animals in a specific screening test round.In the univariable analysis, the prevalence of bTB infection in local breed (Boran) and 50% Boran-Friesian cross was about 10.5 and 4.1 times higher comparing with the prevalence of high-grade animals (75% Friesian-Boran), respectively.

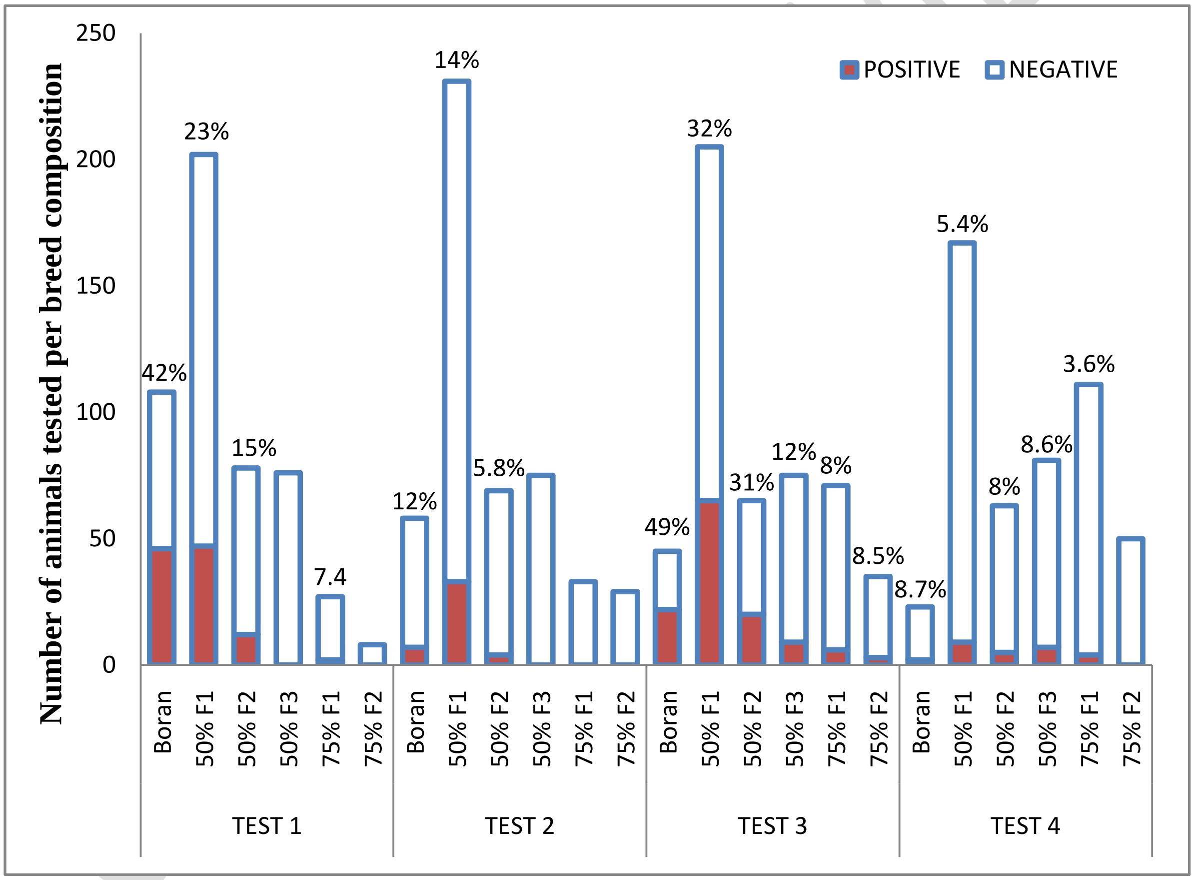

Figure 2bTBprevalence over breed difference of the herd along successive screening test rounds

Impacts of bTB incident on herd demography and structure

Animal entries and exits determined the herd demography, and were responsible for herd age and breed composition variation among the four screening test rounds. Following test and removal measures, a number of cowsleft the herd due to culling, andheifers entered to the herd as replacement stock. Totally, exits were more than entries in all screening programs.

The 50% crossbred animals consistently formed over 63% of the study herd population, of which 50%F1 (BO X F) took the largest proportion in all screening test rounds. However, the proportion of purebred Boran animals declined to 5% while high-grade animals (75% Holstein blood) increased almost five-fold between the first and the fourth test rounds. The proportion of 75% crossbred animal was inversely proportional to that of purebred Boran animals across subsequent herd test rounds (Figure 2).Theaverage age distribution of the study herdand mean age of culled animals for successive SICCTT test roundswas comparedin Table 4. The herd included animals ranged from 6 months to 17.5 years old.

Table 4 Average herd age of new entry and retested animals at each test rounds

Test round	Mean age (yrs) of study herd					Mean age (yrs) of culled animals
	Total	New Entry	Retest-1	Retest-2	Retest-3	
Test 1	5.75	5.75				9.97
Test 2	4.90	2.10	5.82			8.73
Test 3	5.72	2.09	7.11	2.95		8.07
Test 4	4.85	2.09	7.12	3.86	3.04	6.86

In the current study, we defined culled animals asthose animals that had been tested in at least in one of the SICCTT test round and removed before the end of the study. The majority of culled animals were removed due to bTB.Other culling reasons were fertility, accident, or other diseases.The mean age of animals at cullingwas 8.71(range: 0.64 to 17.52) years. Around 23% of culled animals (mean age of5.30 years) were younger than the mean age of the study herd.

Table 5 Description of culled animals by subsequent SICCTT herd screening tests

Test no	**N**	**Mean**	**SD**	**25th percentile**	**50th percentile**	**75th percentile**	**Min**	**Max**
Test-1	110	9.97	4.03	6.98	10.16	14.37	2.82	15.67
Test-2	70	8.73	3.63	6.11	8.51	10.91	0.64	15.77
Test-3	132	8.07	3.88	5.06	6.98	10.79	2.45	17.52
Test-4	30	6.86	2.58	5.01	6.06	8.26	3.23	12.66
Total	342	8.71	3.89	5.65	8.24	11.58	0.64	17.52

Discussion

Despite some limitations,this dairy farm carried out test-and-slaughter measures to control bTB incidence, resulting in a significant impact on herd structure and dynamics. Herd age and breed distribution varied remarkably among subsequent herd tests. With an increased culling rate, the average age of the herd and the average number of lactations per cow decreased (Stewart, 1995), which was not favorable for herd demography maintenance.

Conventionally, SICCTTare recommendedas 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(Kao et al., 1997). Only test-and-slaughter techniques have proven capable of eradicating tuberculosis from domestic animal populations. In this study, however, the prevalence of the disease was not reduced steadily and failed to eliminate the disease from the herd, despite fourrounds of test-and-slaughter. High incidence of the disease was recorded in thethird roundscreening test, althoughrelatively low incidence was identified in the fourth round screening test.Previous trials in Ethiopia found that test and slaughter as practiced by a few dairy farmsshowed an apparent improvement in incidence(AMENI *et al.*, 2007; Shitaye *et al.*, 2007).Ameniet al.(2007)reported that test and slaughter resulted in a pronounced incidence reduction. Moreover, the study byProud (2006) showed a trend toward meaningfulreduction ofcattle-to-cattle transmission as soon as reactors and non-reactors werephysically separated.

Failures of the test and slaughter approachin the current study might be due to excessivelyprolonged and inconsistent tuberculin test time intervalsfor repeated herd retesting.The previous study by Ameniet 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 five years afterthe first test, and the time interval between successive SICCTT tests varied from 0.95 years to 1.84 years.This prolonged inter-test interval would have allowed for continued transmission within the herd.

Domestic cattle and species of the *Bovidae*family (i.e., buffalo and bison) are the most susceptible and represent the main animal reservoirs (Brosch *et al.*, 2002); albeit, possible differencesin susceptibility between different cattle subspecies have been hypothesized (i.e., *Bostaurus*and*Bosindicus*) (Rodríguez-Campos *et al.*, 2014). Different studies showed that indicene (Boran) and taurine(Holstein) cattle have differences in their relative susceptibilities to bTB.

In the current study, the prevalence was significantly higher in purebred Boran(Bosindicus) than in high-grade Holstein cattle (B. taurustaurus) 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 Borananimals 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 diverse 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 in relation to cattle breeds was dramatically lower in Zebu cattle compared to taurineAnkole cattle, indicating that Zebu calves showed remarkable resistance compared to Ankole calves.

This study measured also 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 penultimate inconclusive SICCTT test 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).Reactors that had a doubtful or inconclusive result in the previous SICCTT were more likelyto have visible lesions at slaughter than those with a negative penultimate SICCTT test result. 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. Similar results in Irish cattle evidenced our findings through a greater proportion of inconclusive animals slaughtered and confirmed as bTB-positive at slaughter compared to negative animals(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 test and slaughter intervention in this study herd was not sufficient to steadily reducethe prevalence or eliminates infection from the herd. The study identified an important knock-on effect on herd demography due to test-and-slaughter measures; the higher the culling rate, the lower the average age of the herd.Animals that had inconclusive/doubtful previous test result were found to have a higher probability of being reactor. These findings suggested the importance of time between consecutive SICCTT tests in test-and-slaughter strategy. The inconsistent and extremely prolonged retest-and-slaughter schemes in the current study were likely responsible for the failures to reduce the number of new bTB cases and ultimately reach the goal of bTB elimination from the herd.

List of abbreviations

BCG: Bacillus Calmette and Guérin; BO X F: Boranvs 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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