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MODELING BOVINE TUBERCULOSIS TRANSMISSION DYNAMICS AND DISEASE CONTROL INTERVENTIONS IN SELECTED DAIRY FARMS OF ETHIOPIA

PhD Thesis

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PhD Program in Veterinary Public health

May, 2024
Bishoftu, Ethiopia

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A Dissertation Research Submitted to the College of Veterinary Medicine and Agriculture, Addis Ababa University in Fulfillment of the Requirement of the Degree of Doctor of Philosophy in Veterinary Public health

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As members of the Examining Board of the final PhD research dissertation open defense, we certify that we have read and evaluated the research proposal prepared by Berhanu Abera titled: '**Modeling bovine tuberculosis transmission dynamics and disease control interventions in selected dairy farms of Ethiopia'** and recommend that it be accepted as fulfilling the dissertation requirement for the degree of **Doctor of Philosophy in Veterinary Public Health.**

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Chairman (title and name)	Signature	Date
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Examiner (title and name)	Signature	Date

I hereby certify that I have read the revised version of this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation research requirement.

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Statement of authors

I, Berhanu Abera, hereby declare that this thesis titled, "Modeling bovine tuberculosis transmission dynamics and disease control interventions in selected dairy farms of Ethiopia" and the work presented in it are my own. I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at the College of Veterinary Medicine and Agriculture, Addis Ababa University, Addis Ababa University.
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated.
3. Where I have consulted the published work of others, this is always clearly attributed.
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work.
5. I have acknowledged all main sources of help.
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

Signed: ________________________

Date: ___________________________

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AHT Animal Health Technician

LIST OF ABBERVIATIONS

BCG	Bacillus Calmette and Guérin
bTB	Bovine Tuberculosis
CMI	Cell-Mediated Immunity
CIDT	Comparative intradermal tuberculin test
CIDT	Comparative intradermal tuberculin test
DTH	Delayed-Type Hypersensitivity
EIAR	Ethiopian institute of agriculture research
HARC	Holeta Agricultural Research Center
HPD	Highest Posterior Density
IFN-γ	interferon-gamma assay
LPA	Low Prevalence Areas
MCMC-ABC	Markov Chain Monte Carlo-Approximate Bayesian Computation
MoH	Ministry of Health
MTC	Mycobacterium tuberculosis-complex
NAIC	National Artificial Insemination Centre
NPV	Net present value
OTF	Officially Free of Bovine Tuberculosis
PCR	Polymerase Chain Reaction
PD	Pregnancy diagnosis
PPDs	Purified Protein Derivatives
PSM	propensity score matching
SICCTT	Single Intradermal Comparative Caudal Tuberculin Test
SITT	Single Intradermal Tuberculin Test

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SUMMARY

Deepening poverty; sluggish performance of the agricultural sector in general, livestock development in particular; accelerated resource depletion and emerging zoonotic disease in Ethiopia have always concerned policy makers and researchers and urged them to search for effective research and policy tools. The present PhD research work; therefore, aims to reveal peculiarities of information on bTB transmission dynamics and control, relevant data for the implementation of a model-based disease control system and factors that might hamper the control of the disease in order to optimize resources. The studies included in my PhD thesis are summarized below:

1. In the first study, non-biological factors affecting bovine tuberculosis control and prevention in dairy herds was analyzed. The results indicated that the KAP level among dairy farmers varied depending on herd size, milk-shed, training availed, veterinary consultation and their previous farming experiences. Farmers from medium and large-scale farms knew more about bTB than those from small scale farms, by a factor of 2.8 and 7.7 respectively. Similarly, farmers who had been farming for more than 6 years and farmers from Selale milk-shed had higher odds of being knowledgeable about bTB, by a factor of 5.7 and 10.4 respectively, compared to others. Only 12% of the study participants were aware of test and slaughter method, likewise, 18% of farmers tended to avoid buying cattle from risky sources. The finding revealed a dearth of knowledge on the production loss incurred (12%) and the probability of human infection (1.9%), instead a substantial number of farmers (25%) believed that bTB infection could badly affect the dairy market. These limitations may explain how bTB will continue to be a major threat. All these negatively impact disease surveillance and control intervention programs.

2. The second study assessed the incidence of bTB in a dairy herd with repeated irregular skin test and slaughter programme, and found that these incidences exhibited an oscillating pattern over subsequent time period. The study highlighted the importance of consistent testing and control measures to manage the risk of bTB in dairy herds. Penultimate test result, 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. Moreover, animals that had inconclusive results in previous tests were more likely to be bTB reactor, and high probable to have visible lesions at slaughter than those with a negative penultimate SICCTT result, emphasizing the need for careful monitoring and follow-up. It's crucial for dairy farms to implement regular and systematic skin testing and slaughter control programs to effectively control the spread of bTB. This not only protects the health of the herd but also ensures the safety of milk products for consumers.

3. In the third study, the impact of repeated test and slaughter for bovine tuberculosis control was examined. The effect of the intervention measures on resultant incidences, and its consequence on herd demographic changes were evaluated. Despite repeated testing and removal measures, the incidence did not exhibit a substantial reduction trend along the successive test and slaughter rounds**.** The time interval between successive SICCTT tests varied from 0.95 years to 1.84 years, which were excessively prolonged and inconsistent time intervals. Apart from the incompetence on incidence reduction, the study identified a vital knock-on effect on herd demography due to culling of a substantial number of cows (n=342) 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. Similarly, animal entries and exits also influenced the breed composition of the herd. The proportion of purebred Boran animals declined to 5%, while high-grade animals (75% Holstein blood) increased almost five-fold between the first and fourth test rounds. It is recommended that culling should be carried out with no or minimal significant impact on herd demography change. Compliance to conventional test and slaughter procedural protocol is supposed to play an important role in succeeding bTB control measures. Therefore, at least a minimum of 2-month a maximum of 6-month testing interval, and two consecutive negative whole herd testing should be carried out in order to declare a herd free of bTB.

4. The fourth study, “Bayesian modeling of bovine tuberculosis prevalence”, estimate the true prevalence and test characteristics (sensitivity and specificity) of SICCTT from the apparent prevalence in semi-intensive dairy farm over time. True disease states are uncertain

in practice due to imperfect specificity and sensitivity, and so the true prevalence and characteristics of SICCTT were inferred using Markov Chain Monte Carlo estimation with prior distributions. Median sensitivity estimates using standard and severe interpretations were 68.6% (BPI; 50.3-84.3%) and 78.1% (BPI; 62.5-90.9%), while the specificity median were 96.8% (BPI; 94.3-98.9%) and 94.5% (BPI; 91.5-97.1%), respectively. Furthermore, adjusted true prevalence estimates (median and 95 %; BPI) were produced for each testing round using the Rogan-Gladen estimator (RGE). Bayesian estimation with informative priors exhibited much wider credible intervals and strong coverage compared to uninformative priors and frequentist method (RGE). Classic apparent prevalence estimates are overly precise when uncertainty around test performance is high. These Bayesian approaches provided a more accurate estimate of bTB prevalence in the study herd and provide a baseline data for the future true prevalence estimates using linked combined data.

5. The fifth study estimated the within-herd transmission dynamics of bovine tuberculosis in a commercial dairy herd, and predict the efficiency of the envisaged control interventions. The study developed a stochastic compartmental SORI (Susceptible, Exposed (latent), and Infectious) model to simulate within herd transmission dynamics of *M. bovis* in intensively managed dairy farm. Several parameters related to bTB spread, in particular the cattle-to-cattle transmission rate (β) and the rate at which infected cattle become infectious (α) was inferred. Data for parameter inference was obtained from farms where there were epidemiological evidences of bTB introduction into the herd through the purchase of infected animals, which allowed us to have data on: a) the date of introduction of infection into the herd, b) initial number of infected animals introduced and c) final number of infected animals (when infection of the herd is detected). A Markov Chain Monte Carlo-Approximate Bayesian Computation (MCMC-ABC) method was used to generate posterior distributions of bTB transmission parameters.

The results from the studies-I has been accepted by Ethiopian Veterinary Journal, study II has been published on Asian III, IV and V included in the present PhD Thesis have been published or accepted or submitted for publication in international scientific peer-reviewed journals:

CHAPTER ONE: INTRODUCTION

Tuberculosis (TB) is a chronic disease caused by infection with a member of the *Mycobacterium tuberculosis* complex /MTC/ which comprises *M. tuberculosis* (the cause of most human tuberculosis), *Mycobacterium bovis* (incl. BCG and *Mycobacterium caprae*), *Mycobacterium microti, Mycobacterium africanum, Mycobacterium canettii* and *Mycobacterium pinnipedii* (AHT, 2016). Bovine tuberculosis (bTB) primarily cause in disease in animal species and has a potential economic and public health burden to society whose livelihood depends on their livestock like a pastoralist community (Mamo *et al*., 2013). However, investigation of *M. bovis* both in animal and human populations' still leftover low in such underdeveloped regions particularly in Africa despite the disease remains a possible health risk.

Only test-and-slaughter techniques are guaranteed to eradicate tuberculosis from domesticated animals. However, this method is too costly to apply to most of the developing world and even it is difficult to force culling of infected cows in Africa-because the cattle value is deeply interwoven with the social system and they are the savings of the rural poor (Michel *et al*., 2004). Yet a large proportion of the countries in Africa do not have control measures in place. Only about 15% of the cattle population in Africa is found in countries where bovine tuberculosis is a notifiable disease and a test-and-slaughter policy is used. Thus, approximately 85% of the cattle and 82% of the human population of Africa are in areas where bTB is either partly controlled or not controlled at all (Cosivi *et al*., 1998). It is fair to say that test and slaughter only apply to a few countries in Africa. Therefore, other alternative control strategies without test and slaughter policy have to be developed. Some possibilities worthwhile considering are the development of a vaccine, the use of the interferon gamma test to identify herds or areas with a high prevalence which can then be targeted for a short-term, high-impact control strategy, but most important of all, to inform owners of cattle about the risks of bovine tuberculosis and the necessity for pasteurization of milk and inspection of carcasses after slaughter (McCrindle and Michel, 2007). Moreover, testing and segregation of positive animals is another control option in resource limited countries. Bovine tuberculosis (bTB) is a known endemic disease of cattle in Ethiopia;

however, there is lack of information on the measures taken to control the disease in the country.

1.1. Commercial Dairy Production in Ethiopia

Ethiopia has the largest livestock population in Africa. According to a CSA (2021) livestock sample survey, the country possesses 70.3 million cattle, 42.9 million sheep, 52.5 million goats and 8.1 million camels. Livestock are an integral part of agriculture and the daily life of the Ethiopian people. The livestock sector contributes about 45% of the agricultural GDP, 18.7% of the total national GDP, and 16–19% of the total foreign exchange earnings of the country (Behnke and Metaferia, 2011).

Commercial dairy production refers to the large-scale farming and management of dairy animals, primarily cows, for the purpose of producing milk and milk products for sale in the market. There are 10 million dairy cows in Ethiopia producing approximately 3.2 billion liters on average of 1.54 liters per cow per day over a lactation period of 180 days (Getabalew *et al.*, 2019; Tefera *et al.*, 2010). The farm-level value of the milk is an estimated Birr 16 billion. This production process typically involves specialized facilities, equipment, and management practices aimed at maximizing milk yield, quality, and profitability (Gebresellasie, 2019).

As indicated by Gebresellasie (2019) and Bereda *et al*. (2014), commercial dairy production system is challenged with high feed cost, infectious diseases, land shortage and space limitation, feed quality, availability and cost problems as well as inadequate extension and veterinary services in the Urban and Peri-Urban areas of central Highlands of Ethiopia. Infectious diseases such as mastitis, brucellosis and bovine tuberculosis are the common diseases for commercial dairy production in Ethiopia which are risk to public health.

1.2. Background of Bovine tuberculosis

1.2.1. Definition of bovine tuberculosis

1.2.1.1. Etiology

Tuberculosis (TB) is a chronic disease caused by infection with a member of the *Mycobacterium tuberculosis* complex /MTC/ which comprises *M. tuberculosis* (the cause of most human tuberculosis), *Mycobacterium bovis* (incl. BCG and *Mycobacterium caprae*), *Mycobacterium microti, Mycobacterium africanum, Mycobacterium canettii* and *Mycobacterium pinnipedii* (AHT, 2016). Bovine tuberculosis is a gram-positive, acid-fast bacterium in the *Mycobacterium tuberculosis* complex of the family Mycobacteriaceae. *M. bovis* is the main cause of tuberculosis in cattle, deer, and other mammals and shows some features distinct from *M. tuberculosis* in human hosts. Mycobacteria have DNA with high proportion of guanine and cytosine which increases DNA stability. The thick and lipid-rich cell wall of mycobacteria protects DNA from attack of lytic enzymes after autolysis and necrosis of the host cell (Guta Debela, 2013).

Table 1. 1. Susceptibility of animals to the three Mycobacterium strains

Animal	*M. bovis*	*M. avium*	*M. tuberculosis*
Cattle	XXX	X	X
Fowl	-	XXX	
Human	XXX	XX	XXX
Pig	XXX	XX^{I}	XX^{I}
Sheep	XX^{I}	XX^{I}	XX
Goat	XXX	XX	XX
Horse	XX^{I}	XX	XX
Cat	XXX	XX	
Dog	XX^{I}	XX	XXX

XXX susceptible - visible lesions develop
XX^{1} susceptible - visible lesions sometimes develop
XX susceptible - visible lesions seldom develop
X visible lesions usually do not develop but animals react to the tuberculin test
Source: (AHT, 2016)

1.2.1.2. Pathology, pathogenesis and lesions

Tuberculosis spreads through the body in two stages, the primary complex and post-primary dissemination. The primary complex consists of the lesion at the point of entry and the local lymph node. Post-primary dissemination from the primary complex may take the form of acute miliary tuberculosis, discrete nodular lesions in various organs, or chronic organ tuberculosis (Francis, 1947; Radostits *et al*., 2000).

After the entry of the mycobacteria into the animal, the inflammatory signal pathways will be activated, leading to the phagocytosis of the bacteria by macrophages (Domingo *et al*., 2014), which are the primary host cell for intracellular growth. Despite MTC species lacking toxins; they have several virulence genes, which mostly encode for enzymes of lipid pathways, cell surface proteins, regulators, or proteins of the signal transduction system. Moreover, other genes are involved in mycobacterial survival inside the host macrophages, encoding for proteins inhibiting the antimicrobial effect of macrophages, including phagosome arrest and inhibition of apoptosis (Forrellad *et al*., 2013). The gradual accumulation of macrophages in the lesion and the formation of a granulomatous response contribute to the development of a tubercule (Quinn *et al*., 2002) where bacteria have localized. The granuloma (tubercle) prevents the spreading of bacilli resident within macrophages. However, the latent bacilli could be later released if the immunological balance is broken, triggering disease reactivation. These granulomas are usually yellowish and either caseous, caseo-calcareous or calcified. They are often encapsulated. The inhalation of infected droplets is described as the most common route of infection in cattle (Neill *et al*., 2001) causing initial granulomatous lesions in the nasopharynx and lower respiratory tract, including the lungs, and associated lymph nodes, particularly those of the head and thorax. They are also common in the spleen, liver and the surfaces of body cavities. Within the granuloma, the mycobacteria may remain dormant for decades without any clinical disease (i.e., latent tuberculosis) (Domingo *et al*., 2014; Pollock and Neill, 2002). Subsequent immune suppression could allow activation of the dormant bacteria, followed by replication and spread; consequently, a proportion of infected cases may not develop any active tuberculosis. The mechanisms responsible for latency in tuberculosis are not well understood; however, potential latent infections are suspected in cattle (Domingo *et al*., 2014; Pollock and Neill, 2002).

1.2.2. Epidemiology of bovine tuberculosis

1.2.2.1. The biology transmission of M. bovis

The biology of transmission of *M. bovis* includes a multispecies interface involving wildlife, livestock, and humans. Studies on *M. bovis* transmission in Ethiopia have so far not identified any wildlife source (Tschopp *et al*., 2010a, 2010b) and *M. bovis* seems to be almost absent in Ethiopian goats and camels (Camelusdromedaries). *M. bovis* has not been found to be a major TB agent in humans in Ethiopia, except for a few cases in southeastern Ethiopia where three *M. bovis* out of 164 MTC strains were isolated from humans (Gumi *et al*., 2012). In contrast, cattle and small ruminants may occasionally be infected with *M. tuberculosis* (Berg *et al*., 2009; Tschopp *et al*., 2011). Based on the current understanding, *M. bovis* transmission in Ethiopia seems to be largely restricted to cattle with only rare cases in humans. Therefore, a cross-sector study on the cost-effectiveness of brucellosis control involving human and animal health as was conducted in Mongolia, does not currently seem appropriate for bTB in Ethiopia.

1.2.2.2. Cattle to cattle transmission

The pattern of lesions observed in slaughtered animals represents good evidence of the transmission route of *M. bovis* to cattle (Phillips *et al*., 2003). Therefore, the high frequency of lesions found in the nasopharynx, lower respiratory tract and associated lymph nodes in cattle (Courtenay *et al*., 2006; Domingo *et al*., 2014; Goodchild and Clifton Hadley, 2001; Johnson *et al*., 2007; Phillips *et al*., 2003) is indicative that the respiratory route (via the inhalation of infected aerosol droplets) can be considered as the primary mechanism of infection, (Courtenay *et al*., 2006; Menzies and Neill, 2000). Moreover, experimental studies have shown that low numbers of bacilli are needed to experimentally infect animals via the respiratory tract, as compared to the large doses needed to infect animals via the digestive route, which appears to be a less effective method of disease transmission (Palmer *et al*., 2012; Phillips *et al*., 2003). In addition, it has been suggested that some intestinal lesions in cattle may be the result of swallowing their own *M. bovis*-contaminated sputum (Menzies and Neill, 2000).

Indirect horizontal transmission through ingestion or by inhalation of infected aerosols present in the environment relies on the survival of the mycobacteria as it must remain viable in order to be infectious. The survival of the mycobcateria in the environment is dependent mainly on the temperature, moisture and exposure to ultraviolet light. low temperature, high humidity and protection from sunlight will provide an ideal environment for the persistence of the mycobacteria (Phillips *et al*., 2003). Infection through the ingestion of infected milk from tuberculous udders together with vertical transmission, as a result of tuberculous endometritis, can be described also as a way of direct transmission in calves (Goodchild and Clifton Hadley, 2001; Neill *et al*., 1991). However, those transmission routes are very uncommon in developed countries.

1.2.2.3. State of infection

Bovine tuberculosis is considered as a chronic disease progressing through time, though the dynamics of *M. bovis* transmission are not completely understood, and the conditions under which infected cattle become effectively infectious are not fully defined (Goodchild and Clifton Hadley, 2001; Pollock and Neill, 2002). Following infection, bTB develops through several stages represented by latency stage, infectious, and anergic stage (Kleeberg, 1984). Latency state has two sub-stages including an unresponsive period (the animal is infected but neither infectious nor reactive to the tests), and a non-infectious reactor period in which the animal tests positive but is not yet infectious (no excretion of *M. bovis*) (Goodchild and Clifton-Hadley, 2001). During the initial period of latency, the cattle have not yet mounted a cell-mediated response to invading *M. bovis* bacteria. It takes 8-65 days before infected cattle achieve the maximum immune response (Kleeberg, 1984). This early latent state, occult state (O) therefore represents a state of limited responsiveness and low sensitivity to the test. The next state of disease, late latent stage (R), represents animals with non-infectious disease but which are responsive to the skin test so test sensitivity is higher for this state (Figure 1.1). The total duration of latency is thought to lie within the range of 87-226 days (Neill *et al*., 1991), in experimentally infected calves) to 7 years. However, a realistic range for most New Zealand cattle is thought to be 6-20 months, which corresponds to the sum of the lengths of states O and R. In practice, the durations of these states vary between cattle, depending on the route of infection and the initial dose (Neill *et al*., 1991). Stress resulting from pregnancy, lactation, poor nutrition, or other disease depresses the immune response in cattle, thus also affecting the test sensitivities and durations of the states (Kleeberg, 1984). The final state, I, corresponds to infectiousness, and cattle are assumed to remain infectious until detected by the skin test and removed (Cosivi *et al*., 1998).

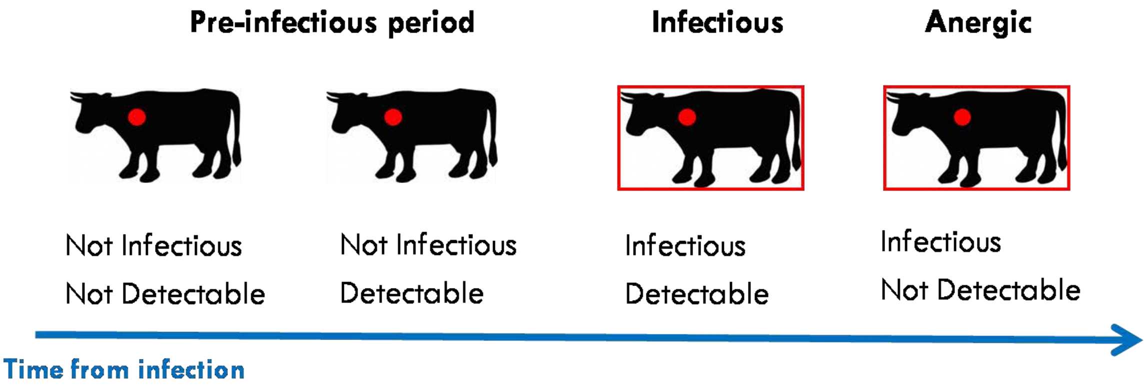


Figure 1. 1 BTB infection dynamics in cattle

Source: Barlow, 1993.

1.2.3. Control and prevention of bovine tuberculosis

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 the slaughter of diseased animals removes the infection from within the herd (Kao *et al.*, 1997). Based on these strategies, high-income countries have established national bovine tuberculosis control and prevention programs, and thus nearly eradicated or drastically reduced bTB in farm animals (Ayele *et al.*, 2004; Cosivi *et al.*, 1998; Pavlic *et al.*, 2002). Despite being considered impractical to adopt in Africa, seven nations (Morocco, Algeria, Burkina Faso, Cameron, Zambia, Namibia, South Africa) applied disease control measures as part of a test-and-slaughter policy (Figure 1.2), making bTB a notifiable disease; the remaining 48 countries in Africa control the disease inadequately or not at all (McCrindle and Michel, 2007). There is no single intervention that will on its own achieve control of the bTB epidemic unless able to block all routes of transmission of the disease (https://veterinaryrecord.bmj.com/content/174/15/367).

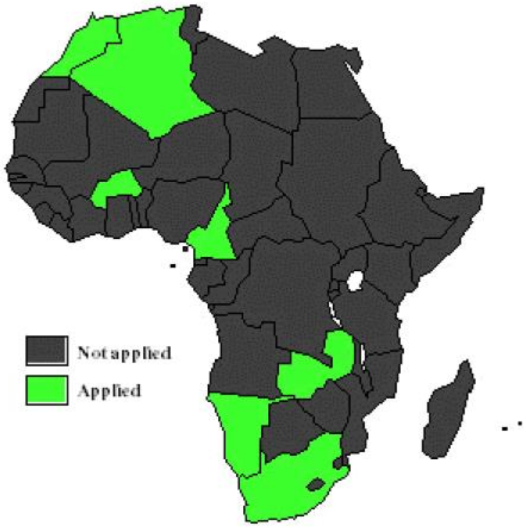


Figure 1. 2 African countries which applied test-and-slaughter policy as their control measures for bTB

Source: After Cosivi *et al* (1998) modified by McCrindle and Michel, 2007

1.2.4. Global estimate of zoonosis tuberculosis

As per Natasha (2007) report, an obscure number of human TB cases may be because of the *M. bovis* in Africa, proven in Tanzania, as of late as 2006, 88 percent of towns screened had at least one animal that tested positive for bTB and 10.5 percent of individuals with stomach or lymph gland tuberculosis were infected by *M. bovis.* One of the major difficulties in knowing how much human disease is caused by *M. bovis* is that some of the most commonly used laboratory processes can't distinguish between disease caused by *M. bovis* and disease caused by *M. tuberculosis*. However, the World Health Organization (WHO) does have some estimates of TB disease in humans caused by *M. bovis.* So, the figures below cover all TB diseases in humans that are estimated to have started in diseased animals whose disease is caused by the bacteria *M. bovis*. The WHO Program to End TB calls for the diagnosis and treatment of every TB case in humans, including cases of TB in humans caused by *M. bovis* (WHO, 2019).

Table 1. 2 Estimated incidence and mortality in humans due to *M. bovis*

Regions	Incidences	Deaths

Africa	69,800	9100
America	821	44
Eastern Mediterranean	7940	655
Europe	1150	91
South-East Asia	44800	2110
Western Pacific	18600	310
Global Total	143,000	12300

Source :(WHO, 2019)

1.2.5. Approaches to control zoonosis tuberculosis

Huge global efforts are being made in tuberculosis control and outbreak containment in industrialized countries. However, many resource-limited and transitioning countries, especially in pastoral areas, lack such necessary control efforts (Ashford *et al*., 2001; Cosivi *et al*., 1998; Malama *et al*., 2013; McCrindle and Michel, 2007). One potential drawback for the global initiative to combat human TB is that the problems that need to be addressed differ between countries, limiting anyone-size-fits-all global solutions. Of interest here is the importance of zoonotic tuberculosis, which varies greatly depending on a number of factors outlined above. The key for controlling zoonoses is often to focus on the animal reservoir, but ministries of health sometimes question whether the public health sector really benefits from interventions for livestock (Zinsstag *et al*., 2007). Instead, benefits from livestock health and productivity become the goals of interventions in low-income countries, often led by ministries of agriculture. Veterinary public health is not integrated into the mainstream of public health services in sub-Saharan African countries. There are no formal mechanisms within government public health services in Ethiopia through which veterinary skills and resources can be effectively harnessed to bear upon community health (Tibbo *et al*., 2008), but individual institutional attempts have failed to eliminate zoonotic disease. Therefore, control of a disease like bTB, which affects both livestock and humans, requires joint activities between the ministries of Agriculture and Health. The close working relationship developed between them in the National Coordination Committee created for the avian human influenza and Rift Valley fever threat could be used for bTB as well (Daginet and Demissie, 2008). Generally, by addressing the total societal benefits, intervention in the animal sector saves money and provides an economic argument for control. This opens new approaches for the control of zoonoses in resource-limited countries through contributions from multiple sectors (Zinsstag *et al*., 2007).

In high-income countries, the occurrence of human tuberculosis due to *M. bovis* has meaningfully declined because of mandatory pasteurization of milk, together with tuberculin skin testing of cattle followed by culling and slaughtering the infected cattle (Palmer *et al.*, 2012). This approach has been largely neglected in Asian and African countries.

1.3. Bovine Tuberculosis in Ethiopia

In Africa, bovine tuberculosis has a wide distribution with a high prevalence in both domestic and wild animals (De Garine-Wichatitsky *et al*., 2013). Despite unavailable national-level all-inclusive studies on the status and epidemiology of the disease in Ethiopia, 5.8% (95% CI: 4.5, 7.5) national pooled bovine tuberculosis prevalence estimate was reported via systematic review and meta-analysis (Sibhat *et al*., 2017).

Bovine tuberculosis is an endemic disease of cattle in Ethiopia; however, there is only little comprehensive information on the status and distribution of the disease in the country which has made the quantification of economic and public health impacts of the disease difficult. Nonetheless, it may be possible to project the enormous losses bovine tuberculosis could inflict on the country's livestock economy, given the high prevalence of the disease reported through several fragmented studies in different parts of Ethiopia *(*Demelash *et al.*, 2009).

Most of these studies were carried out based on tuberculin skin testing (Kemal *et al*., 2019; Romha and Ameni, 2014) and postmortem inspection (Regassa *et al*., 2010), even though few studies were supported by isolation and molecular typing of the causative agents (Alemu *et al*., 2016; Ayana *et al*., 2013) (Table3). Bovine purified protein derivative (PPD) alone or together with avian PPD has been used to determine exposure status in live animals.

Table 1. 3 List of representative study reports on bovine tuberculosis in Ethiopia

Authors	Study setup	Dx. Test	Sample size	APP
(Asseged *et al*., 2000)	Farm/field	SIDT	1241	10.5
(Ameni and Regasa, 2001)	Farm/field	CIDT	416	14.2

Ameni *et al*. (2003b)	Farm/field	CIDT	1168	46.9
(Fikru *et al*.,2005)	Farm/field	SIDT	353	3.4
(Shitaye *et al*.,2006)	Farm/field	CIDT	2098	18.7
(Ameni and Erkihun ,2007)	Farm/field	CIDT	524	11.1
(Ameni *et al*.,2007a)	Farm/field	CIDT	5424	13.5
(Elias *et al*.,2008)	Farm/field	CIDT	1869	23.7
(Fetene and Kebede ,2009)	Farm/field	CIDT	1207	9.7
(Tsegaye *et al*.,2010)	Farm/field	CIDT	1132	34.1
(Tschopp *et al*.,2010a)	Farm/field	CIDT	499	0.8
(Amenu *et al*.,2010)	Farm/field	CIDT	425	6.4
(Tschopp *et al*.,2011)	Farm/field	CIDT	1214	1.6
(Gumi *et al*.,2011)	Farm/field	CIDT	473	5.5
(Dinka and Duressa,2011)	Farm/field	CIDT	625	12.2
(Tigre *et al*.,2012)	Farm/field	CIDT	384	21.4
(Gumi *et al*.,2012)	Farm/field	CIDT	411	2.4
(Firdessa *et al*.,2012)	Farm/field	CIDT	2926	32.2
(Tschopp *et al*.,2013)	Farm/field	CIDT	584	0.3
(Mamo *et al*.,2013)	Farm/field	CIDT	1087	11
(Ayana *et al*.,2013)	Farm/field	CIDT	371	1.3
(Romha *et al*.,2013)	Farm/field	CIDT	484	6.6
(Zeweld *et al*.,2013)	Farm/field	CIDT	423	7.3
(Zeweld,2014)	Farm/field	CIDT	524	2.7
(Zeru *et al*.,2014)	Farm/field	CIDT	480	11.3
(Bussa,2014)	Farm/field	CIDT	508	1.8
(Biru *et al*.,2014)	Farm/field	CIDT	835	11.4
(Romha *et al*.,2014)	Farm/field	CIDT	440	4.3
(Tschopp *et al*.,2015)	Farm/field	CIDT	220	0
(Nuru *et al*.,2015)	Farm/field	CIDT	788	1.3
(Dejene *et al*.,2016)	Farm/field	CIDT	2550	5.5
(Duguma *et al*.,2016)	Farm/field	CIDT	554	3.8

Source: (Sibhat *et al.*, 2017)

Note: Dx: Diagnostic technique, App: Apparent prevalence, CIDT: Comparative Intradermal Tuberculin test, SIDT: Single Intra-dermal Tuberculin test, DMI: Detailed Meat Inspection

Since the report of Hailemariam (1975), several fragmented studies have been under taken to show bTB prevalence in Ethiopia (Aklilu and Ameni, 2007; Ameni *et al*., 2007; Berg *et al*., 2015; Elias *et al*., 2008; Gumi *et al*., 2011; Gumi *et al*., 2012b), dynamics of transmission over time (Ameni *et al*., 2010a, 2010b, 2007b, 2007a; Berg *et al*., 2009; Firdessa *et al*., 2013;

Gumi *et al*., 2011; Tschopp *et al*., 2010) and characterization with molecular typing methods (Ameni *et al*., 2010a; Berg *et al*., 2015).

Many of these reports however, were conducted by graduate and undergraduate students to fulfill their research requirements in the academic programs to which they were enrolled, therefore these studies were noted to have limitations in producing country level picture for one or the other reasons including, the scope of study objectives, methodology used, target population and geographic coverage (Sibhat *et al*., 2017).

Table 1. 4 PPD based prevalence studies in different study location, breed and animal husbandry

Authors	APP	Breed Type	Husbandry	Study locations
(Biru *et al*.,2014)	11.4			central Ethiopia
(Ameni *et al*.,2007)	13.5	Zebu & Holstein	Extensive	central Ethiopia
(Shitaye *et al*.,2006)	18.7	Exotic (Cross/pure)	Intensive &semi	central Ethiopia
(Elias *et al*.,2008)	23.7	Exotic(Cross/pure)	Intensive &semi	central Ethiopia
(Firdessa *et al*.,2012)	32.2	Exotic(Cross/pure)	Intensive	central Ethiopia
(Tsegaye *et al*.,2010)	34.1	Exotic(Cross/pure)	Intensive	central Ethiopia
(Ameni *et al*.,2003b)	46.9	Cross breed	Intensive	central Ethiopia
(Tschopp *et al*.,2015)	0	Local breed	Extensive	Awash fentale/pastoral
(Nuru *et al*.,2015)	1.3	Mixed breed	Intensive & extensive	northwestern Ethiopia
(Gumi *et al*.,2012)	2.4	Local breed	Extensive	Somali regional state, /pastoralist
(Tschopp *et al*.,2013)	0.3	Mixed breed	semi-intensive	South-Eastern Ethiopia
(Tschopp *et al*.,2011)	1.6			Southern Ethiopia
(Romha *et al*.,2014)	4.3			southern Ethiopia
(Regassa *et al*.,2010)	11.6	Mixed breed	semi-intensive	South Ethiopia
(Tschopp *et al*.,2010a)	0.8	Local breed/zebu	Extensive	South Ethiopia/Pastoral area
(Duguma *et al*.,2016)	3.8	Boran breed	semi-intensive	South Ethiopia/Pastoral area
Gumi *et al*. (2011)	5.5	Local	Extensive	South

				Ethiopia/Pastoral area
(Ayana *et al*.,2013)	1.3	Mixed breed	Intensive	West shewa
(Fikru *et al*.,2005)	3.4	Local/zebu	Extensive	Western Ethiopia
(Ambaw *et al*.,2017)	16.53	Mixed breed	Intensive/semi-intensive	Central Ethiopia

As of late attempted investigations demonstrated, the prevalence of BTB is with a range of <1% (in southern Ethiopia and Awash Fentale Pastoral area) (Tschopp *et al*., 2015, 2013) to 47 % (in intensive dairy farms in Central Ethiopia) (Ameni *et al.*, 2003) and at specific localities, depending on breeds of cattle kept and husbandry methods, a range of no detection of infection (0%) (Tschopp *et al*., 2013) to 90 % (Firdessa *et al*., 2012) in different spots of the nation, with rural settings, characterized by small holders owning predominantly Zebus (Berg *et al.*, 2009; Tschopp *et al.*, 2010), showing a lower prevalence compared to intensive dairy farms (Ameni *et al*., 2007a).

Table 1. 5 Prevalence studies reported based on DMI in ascending prevalence value

Authors	Study set up	Test	Sample size	P
(Regassa *et al*.,2010)	Abattoir	DMI	1023	1.1
(Asseged *et al*.,2004)	Abattoir	DMI	1350	1.5
(Gebrezgabiher *et al*.,2014)	Abattoir	DMI	768	2.6
(Bekele and Belay,2011)	Abattoir	DMI	780	2.7
(Teklu *et al*.,2004)	Abattoir	DMI	751	4.5
(Ameni *et al*.,2010a,b)	Abattoir	DMI	1138	5
(Mekibeb *et al*.,2013)	Abattoir	DMI	500	5
(Ameni and Wudie,2003)	Abattoir	DMI	1125	5.2
(Tigre *et al*.,2012)	Abattoir	DMI	1102	5.4
(Woyessa *et al*.,2014)	Abattoir	DMI	1183	5.7
(Ewnetu *et al*,2012)	Abattoir	DMI	720	5.8
(Romha *et al*.,2013)	Abattoir	DMI	582	5.8
(Aylate *et al*.,2013)	Abattoir	DMI	1029	6.1
(Zeru *et al*.,2013)	Abattoir	DMI	768	6.4
(Terefe,2014)	Abattoir	DMI	486	6.8
(Ayana *et al*.,2013)	Abattoir	DMI	487	9.3
(Demelash *et al*.,2009)	Abattoir	DMI	3322	10.1
(Alemu *et al*.,2016b)	Abattoir	DMI	500	10.6
(Alemu *et al*.,2016a)	Abattoir	DMI	500	13.2

Previous prevalence studies based on detailed meat inspection (DMI) have reported bTB in many regions (Asseged *et al.*, 2004, 2000) with prevalence ranging from 1.5 % in Addis Ababa to 13.2% in Gambella town municipal abattoir. High prevalence in central Ethiopia is likely due to the fact that dairy farms in this area keep high numbers of upgraded cattle and crosses, which yield more milk than zebus (Tschopp *et al*., 2013). The disparity of herd prevalence reported among the different studies might be attributed to the differences in the available different epidemiological conditions conducive to the transmission of bTB (such as herd size, the level of intensive husbandry system practiced, the amount of susceptible breeds available in the herd, mobility, and close contact between different susceptible animal species in the study area) (Nuru *et al*., 2015).

1.4. Bovine tuberculosis control measures in Ethiopia

In Ethiopia, where animal TB is widely distributed, control measures are either not applied or applied sporadically, informal milk production with lack of adequate pasteurization is common, and little information is available on direct correlation between *M. bovis* infection in cattle and disease in the human population (Fekadu *et al.*, 2018; Gumi *et al.*, 2011; Gumi *et al.*, 2012a; Jemal, 2016; Kemal *et al.*, 2019; Nuru *et al.*, 2017). These limitations may explain how bovine TB will continue to be a major cause of public health threat (Dabadea *et al*., 2017).

In Ethiopia to date, national control of bTB through test-and-slaughter has not yet been established (Shitaye *et al.*, 2007b), and thus the disease remains largely uncontrolled. Although there is no concerted effort to control the disease at the national level, a few attempts have been undertaken to control bovine tuberculosis at the farm level. Some benefits have resulted from these endeavors. Education efforts regarding the safety of bovine products for human consumption resulted in milk collection associations rejecting milk from infected herds, which encouraged livestock owners to get rid of or segregate their positive animals (Tschopp *et al.,* 2013). Both the published studies and observation of common community actions in Ethiopia revealed that test and slaughter, test and segregation, selling chronically diseased animals, boiling or pasteurization of milk, and improving sanitary and hygienic standards are among the measures that are being untaken for control the disease.

Test-and-slaughter was being practiced by a few dairy herds containing exotic animals and exporting live animals. Some government state farms and private farms showed an apparent improvement in prevalence following tuberculin skin-testing and either segregation of reactors or slaughter as a potential control method (Ameni *et al.*, 2007; Shitaye *et al.*, 2007).

Table 1. 6 Representative state dairy farms practicing major bTB control measures in Ethiopia

Study report	Dairy farms	Control Measures undertaken
Alemu 1987	Mojo state dairy farm	Tuberculin skin test Test and slaughter Test and segregation
Ameni *et al.*, 2007	Holetta National Artificial Insemination Center	Tuberculin skin test Test and segregation Test and slaughter
Grey farm report, 2017	Ethiopian institute of agriculture research (EIAR)	Annual Tuberculin skin test Test and segregation Test and slaughter

Mojo state dairy farm was closed after 50% of the reactors cattle were slaughtered and healthy cattle were transferred to other farms (Alemu,1992). Furthermore, Holetta National Artificial Insemination Center closed its farm as a result of test and slaughter. Likewise, the Ethiopian institute of agriculture research (EIAR) has been practicing test and slaughter regularly in its farms at least once in every one or two years in order to reduce infection incidence (Grey farm report, 2017). Apart from test and slaughter measures, test and segregation resulted in a pronounced prevalence reduction in Holetta farm of the National Artificial Insemination Centre (NAIC) (Figure 1.3), and a high intensity farm in central Ethiopia (Ameni *et al.*, 2007, 2010b).

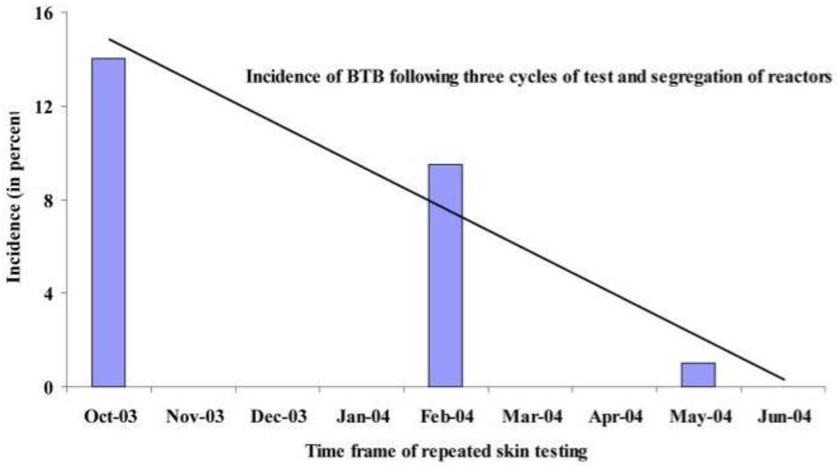


Figure 1. 3 Effect of skin testing and segregation on the incidence of bovine tuberculosis in the Holeta Farm, Ethiopia

Source: (Ameni *et al.*, 2007)

Unlike state dairy farms, in many dairy farms control measures are not frequently practiced. This is especially true in most of the extensive productions systems. Control measures in the traditional extensive production systems are more difficult and complex by virtue of the large numbers of livestock involved, the mobility of animals (pastoral production) and the socio-economic factors involved (Shitaye *et al.*, 2007b). Despite lack of documented studies as to what farmers did with chronically sick animals, sale of apparently sick animals has been reported to be used as alternative control of bovine tuberculosis in small holder dairy farm. Farmers commonly sell of sick and old animals to minimize economic loss due to cull/slaughter (Personal communication). Older animals are more exposed to infection (Cleaveland *et al.*, 2007; Phillips *et al.*, 2002; Tschopp *et al.*, 2009). Culling of old animals (reactors) from a herd might decrease within-herd prevalence, but it is uncommon to test the new animals prior to purchase, so the disease is likely to be spread to emerging dairy herds in regional towns by animal movement. In fact, animal movement is significantly associated with bTB positivity (Tschopp *et al.*, 2009).

Some research has provided valuable information on a cost-effective disease control strategy for reducing this prevalence by implementing BCG vaccination. Vaccination has been found to reduce the proportion of susceptible animals (full protection, direct effect of vaccination) and in likely to reduce onward transmission to other animals (a so-called indirect vaccine effect). A natural transmission experiment was conducted to estimate directly the rate of transmission to and from vaccinated and unvaccinated calves over a 1-year exposure period, and the results show a higher indirect efficacy of BCG to reduce transmission from vaccinated animals that subsequently become infected compared with direct protection against infection with estimated total efficacy of 89% (Fromsa *et al*., 2024). This would have a beneficial impact on disease control in vaccinated farms (Ameni *et al.*, 2018, 2010b). Jemal (2016) recommended that vaccination of calves with BCG, combined with testing and culling, would be an important bTB control and prevention strategy in endemic area like Ethiopia. However, all these measures are found to be insignificant to the cattle population of the country (Shitaye *et al.*, 2007). Hence, there is a need for exploring alternative control strategies such as routine testing and surveillance, pre-movement testing, movement restriction of infected herds, and vaccination, all of which could be combined with better bio-security and farm hygiene (Ameni *et al.*, 2018).

In spite of test and slaughter being the standard control measure for bovine tuberculosis (Schiller *et al.*, 2010), this method is too costly to be applicable in Ethiopia. It is also difficult to enforce culling, because cattle are deeply interwoven with the social system and they are the savings of the rural poor (Michel *et al.*, 2004). The prevalence of bTB is significantly different in various production systems due to environmental and management factors (Collins, 1994; Kiros, 1998; Tweddle and Livingstone, 1994) which makes it difficult to recommend global control and eradication programs. Nowadays significant progress recommendations have been made by different scholars on possible alternative control intervention based on livestock systems (extensive, intensive), breeds (local, exotic, cross-breed), and ecological and geographic factors (Ameni *et al*., 2018, 2010b; Jemal, 2016). Such research underlies the estimate that bovine tuberculosis (bTB) is highly prevalent in intensive dairy farms of the urban “milk-sheds” in Ethiopia.

Even though the increased number of more susceptible exotic breeds together with the increased intensification of production demands has led to prioritization of improved bTB control strategies primarily on intensive dairy farms (Ameni *et al.,* 2018), eradication of tuberculosis on a national scale is possible if there is: full control of all movements of cattle, compulsory identification, payment of compensation for slaughter of positive reactors, compulsory testing of all cattle within specified intervals, establishment of disease free areas, sufficient funds and manpower to fulfill the task it has been stated (Snyman, 1955).

1.5. Human Tuberculosis caused by *Mycobacterium bovis*

Consumption of raw or unpasteurized animal products and contact with infected carcasses plays a large role in zoonotic *M. bovis* infection of humans in Africa and South America (Pirson *et al.*, 2012). In Africa, human TB is widely known to be caused by M. tuberculosis; however, an unknown proportion of cases are due to *M. bovis* (Ayele *et al.*, 2004). None of the national reports submitted to the OIE and WHO by African member states mention the importance of *M. bovis* in human TB cases (Ayele *et al.*, 2004). In Ethiopia, similar to other developing countries, it is difficult to determine accurately bTB occurrence in human due to *M. bovis* as a result of the diagnostic limitations in isolating the micro-organism, and thus the infection in humans is underreported (Ayele *et al.*, 2004; Collins and Grange, 1983). The actual impact of animal bTB on human health is generally considered low in Ethiopia in line with other low-income countries (Amanfu, 2006). Indeed, few studies have also confirmed the disease is zoonotic in Ethiopia, as isolation of *M.bovis* from humans and M. tuberculosis from livestock suggests transmission between livestock and humans (Gumi *et al.*, 2012a).

Table 1. 7 Studies reporting *M. bovis* isolation from humans and M. tuberculosis from livestock in Ethiopia

Affected spp	N	*M. tuberculosis*	*M. bovis*	Authors
Human	42	73.8%	16.7%	(Regassa, 2005)
Cattle	11	18.1%	45.5%	
Human	173	92.5	1.7	(Gumi *et al.*, 2012a)
Cattle	39	2.6	61.5	

There is also evidence of many potential risk factors conducive to the spreading and persistence of bTB in humans. Some of these include eating habits, educational status, demography, living style, socio-economic status, culture, existence of other immunosuppressive diseases, and sharing the same house with animals (Ayele *et al.*, 2004; Girmay *et al.*, 2012; Regassa *et al.*, 2008). Unpasteurized raw milk is preferably consumed than boiled milk in Ethiopian community mainly because of its accessibility, convenience, perceived better taste, and lower price.

A human TB control program has been widely implemented in Ethiopia, but the role of animals in the transmission of the causative agent has been neglected; this neglect could create challenges for an effective control program (Romha *et al.*, 2018). The potential for adverse influence on the country agricultural economics (Pollock *et al*., 2005), particularly the livestock export trade (Demelash *et al*., 2009; Elias *et al.*, 2001), a total loss of income for the dairy farmer due to slaughtering and carcass condemnation (Kleeberg, 1984b; Michel *et al*., 2004), and zoonotic threats to human health (Aklilu and Ameni, 2007; Gumi *et al.,* 2012a; Regassa *et al.*, 2008) warrant the need for incorporating animal TB control programs using a “One Health” approach for effective TB control for both human and animal (Romha *et al*., 2018). Hence, it is an advisable to establish a disease-free area, which enables the country to control zoonotic and economic important diseases including bTB.

1.6. Challenges of bovine tuberculosis control in Ethiopia

There are several factors leading to the failure of low-income countries to control and eradicate bovine TB (Ameni *et al*., 2007): the nature of the disease (chronic nature and long incubation period, absence of an effective vaccine, absence of protective natural immunity to the infection) (Perez *et al*., 2011), socio-cultural factors (lack of knowledge on the actual prevalence of the disease, cultural and traditional beliefs, geographical barriers, social unrest), socioeconomic factors (displacement of human and animal populations, live animal smuggling across boundaries), and host range (re-infection from wildlife reservoirs) All of these factors make control and eradication of bTB a challenge (Ayele *et al*., 2004; Shitaye *et al*., 2007b). Lack of adequate policies and funding are also a factor, such as control program design (failure in the design and implementation of diagnostic and intervention measures, lack of veterinary expertise and communication networks, insufficient collaboration with bordering countries, lack of quarantine) and logistic factors (lack of financial and human resources, high cost of sustainable testing program, lack of veterinary infrastructures).

Furthermore, underreported human infection has hidden the importance of *M.bovis* in human TB cases. Underreporting is a result of the diagnostic limitations of many laboratories in distinguishing *M. bovis* from *M. tuberculosis* (Ayele *et al.*, 2004). Many countries also lack awareness, knowledge, and information on the distribution, transmission, and risk of zoonotic diseases, which has made the control and prevention of diseases like bTB in humans and animals difficult. Risks for zoonoses are considered negligible compared with those for human-specific diseases because the societal consequences of zoonoses are not generally recognized by the individual sectors. However, transmission of zoonoses to humans can be greatly reduced by health information and behavior (Zinsstag *et al*., 2007).

1.7. Economic Estimation of Bovine Tuberculosis to the Society

In Ethiopia, representative studies showing its prevalent (Aklilu and Ameni, 2007; Ameni *et al*., 2007; Berg *et al*., 2015; Elias *et al*., 2008; Gumi *et al*., 2011, 2012b), dynamics of transmission over time for the whole country (Gumi *et al*., 2011, 2012b; Tschopp *et al*., 2010) and characterization with molecular typing methods (Ameni *et al*., 2010a; Berg *et al*., 2015) were undertaken. In addition of being a zoonotic threat, bTB is also an economical and financial burden to society, which may be economically devastating for the cattle industry, especially the dairy sector (Zinsstag *et al*., 2006). Regardless of great burden in developing countries, detailed reviews on the economic burden of bovine tuberculosis and the benefits of its control tend to focus only on experiences from specific industrialized countries, where control and eradication programs have been implemented, and all data suggested that worldwide economic losses due to the disease are significant. Indeed, the economic impact of bTB on livestock production is extremely difficult to determine accurately (Bemrew *et al*., 2015). Apart from projecting the possible economic implications or enormous losses bTB could inflict on the society and country’s livestock economy based on high prevalence of the disease, the economic impact of BTB on cattle productivity, bTB control programs and other related economic effects of the disease are not well enumerated and documented in Ethiopia (Shitaye *et al*., 2007).

Onwards, only a few economic studies tried to show the effect of infection on livestock productivity, on human health and human-health related costs including income loss in order to estimate the cost of bovine TB to the Ethiopian society (Tschopp *et al*., 2013b, Shewatatex, 2015). Though uni-sectoral (Abattoir) economic analysis of the disease, (Abel,

1989) reported that out of 29 956 slaughtered cattle in Dire-Dawa city abattoir, a total of 31.2% and partial of 16.4% condemnation rates that may result in economic losses significantly. Lately (Gezahegne, 1991) demonstrated that from 1.2 million slaughtered cattle in eight export abattoirs had an estimated cost of more than 600 000 ETB (300 000 USD) during a respective time, resulted due to condemned carcasses and organs (Asseged *et al.*, 2004) demonstrated that, based on the ten years retrospective analysis of the detection of bTB lesions in the Addis Ababa abattoir, there was a cause of 0.024% for whole carcass condemnation.

Recently Tschopp *et al.*, (2013b) estimated the cost of bTB to Ethiopia with the aim of informing Ethiopian policy on options for bTB control. The study shows no measurable loss in asset value or cost of disease due to bTB in rural and urban production systems in Ethiopia. This does not mean that there is not a real cost of disease, but the variability of the productivity parameters and prices are high and would require more precise estimates. This was the first comprehensive cost estimate of bTB to African livestock production systems. The weakest element of this study was that there was no African estimate of losses of bTB to African cattle production. Furthermore, (Shewatatex, 2015) tried to estimate economic impact of bTB using propensity score matching (PSM). Accordingly, in which the Propensity Score Matching (PSM) estimates had a difference between bTB positive and bTB negative animals on milk yield, 3.84 litre milk yield loss per day per cow is due to bTB infection and the dairy farm owner loses 9,992 birr annually due to bTB infection.

1.8. Mathematical Modeling of Bovine Tuberculosis

Use of mathematical models to study the transmission dynamics of infectious diseases is becoming increasingly common in veterinary sciences. However, modeling chronic infectious diseases such as bovine tuberculosis (bTB) is particularly challenging due to the substantial uncertainty associated with the epidemiology of the disease (Álvarez *et al.*, 2014). Bovine tuberculosis remains largely uncontrolled in the country level in Ethiopia. With no control and preventive measures in place, bovine tuberculosis continues to cause substantial economic and public loss to the poor farmers and to the nation from ban of livestock and livestock product trade. Studies in South Africa showed that social contact prompted the fast transmission of bTB infection from one animal to another (De Vose *et al.*, 2001; Renwick *et al.*, 2007). Transmission of bTB infection from one animal to another was increased when animals from different herds were put into one grazing area where some animals were infected *http://www.krugerpark.co.za/krugerpark-times-4-6-bovine-survey-24294.html*. The mixing of infectious animals with non-infectious animals was the most prominent mode of transmission of bTB infection.

There is no single intervention that will on its own achieve control of the bTB epidemic unless construct disease control model around all routes of transmission of the disease (https://veterinaryrecord.bmj.com/content/174/15/367), and apply disease eradication and control program. A typical strategy for disease control in domesticated 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 within the herd (Kao R. R. *et al*., 1997). Mathematical modeling is used for the study of complex phenomena such as the dynamics of infectious agents (de Jong, 1995). Diseases with a long incubation period, such as bTB, are difficult to study experimentally or in the field because of prolonged waiting times for obtaining results and the high costs of conducting experimental studies (Perez *et al*., 2002). Mathematical simulation models offer the possibility to test a range of control programs in a short time and to identify the most effective one (Perez *et al*., 2002). Epidemiological models are key tools for designing and evaluating detection and control strategies against animal infectious diseases (Bekara *et al.*, 2014). The ability of countries to control and eradicate bovine tuberculosis (TB) has been jeopardized by various epidemiological and ecological features of the disease (Perez *et al*., 2011). Through epidemiological modeling, the risk of a major outbreak can also quantified, using different surveillance strategies (Fischer *et al*., 2004). Mathematical models of different forms incorporating the environmental factors and movement factors are developed and analyzed to help us to extract insights into the transmission dynamics of the disease. The knowledge gained will help policy makers to apply the correct intervention strategies to minimize the effects of the infection (Patrick, 2015).

CHAPTER TWO: RESEARCH OBJECTIVES

2.1. Research Rationale

In Ethiopia bovine tuberculosis have rapidly spread and mainly affected cattle in the last recent years than ever. Despite its endemicity, inadequate disease surveillance system, lack of diagnostic facilities, inadequate financial resources and qualified manpower and lack of prioritization from government made little to known about the dynamics of the disease in cattle. This has also made bTB control and quantification of economic and public health impacts of the disease difficult. Thus, the situation has led to a significant human health problem as well as a liability for the cattle industry throughout the country, and the control of tuberculosis in both humans and cattle would benefit from the design of a model and its cost estimation on feasibility of bTB control in Ethiopia. Therefore, this research tried to address some of the listed research gaps in one or another way.

2.1.1. Scope and hypothesis of the research

The study was designed to evaluate the existing and the proposed alternative control interventions using mathematical modeling as a decision support tool following test and slaughter intervention, by testing the hypothesis that the envisaged alternative control interventions clear the infection with low number of whole herd tests (WHTs), days to infection clearance, and minimal knock-on effects on herd demography. The hypothesis that herd demography changes (new entry and exit animals) contribute to a poorer response to infection clearance was also tested.

The study also hypothesized that gaps in biological factors (inconclusive SICCTT test results, inconsistent test and slaughter, prolonged test intervals) and non-biological factors (knowledge, attitude and practices) do impact the control of bTB, if addressed, could help the policy makers and researchers to design effective and adaptable intervention strategies.

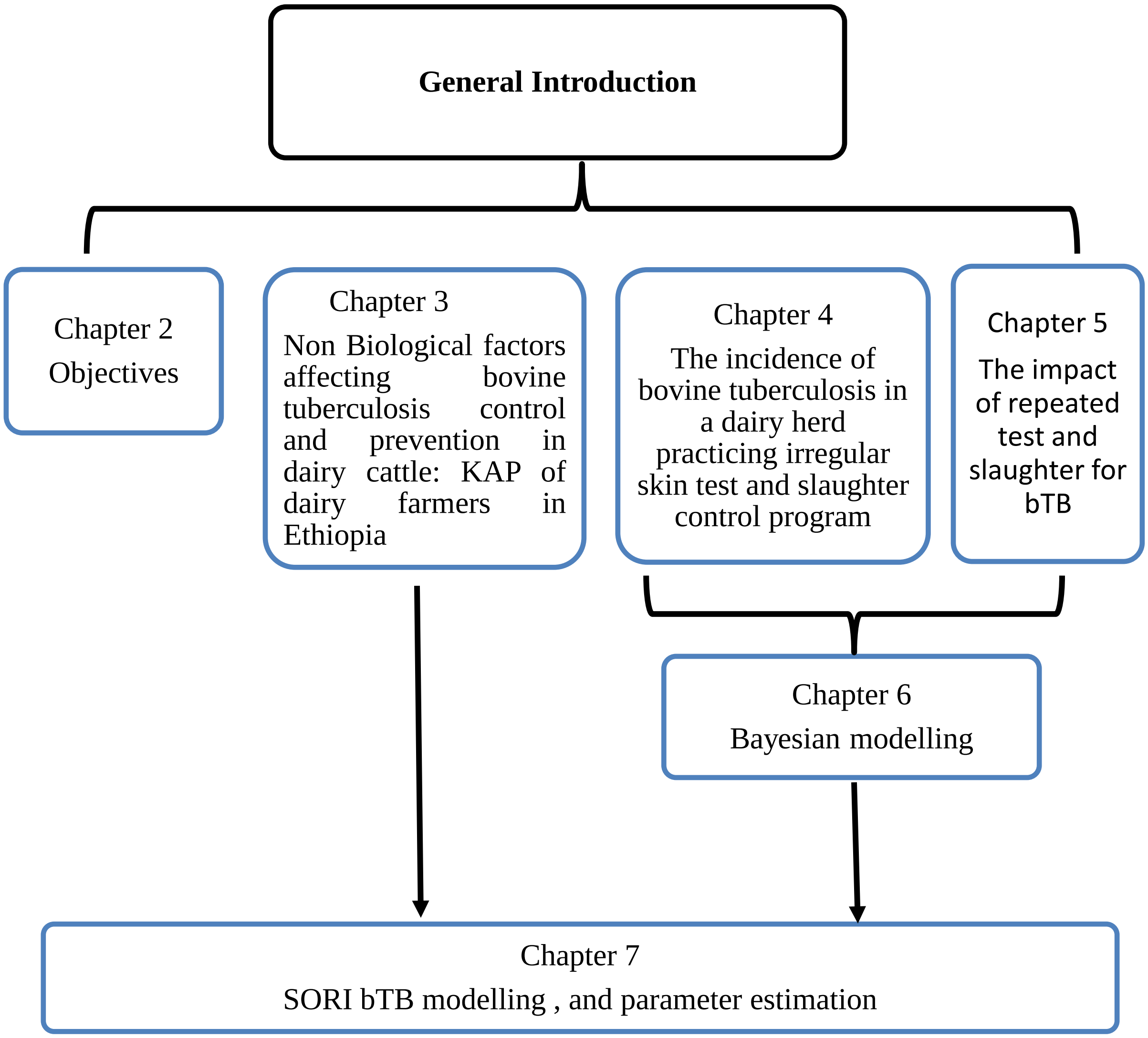
Moreover, the scope of this research was parameter estimation using simulation for Ethiopian dairy herds from selected dairy herds. Consequently, developing adaptable and tailored bovine tuberculosis disease intervention models.

2.2. Objectives

The general aim of this PhD research is to generate information using modeling as a decision support tool for future bTB control options and to evaluate factors that might hamper the control of the disease. This PhD work aims also to provide relevant data for the implementation of a model-based disease control system in order to optimize resources. In order to accomplish this general aim five specific objectives are developed:

- ✓ To assess farmers' knowledge and attitude associated with bTB, and identify practices that potentially contribute to remain the disease endemic in selected milk-sheds of Ethiopia
- ✓ To assess the incidence of bTB, and identify some determinants associated with the odds of becoming a reactor in a dairy herd practicing irregular test and slaughter
- ✓ To examines the existing control measure would take over *M.bovis* infection from the studied herds, identify any limitations for its incompetence, and assessed its impact on herd demographic change
- ✓ To estimate the true prevalence of bTB in the study herd and test characteristics (sensitivity and specificity) of SICCTT
- ✓ To estimate parameters related to bTB transmission, model within herd bTB transmission dynamics, alternative control intervention options and their feasibility

2.3. Thesis outline



CHAPTER THREE: NON-BIOLOGICAL FACTORS AFFECTING BOVINE TUBERCULOSIS CONTROL AND PREVENTION IN DAIRY CATTLE: KNOWLEDGE, ATTITUDE, AND PRACTICES (KAP) OF DAIRY FARMERS IN ETHIOPIA

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Abstract

Bovine tuberculosis (bTB) is a serious animal health problem in Ethiopia, ranking among the top three livestock diseases. If conditions that favor the transmission and persistence of bTB are not addressed, the situation will get worse; hence bTB control should be priority. However, interventions are influenced by several "non-biological" factors. Cross-sectional study was conducted to assess farmers` knowledge, attitude and practice (KAP) using a structured questionnaire-based approach. Multi-stage sampling procedure was employed to select 307 study participants. Accordingly, 55% of participants knew about bTB; of which 36.4% knew basic information, and the remaining (18.6%) knew nothing except the term bTB. Less than 2.0% of the farmers knew about zoonotic importance of bTB. The knowledge among dairy farmers varied depending on herd size, milk-shed, training availed, veterinary consultation and their years of farming experience. Using multivariable analysis, farmers from medium and large-scale farms knew more about bTB than those from small scale farms, by a factor of 2.8 and 7.7 respectively. Similarly, farmers who had been farming for more than 6 years and farmers from Selale milk-shed had higher odds of being knowledgeable about bTB, by 5.7 and 10.4 times respectively, compared to other milk-shed. Only 12% of participants were aware of test and slaughter method, likewise, 18% of farmers tended to avoid buying cattle from risky sources. The finding revealed a dearth of knowledge on the production loss incurred (12%) and the probability of human infection (1.9%), instead a substantial number of farmers (25%) believed that bTB infection could badly affect the dairy market. These limitations may explain how bTB will continue to be a major threat. All these negatively impact disease surveillance and control programs. In conclusion, the present study highlighted knowledge, attitude and practice gaps which, if addressed using tailor-made training, might assist in reducing the disease consequence.

Keywords: Bovine tuberculosis; Control; Ethiopia; Farmers; KAP; Milk-shed

3.1. Introduction

Although still present in some industrialized countries, nowadays bovine tuberculosis mostly affects developing countries lacking the resources to apply expensive test and slaughter schemes (Bemrew *et al.,* 2015). Recently, bovine tuberculosis is one of the top three, high-priority, livestock diseases in Ethiopia (Lakew *et al.,* 2022). The existence of potential associated factors conducive for the spreading and persistence of bTB would make the situation worst (Ayele *et al.,* 2004; Girmay *et al.,* 2012; Regassa *et al.,* 2008), and hence control of bTB should be a priority.

Bovine tuberculosis is a poorly studied and widely underreported zoonoses but is believed to be a significant contributor to animal and human losses (Grace *et al.*, 2012; Liverani *et al.*, 2013). In Ethiopia, the number of *M.bovis* infections in humans has been reported to be low, despite likely to be substantial and high possible health risk because of the high prevalence of the diseases in livestock (Ayele *et al.*, 2004; Kemal *et al.,* 2019).

According to the World Health Organization (WHO), there is no single intervention that can effectively control bTB by its own, unless able to block all routes of transmission of the disease (WHO, 2023). Conventionally, a typical strategy for bTB control in domesticated animals involves regular field tests and slaughter of infected herds. However, beyond high cost incurred , these interventions are influenced by several “non-biological” factors (Ciaravino *et al.,* 2017). For instance, farmers are the actors who regularly interact with veterinarians, and who comply with (or resist) the legislative basis and biosecurity practices recommended or enforced for disease control. They own the animals which succumb to infection; determine their husbandry and welfare; buy and sell them; present them for disease testing; and take responsibility for financial loss for affected animals (Robinson, 2017). Hence, the knowledge, attitudes and behaviors of dairy farmers is a key factor for successful disease control and surveillance systems (Brennan *et al.,* 2016; Pfeiffer, 2006).

Most high-income countries have successfully controlled bTB based on test-and-slaughter programs, alongside slaughterhouse surveillance, and trade and movement restrictions of affected herds (Napp *et al.,* 2019; OIE, 2019). This can be considered impractical to adopt in developing countries due to extensive socioeconomic significance where replacement of equivalent breeding stock might be excessively unaffordable (McCrindle and Michel, 2007). Therefore, the need for evaluation of potential control strategies is critical to minimize the impact of the disease (Lakew *et al.,* 2022). In previous years, social factors have not been often given enough attention in the implementation programmes of animal health. Inversely, the impact of social factors on public health interventions is well known and these aspects have been accounted for in several human medicine studies (Bach *et al.,* 2017; Berkman *et al.,* 2014).

Recently, the situation has changed and social factors have become more relevant for the control of animal diseases. Studies have shown that it is crucial to understand how the different actors involved think and act, since their attitudes and behaviors affect the effectiveness and sustainability of such programmes (Brunton *et al.,* 2018; Catley *et al.,* 2012; Enticott *et al.,* 2015).

Nowadays bovine tuberculosis is an endemic disease in Ethiopia because of dearth of awareness, policies and resources. Measuring the knowledge, attitudes and practices (KAPs) of farmers is a significant step when developing and implementing disease control and prevention strategies (Balkhy *et al.,* 2010; Brennan *et al.,* 2016).

The knowledge, attitudes, and practices (KAPs) of bovine tuberculosis are not adequately investigated in Ethiopia. Many of previous studies however, were fragmented report conducted by different scholars to address only some specific problems, therefore these were noted to have limitations either in producing a more comprehensive and representative pooled country level picture, or to generate detailed information that could remarkably impact the application and effectiveness of interventions against bTB, for one or the other reasons including, the scope of study objectives, methodology used, target population and geographic coverage. As a result, additional all-inclusive investigations on the potential source of infection, public health and economic burden of the disease, the transmission ways, and its control measures are essential to attain a comprehensive and accurate insight into the subject (the gaps in knowledge, attitude and practice) which, if addressed, could help the policy makers and researchers to design effective and adaptable intervention strategies. With this understanding, a cross-sectional study was designed to assess farmers' knowledge and attitude associated with bovine tuberculosis, and to identify farm practices that potentially result in the disease remaining as endemic in selected milk-sheds of Ethiopia, where more commercial dairy farms located.

3.2. Material and methods

3.2.1. Study setting and sampling procedure

A multi-stage sampling procedure (purposive and random) was applied for the study. Given excelling up and intensification of dairy farming, the four milk-sheds (MS) of Asela-MS, Debre-birhan-MS, Selale-MS and Others-MS were selected for their substantial dairy farm population, different socio-economic and cultural features and facilities, operational safety and convenience. Since farmers` knowledge towards bTB and their possible determinants may vary in different areas, the study was conducted in the above four different settings. Here Selale-MS covers the areas from Addis Ababa following major highway passing through these districts connecting to Muke-turi; Debrebirhan-MS extends from the national capital to the city of Debre-birhan in the north; Asela-MS spans the area from Addis Ababa through Bishoftu and Adama to the town of Asela.Others-MS consists of commercial dairy farms from various regions of Ethiopia (Diredawa, Bahirdar, Gonder, Hawasa, Kombolcha, Harbu /South Wollo). The number of selected farms from each milk-shed ranged between 35–108 depending on the number of commercial dairy farms (semi-intensive and intensive dairy farms) found on proximity to the main highway with good road access. With regard to herd size, a balanced number of small, medium and large scale farms were selected. The classification of herd size into small (1–3 dairy animals), medium (4 − 10 dairy animals) and large farms (more than 10 dairy animals) was made according to the classification made by an FAO report (Sharma *et al.*, 2003). Hence, as part of the survey, we interviewed 111 large scale, 101 medium scale and 95 small scale commercial farms. A simple random sampling technique was applied to choose dairy farms from each market-sheds.

Number of the study participants was calculated using the formula

$$N = \frac{Z^2 P(1-P)}{d^2}$$

on the basis of the 24.1% awareness of dairy farmers with 95% level of confidence (CI) and 5% precision in the estimates. Then, the total sample size was determined as 282 dairy herds, and to account for an assumed small design effect, we aimed at 307 farmers.

The participants were identified from different randomly selected villages along the study milk-sheds based on their availability and willingness to participate.

3.2.2. Data collection and interview procedure

Using set of questions, we gathered the socio-demographic and epidemiological data on the determinants that influence the knowledge, attitude and practice of farmers regarding bTB. This included the age, gender, educational level, participants` role in the farm, training availed, veterinary consultation and farming experience of the participants.

Knowledge and attitude on bTB were assessed by asking whether they had heard or knew about bTB disease or not. The farmers, who knew about bTB were further asked some additional questions related to bTB detection, its impacts, transmission, the potential source of infection for their herds, and control measures through a set of structured questions. Their practices relevant to prevent their herd from getting bTB infection, frequent measures they had taken on reactors and farmers` priority level/ concerning for disease intervention comparing to other diseases were also assessed accordingly. Furthermore, information on the study herd size, animal husbandry practices, history of exposure to bTB, their prevailing knowledge and practices towards bTB management, and animal movements entering and exiting the herd were collected.

The assessments were through open questions. The questionnaire was pretested, and interviewing method was validated to have a good insight in commercial dairy farming in the study area. During the pre-test, the questions were evaluated to make sure that the farmers understood them correctly. Using enumerators, the dairy animal managers or the responsible person from the farming community were questioned in their local language. The enumerators also filled an observation checklist during the visit. The farmers were informed about the purpose of the study at the beginning of the interview and the interview began once we got their consents.

3.2.3. Data analysis

The final data was recorded and analyzed using SPSS 16.0. Descriptive statistics were generated for each variable of interest. The percentages and their 95% confidence intervals (95% CI) were calculated. Characteristics of the respondents in terms of farm size, age, gender, level of education and farming experience were examined and presented as percentage and frequency. The selection of variables to be included in the multivariable model was carried out in two steps. Initially a univariate logistic regression model was built to identify important covariates or determinants that are at least moderately associated with farmers' knowledge, attitudes and practices where the candidate variables for which $p \leq 0.2$ were selected for further analysis based on the wald test. The multicollinearity between pairs of explanatory variables was assessed by a correlation matrix. The significance of this association was examined using chi square test. In the case of a pair of variables with a significant association ($p < 0.05$), the variable judged as the most biologically plausible was used as a candidate in the multivariable analysis. All variables passed the previous 2 steps were incorporated in the final multivariable logistic regression model. A manual stepwise selection approach was used for the selection of variables in that model using backward variables selection techniques to keep only variables with $p < 0.05$ in the final model.

3.3. Results

3.3.1. Demographic characteristics of the respondents

The socio-demographic profile of the respondents is summarized in Table 3.1. Almost two third (65%) of the study participants were found on the age ranged between 31–60 years. Male respondents constituted 69.4%, of which 59.6% (127/213) engaged actively on major dairy farming practices. Only 27.7% of respondents were currently unmarried. Regarding to occupational status of the participants, majority of the respondents, 43.3% (133/307), were owners of the dairy farm, followed by 31.3% (96/307) employed staff. However, a notable percentage of 25.4% (78/307) were unemployed family member.

Table 3. 1 Socio-demographic characteristics of study participants

Variable	Category	Frequency	Percent
Age (years)	18-30	71	23.1
	31-45	98	31.9
	45-60	101	32.9
	>60	37	12.1
Gender	Female	94	30.6
	Male	213	69.4
Marital status	Married	222	72.3
	Unmarried	85	27.7
Occupational status	Employed	96	31.3
	Unemployed/family	78	25.4
	Owner	133	43.3
Roll in the farm	Actively engaged	179	58.3
	Supportive staff	116	37.8
	Partime/occasionally	12	1.6

3.3.2. Farmers` awareness on bovine tuberculosis

Nearly 45% of the dairy farmers were not aware of bTB disease or never heard the name of the disease. 36.4% farmers reported that they knew some fundamental information about bTB, and the remaining 18.6% farmers had heard about bTB but did not know anything in detail (Figure 3.1). The association between independent variables and knowledge of participants on bTB are shown in (Table 3). Milk-shed, herd size (scale of the farm), previous experience, training and veterinary consultation had significant association with participants` awareness towards bTB (Table 3.2). In Selale milk-shed, more farmers 80% (67/83) knew about bTB than Debre-birhan milk-shed (75%) or other milk-sheds (34%). The most common knowledge reported was that bTB could affect cattle and it could transmit from infected animal to healthy one. The source of knowledge was mainly through training (46%), previous experience in dairy farming, and consultation from veterinarians (52%).

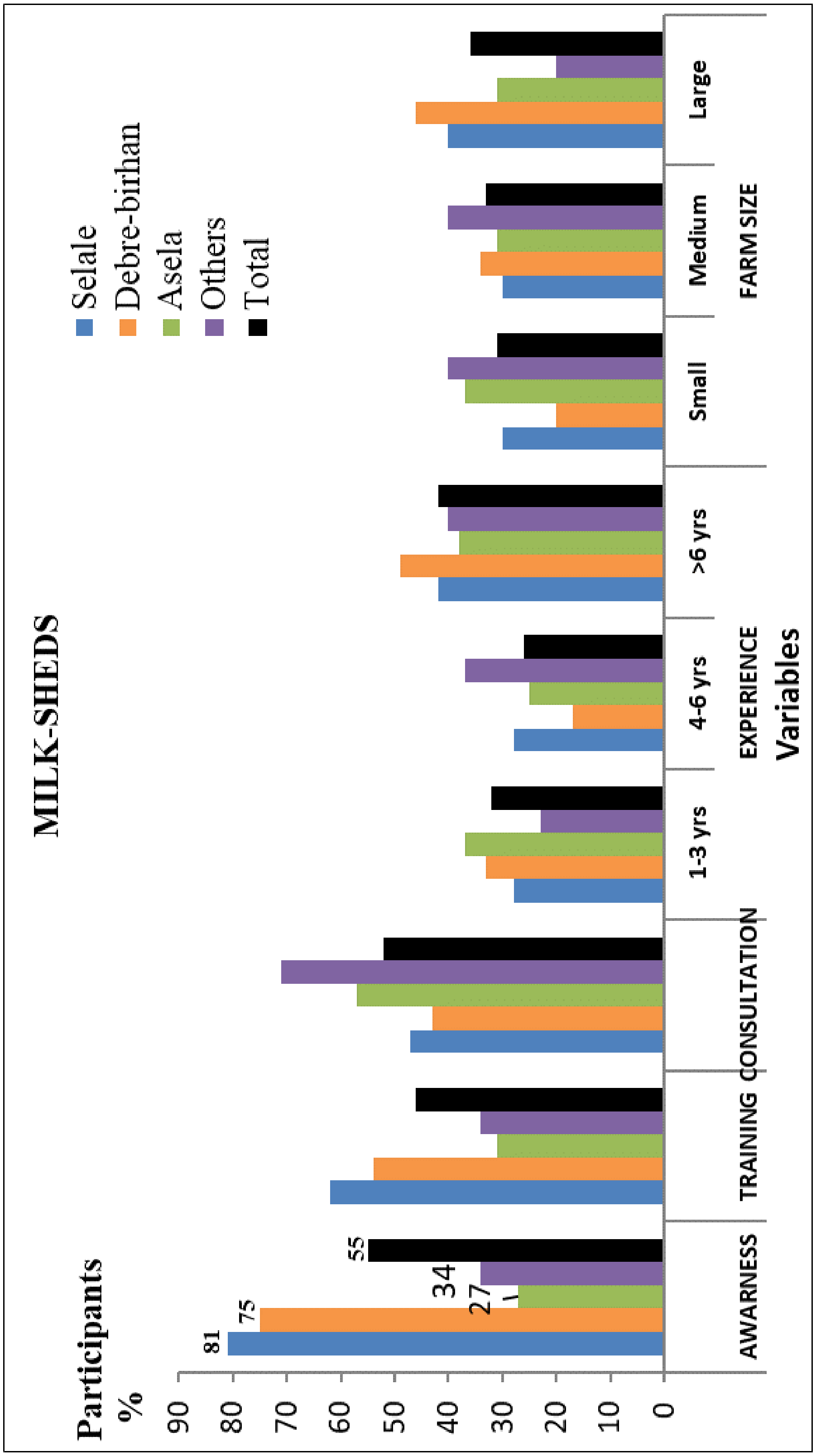

Figure 3. 1 Determinants along the study milk-sheds

Table 3. 2 Variation of farmers' knowledge among milksheds, educational level, herd size variation, training exposure, previous experience, and veterinary consultation

Variable	N	χ^2-Value	df	P value
Milk shed	307	76.34	3	0.000
Educational level	307	24.94	2	0.000
Herd size	307	42.2	2	0.000
Training	307	67.98	1	0.000
Veterinary consultation	307	10.22	1	0.001
Previous dairy farming experience	307	28.55	2	0.000

Multivariable logistic regression analysis revealed that farmers` knowledge towards bTB in medium and large sized farms was almost 2.8- and 7.7-times higher comparing with small sized dairy farms, respectively (Table 3.3). Dairy farmers who had > 6 years of farming experience and farmers from Selale MS was also significantly associated with the knowledge about bTB (odds 5.7 and 10.4 times higher in case of more experienced farmers and farmers from Others MS).

Table 3. 3 Results of multivariable analysis of selected determinants having association with farmers' knowledge towards bTB

Determinants	**P-value**	**OR**	**95% CI**	
			Lower	**Upper**
Farmers from Selale MS compare to Others MS	0.000	10.4	3.8	28.9
Farmers from Debre birhan MS compare to Others MS	0.001	5.8	2.2	15.7
Medium size (4–10 dairy animals) farms compared to small size (1–3 dairy animals) farms	0.005	2.8	1.4	5.9
Large size (>10 dairy animals) * farms compared to small size	0.000	7.8	3.6	16.5
Farmers with 3 ≤ 6 years of farming experience compare to ≤3 years of experience	0.008	3.0	1.3	6.8
Farmers with> 6 years of farming experience compare to ≤3 years of experience	0.000	5.7	2.8	11.5

3.3.3. Farmers` knowledge regarding bTB control measures, ways of transmission, its impacts and diagnosis.

About 38.8% (117/301) of the participants knew at least something to control and prevention methods, of which 30% (36/117) knew test and slaughter method as a typical strategy for bTB disease control and prevention. Farmers` knowledge on bTB control was significantly associated with market sheds, herd size and previous farming experience ($p < 0.05$).

Concerning its modes of transmission, 42.3% (130/307) of participants had some knowledge on how bTB spreads. Knowledge on ways of bTB transmission is summarized in Figure 3.2. Participants from Selale MS, as well as participants from large scale dairy farms had more level of knowledge on bTB disease transmission ($p<0.05$). Similarly dairy farm experience had significant association with disease transmission knowledge.

Less than 2% (1.88%) knew the possibility of human infection from infected animals. Only 1.6% farmers knew skin test as common diagnostic tool for bTB. Similarly, majority of the respondents did not know its impact on milk and meat production loss (Figure 3.2).

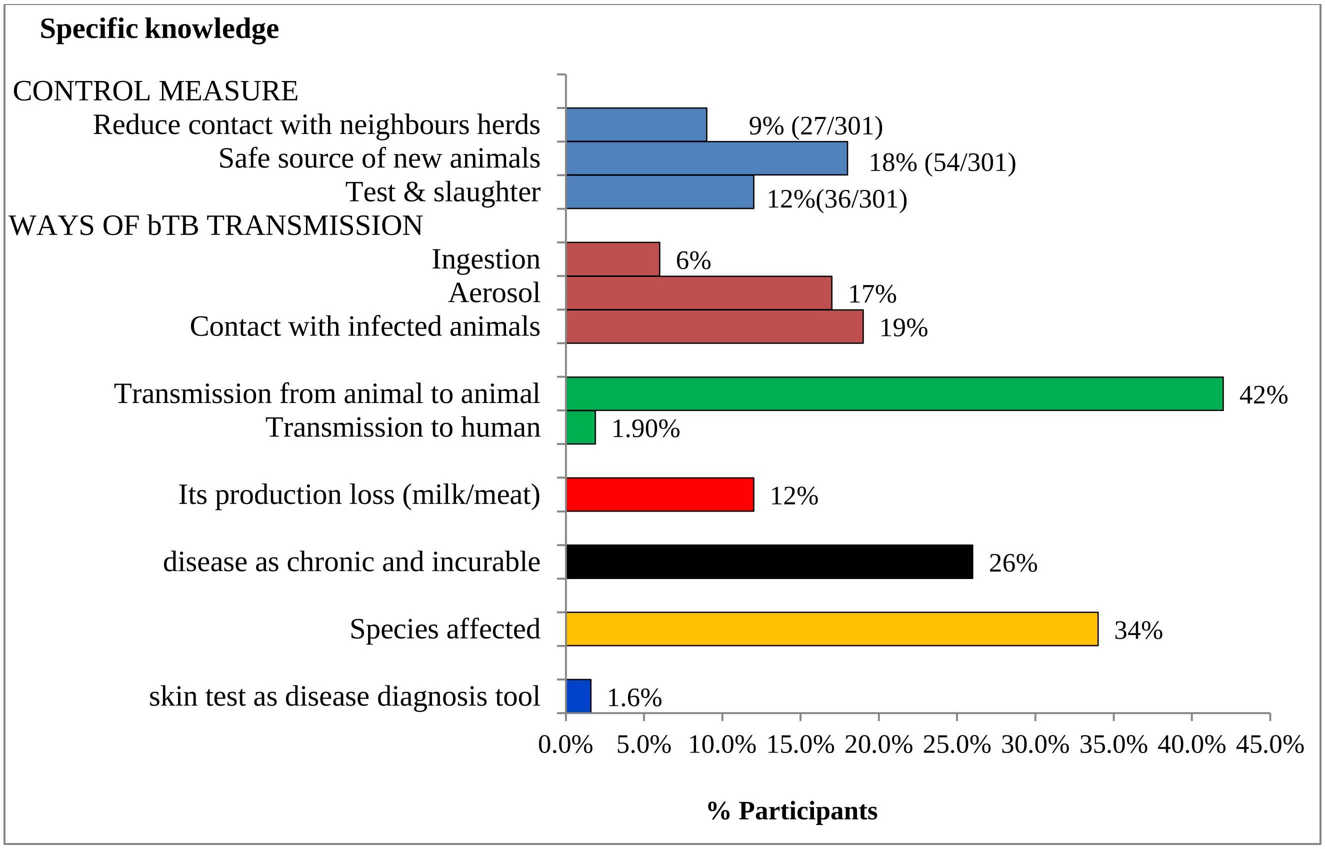

Figure 3. 2 Detail knowledge of participants on bTB control measures, transmission ways, and feature of the disease

Moreover, participants responding aerosol as modes of bTB disease transmission comparing to those who replied as transmission through contact was approximately 3 times higher in trained participants compared to those not receiving training.

Table 3. 4 The influence of farmers` previous training exposure on their response to the type of mode of transmission

Farmers' knowledge about mode of transmission	N	OR	CI	P-value
Contact with infected animal	59	-	-	-
Aerosol	51	3.02	[1.27, 7.14]	0.012
Ingestion	20	4.17	[1.10, 15.78]	0.036

3.3.4. Attitudes, and practices towards bTB

The results for the responses to questions relating to farmers' attitudes and practices are presented in Table 5 and Table 6. Despite only 12% of participants being aware of the production loss incurred by bTB infection, one fourth of the farmers (25%) believed that bTB infection could have a negative impact on the dairy markets.

Surprisingly, almost none of the respondents (0.98%) believed test and slaughter methods as feasible and applicable control and prevention measure in reality. Approximately one fifth of the farmers (22%) thought that improving the awareness of the community towards bTB is an important element for effective prevention and control measures. Similarly, 16.3% of respondents mentioned that government or other legal bodies should provide disease free heifers for sale in order to avoid purchasing of cattle from unknown or potentially infected sources. Even if very few farmers were aware of skin test as a diagnostic tool to know whether their animals being infected or not, none of them reported skin test as reliable.

Roughly, 21% (64/307) of the farmers listed new animal entry, neighboring dairy farms, and people, equipment and vehicles entering from infected sources as potential sources of infection for their herds, of which introduction of new animal (62.5%) taking a lion share.
Using univariable analysis, farmers' perception as the primary cause for the introduction of disease into their herd through new animal entry comparing to farmers who do not know the cause was approximately 12.8 times higher in trained farmers. Similarly, farmers recommending nothing to do for bTB reactors or to sell for others comparing to those advice to slaughter for meat consumption was approximately 11.8 times and 4.95 times higher in untrained farmers, respectively.

Table 3. 5 Results of univariable model for the association between farmers' attitude and previous training exposure (N=307)

Attitudes	Response	OR	P	95% CI

Potential source of infection for your herds			
Don`t know			
Neighboring herds	5.03	0.001	[2.11, 11.96]
Contaminated utility from infected sources	2.9	0.032	[1.09, 8.16]
New animal introduction	12.8	0.000	[6.40, 25.82]
What do you suggest to prevent or control bTB as a country wise?			
Government should provide disease free heifers for sale	2.88	0.001	[1.51, 5.49]
Strict animal movement restriction governed by rules and regulations	15.85	0.000	[3.48, 72.2]
Awareness to dairy farmers about the disease	3.66	0.000	[2.04, 6.57]
What do you recommend to reactors?			
Slaughter cattle for meat	-	0.001	-
Slaughter & buried/burn	2.59	0.019	[1.17, 5.74]
Sell the cattle	4.95	0.002	[1.81,13.51]
Nothing done	11.88	0.024	[1.39,101.33]
How could bTB affect your farming in your opinion?			
Reduce milk production	-	0.48	-
Loss from culling animals	1.11	0.88	[0.275,4.51]
Marketing	1.65	0.24	[0.71,3.85]

Among those who knew bTB, only 1.6% dairy farmers considered bTB as a prioritized animal health problem, which made farmers (54%) nothing to apply as control and prevention measures. On the other hand, mastitis (50.8%) and abortion (35.5%) were their concern to tackle. Almost none of the farmers experienced skin test before new entry animals to protect their herd from getting bTB infection; instead, 26.5% farmers preferred not to buy cattle from unknown or potentially infected sources.

Disregard of their knowledge and attitude for control and prevention measure (test and slaughter), sale of reactors are the common measures taken by dairy owners. In addition, 77% of farmers did nothing for those reactors.

Table 3. 6 Farmers' practices relevant for control and prevention of bTB

Variable	Frequency	Percentage
Have you ever done bTB test? (n=307)		
Yes	36	11.7
What measure did you take on reactor animals? (n=9)		
Nothing done	7	77.8
Sale	2	22.2
Which disease/problem is your priority of concern to intervene in your farm? (n=307)		
Mastitis	156	50.8
Abortion	109	35.5
Bloating	35	11.4
bTB	7	2.2
What prerequisite practices you usually do to protect your herd from getting bTB infection? (n=307)		
Nothing done	218	71
Screening test/skin test	2	0.6
Not buying cattle from unknown/risky sources	87	28.3

3.4. Discussion

Robinson (2017) tried to express how important are the attitudes and behaviors of farmers in relation to disease control. Non-biological factors such as farmers' knowledge, attitudes and practices, which are useful to identify knowledge gaps, cultural and behavioral differences (Zahedi *et al.*, 2014), may remarkably impact the effectiveness of interventions against bovine tuberculosis (bTB) (Ciaravino *et al.*, 2017). Therefore, the data collected provided a valuable intuition into the awareness and approaches of farmers towards bTB.

Our result indicated that 55% of participants knew about bTB, which is nearly in line with the finding of Hailu *et al.*, (2021), conducted in Bahir Dar City, who reported that 57.2% of the respondents were knowledgeable about bTB and its derivatives. Unlike our findings, high percentage (65.0%) of commercial dairy farmers in Zimbabwe had knowledge about bTB (Mosalagae *et al.*, 2011).

Comparing to the previous study (13.9% of respondents reported familiarity with bTB) conducted in Addis Ababa (Kidane *et al.*, 2015), our finding is a high proportion even if the participants in the later one were high school students, who might lack many source information for bTB such as technical training and vet consultation. This result could be one of the top reports on bTB awareness in Ethiopia comparing to the previous studies reported as 24.1% by Bihon *et al.*, (2021); 35% by Ameni and Erkihun (2007) in Adama; 45.6% by Kuma *et al.*, (2013) in Jimma zone; and 37.1% by Tigre *et al.*, (2011). These may be attributed to the respondents' vicinity to Addis Ababa, large and medium scale intensive farm inclusion, and participants with long years of dairy farming experience. All the aforementioned conditions create conducive environment for networking with governmental and non-governmental organizations (NGOs) that offer diverse alternative information avenue, such as technical training and consultation from animal health experts, farmers` experience sharing and educational campaigns.

Clearly, 18.6% of our participants did not know anything except the term bTB. Therefore, considering only 36.4% of the participants who had some basic knowledge about bTB, our result agrees with the research by Ameni and Erkihun (2007) in Adama, and by Tigre *et al.*, (2011) in Jimma with 35% and 37.1% awareness level, respectively.

Furthermore; knowledge on the potential source of infection, public health and economic burden of the disease, the transmission ways, and its control measures are critical for developing and implementing disease control and prevention strategies (Balkhy *et al.,* 2010; Brennan *et al.,* 2016). Farmers are supposed to play an important role in utilizing this knowledge, and the one who comply with (or resist) the legislative basis and biosecurity practices recommended (Robinson, 2017). However, majority of dairy farmers do not know a typical strategy for bTB control involving regular field tests and slaughter of infected herds (Kao *et al.,* 1997). Others (Massó Sagüés *et al.,* 2019) also identified that the animal movement, specifically introduction of new animals was one of the significant risk factors for disease entry into dairy herds. In the current study, only 12% of participants were aware of test and slaughter method, likewise, 18% of farmers tended to avoid buying cattle from risky sources (potentially infected sources or areas with previous reports of bTB). It is widely accepted that the movement of infected animals is the most critical method for the spread of bTB. Poor restriction of infected animal movements and quarantine were considered to be the main barriers to the control of bTB (The ETHICOBOTS consortium *et al.,* 2019).

Bovine tuberculosis is a zoonotic that has been widely underreported and understudied but is believed to be a significant contributor to animal and human losses (Grace *et al.,* 2012; Liverani *et al.,* 2013). Many farmers in these settings do not know about tuberculosis in cattle, rather farmers' knowledge was limited to human tuberculosis and had strong perception that tuberculosis was a disease that 'only' humans could get. This poor awareness may be attributed to farmers` perceptions on benefits of bTB control and prevention, which was believed mainly commercial, as bTB was not considered having an impact on public health neither a disease causing production losses (Ciaravino *et al.,* 2017). Furthermore, farmers mainly perceived the control of bTB as an imposition rather than a necessary activity to protect their animals (Ciaravino *et al.,* 2017). Our findings back this up, revealing a dearth of knowledge on the production loss incurred and the probability of human infection by bTB infection, instead a substantial number of farmers (25%) believed that bTB infection could badly affect the dairy business through disturbing their milk markets. These limitations may explain how bTB continues to be a major threat.

Among the dairy farmers who were aware of bTB, a very small percentage (1.6%) considered bTB as a prioritized animal health issue, leading to a lack of interest in taking preventive and control actions. All these gaps may exacerbate the public health and economic burden of bTB not only in the community but in the country as well.

Skin test was a common diagnostic tool for bTB, but only 1.6% farmers were aware of it. Actually, farmers do not want to have any bTB-infected animal in their herd, but they want to be sure that the test-positive animal is truly infected. In the present study none of the participants reported skin test as reliable. Even veterinarians and farmers expressed strong uncertainties on the reliability of skin test results (Ciaravino *et al.*, 2017). According to our findings, therefore, in the absence of common conscience on the conventional bTB diagnostic tool and a clear policy to compensate for culled animals, almost none of the respondents (0.98%) thought that test and slaughter method was a feasible and applicable way to control bTB. Instead,16.3% and 22% of respondents mentioned that educating the community about bTB, and providing healthy heifers from reliable sources were key factors for a successful bTB prevention and control plan, in a similar pattern that was reported by other scholar (Kawsar, 2022).

3.5. Conclusions

The present study investigates farmers' knowledge, attitudes, and practices (KAPs) of the study population and some important issues that could remarkably impact the application and effectiveness of interventions against bTB in the specific area we examined and in Ethiopia overall. Our result could be one of the top reports on bTB awareness in Ethiopia compared to other previous similar studies. Milk-shed, herd size, previous years of experience, training availed and veterinary consultation had significant association with awareness towards bTB.

The study indicated that training and regular consultation from veterinarians could boost the farmers' understanding of bTB. The knowledge could then help farmers change some of their practices for the better. Hence, a tailored education or training programme could be developed to raise the farmers' awareness and encourage them to adopt bTB prevention practices. In doing so, important determinants identified in this study should be taken into account.

3.6. Limitation of the study

The scope of this study is limited to commercial dairy farms in selected milk-producing areas of Ethiopia. To gain a more comprehensive and accurate insight into the subject, further and broader studies are needed to examine the gaps in farmers' knowledge and attitude in both commercial and extensive dairy farming system.

The analysis of this KAP would be better if done by scoring. The author put this gap as its limitation. The scores used to categorize the participants knowledge and attitude into binary result so that the proportion of farmers with good knowledge and poor knowledge can be identified and associated with the explanatory variables.

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CHAPTER FOUR: THE INCIDENCE OF BOVINE TUBERCULOSIS IN A DAIRY HERD PRACTICING IRREGULAR SKIN TEST AND SLAUGHTER CONTROL PROGRAM

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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; Ethiopia; Herd; Incidence; Inconclusive; Reactor

4.1. 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.

4.2. Materials and methods

4.2.1. Study area

The study was conducted at Holetta Agricultural Research Center (HARC) with altitude: 2400 masl; annual rainfall: 1100mm; average temperature minimum: 6°C, maximum: 24°C) located at 35 km west of Addis Ababa, Ethiopia (9.069206 N and 38.49589 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.

4.2.2. 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.

4.2.3. 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 the suckler/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 dam-line are the main entry animals. However, retested animals were engaged in two or more previous skin testing.

4.2.4. Single intradermal comparative cervical tuberculin test /SICCTT

The SICCTT was used as described in the World Organization for Animal Health (WOAH) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (WOAH, 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 (Mechanical with accuracy/resolution: 0.55mm and up to 40 mm measuring range); 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.

4.2.5. Post mortem examination

Reactors were slaughtered and subjected to post mortem examination in order to inspect whether visible lesions were 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.

4.2.6. 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.

4.3. Results

4.3.1. Herd composition based on previous skin testing

The study had completed four screening test 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 4.1, 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 animals, respectively, were new entry animals. These new entries had no prior history of tuberculin testing.

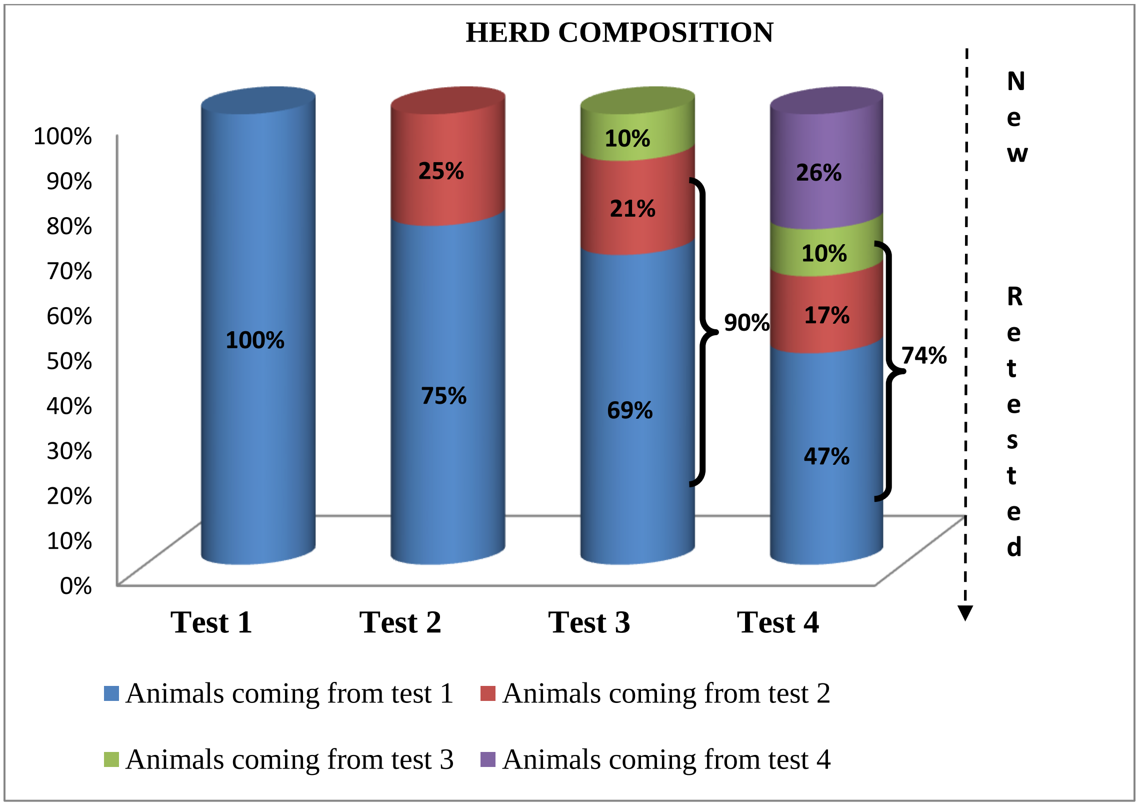

Figure 4. 1. Profile of study herd composition at subsequent test rounds

4.3.2. Herd incidence

The result of the bTB screening test is summarized in Table 4.1. 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 4.2).

Table 4. 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	84.6	8.5	6.9
Test-3	503	71.8	24.8	3.4
Test-4	498	90.2	5.4	4.4

Table 4. 2. Test results based on herd compositions

Tests	Herd composition	n	Reactors%	Doubtful%
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 4.3 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 4. 3. Skin test results based on their penultimate test results

Tests (N)	Penultimate test result	n	Reactors%	Doubtful%
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 multivariable logistic regression analysis are shown in (Table 4.4). 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. 4. Analysis of selected determinants having association with bovine tuberculosis infections using multivariable 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

4.3.3. 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 4.5). 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 4. 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	No test	71	47	0.662
Test-2	43	Negative	29	13	0.448

		Doubtful	8	7	0.875
		New/ no retested	6	4	0.667
Test-3	54	Negative	42	28	0.667
		Doubtful	12	10	0.833
Test-4	27	Negative	21	11	0.524
		Doubtful	6	2	0.333

4.4. 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 our 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. Doubtful animals, animals in latent stage and infectious animals which were not detected on the first test could contribute to the increased incidence in the next test rounds during the prolonged test intervals. 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 (Ameni *et al.*, 2006; Vordermeier *et al*., 2012).

In the current study, the incidence 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 doubtful status of animals and the odds of them becoming a reactor to the SICCTT at a subsequent test. In our findings, the doubtful 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 doubtful animals more likely to become reactor animals than animals which tested negative.

Apart from this, reactors that had a doubtful or doubtful 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.

4.5. Limitations of the Study

The study has limitation on accounting for the potential confounding effect of age to breed effect. Age is an important factor that can influence the exposure rate of animals to mycobacterium and create high incidence. In this study, the relationship between breed and bTB incidence might be impacted by differences in age among different breeds involved. Boran breeds were kept for a long period of time in the farms due to serving as dam line for the breeding purpose so that our samples from Boran had older age. Without adjusting for age in the analysis, some of the observed associations might be due to age differences between breeds rather than the breed itself. Future studies should consider age as a potential confounder and either match or adjust for it in the study design and analysis. This would help isolate the independent effect of breed on the incidence of bTB incidence in the study dairy farms.

4.6. 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.

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CHAPTER FIVE: THE IMPACT OF REPEATED SKIN TESTING AND SLAUGHTER FOR BOVINE TUBERCULOSIS CONTROL IN HOLETA DAIRY FARM: AN UPDATE ON SUBSEQUENT INCIDENCES 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. Its economic impact is primarily driven by direct effects**,** particularly due to test and culling of infected animals. If the factors contributing to the spread and persistence of bovine tuberculosis are left untreated, the situation gets worse. Therefore, prioritizing bTB control is essential. A retrospective study was conducted to evaluate the effect of repeated skin testing and slaughter on subsequent incidences and its consequence on herd demographic changes. Holstein–Friesian Boran crosses and pure Boran animals were involved in the study; all animals except calves $\leq$6 months of age were tested using SICCTT. On the basis of 21.3% prevalence in the first test round in December 2014, three successive rounds of test and slaughter were conducted. As a result, the incidence of bovine tuberculosis (bTB) infection exhibited an oscillating pattern. It initially declined to 8.4% (n = 521) during the second test round, but then experienced a resurgence, reaching 24.8% (n = 503) in the third round. Finally, the incidence decreased to 5.4% in the fourth test round. Despite repeated testing and removal measures, the incidence of the disease did not exhibit a substantial reduction trend along the successive test and slaughter rounds. The time interval between successive SICCTT tests varied from 0.95 years to 1.84 years, which were excessively prolonged and inconsistent time intervals. The existing applied control intervention, apart from the incompetence on incidence reduction, it took decisive action of culling a substantial number of cows (n=342). Consequently, the study identified a vital knock-on effect on herd demography due to removing of animals 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. Similarly, animal entries and exits also influenced the breed composition of the herd. 50% crossbred animals consistently formed over 63% of the herd population, while purebred Boran and 75% Holstein cross took the second and least proportion in the first test round, respectively. 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 fourth test rounds. The proportion of 75% of crossbred animals was inversely proportional to that of purebred Boran animals across subsequent herd test rounds. In conclusion, compliance to conventional test and slaughter procedural protocol is supposed to play an important role in succeeding bTB control measures. Therefore, at least a minimum of 2-month a maximum of 6-month testing interval, and two consecutive negative whole herd testing should be carried out in order to declare a herd free of bTB. It is also recommended that culling should be carried out with no or minimal significant impact on herd demography change.

Keywords: Bovine tuberculosis; Disease control; Incidence; Reactor; Test-and-slaughter.

5.1. Introduction

Deep-rooted poverty and the rapid depletion of resources in various countries have prompted policy makers and researchers to shift their focus toward enhancing the effectiveness of the livestock sector. This shift aims to address critical challenges related to poverty alleviation, sustainable resource management, and food security (UN, 2023; World Bank, 2023, 2022a, 2022b). Therefore, efforts to improve livestock practices, enhance livestock productivity, and support livelihoods within this sector are crucial for achieving inclusive and sustainable development.

Ethiopia has one of the largest livestock inventories in Africa (CSA, 2013). However, the prevailing approach of relying on indigenous cattle and extensive rural production may not adequately satisfy the increasing domestic demand for animal products (Marcotty *et al.*, 2009; Vordermeier *et al.*, 2012). Consequently, 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, bovine tuberculosis has indeed been associated with intensive farming practices and peri-urban animal production systems (Marshet, 2020; Vordermeier *et al*., 2012).

Although still present in some industrialized countries with well-developed veterinary control systems, nowadays, the worst impact of bTB is 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 that indicates the existence of potential risk factors conducive to the spreading and persistence of bTB (Ayele *et al.,* 2004; Girmay *et al.,* 2012; Regassa *et al.,* 2008).

Bovine tuberculosis (bTB) is among top three serious animal health problem in Ethiopia (Lakew *et al.,* 2022). Its economic impact is primarily driven by direct effects, particularly due to test and culling of affected animals. It is not recommended to treat animals with tuberculosis using medication (anti-tuberculous chemotherapy) because of the potential negative consequences for both economic and public health hazards. So the best controlled 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 remaining uncontrolled and no concerted effort at the national level to date, a few attempts have been undertaken to control bovine tuberculosis at the farm level in Ethiopia.

Farmer’s choice to cull or keep dairy cows is likely a complex decision influenced by intrinsic and extrinsic factors (Bergeå *et al.,* 2016; Owusu-Sekyere *et al.,* 2023; Rostellato *et al.,* 2021). Cows were 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 (Adriaens *et al*., 2020; Grandl *et al*., 2019; 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, 2017, 2013; Zehetmeier *et al.,* 2014). Decisions on voluntary culls are made to maximize profit. Usually, voluntary culling is not effective, resulting in all cows eventually leaving the herd due to health or fertility issues, 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, milk production, reproduction, or genetic improvement may be impaired (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 disease. Likewise, the impact of test- and-slaughter control option for bovine tuberculosis also go 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 common ante-mortem bTB diagnostic test (Marassi *et al.,* 2013). Another test that targets quantifying the amount of the most stable cytokine (INF γ) produced in response to bTB antigen exposure has been into use. Unluckily, they have limitations in terms of sensitivity and specificity, which means some infected animals are missed from being culled, 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 a significant role in the transmission of the disease; the available antemortem tests are not able to distinguish these animals.

Despite some limitations, Holeta dairy farm had been practicing a test-and-slaughter measure for the previous years to control and eliminate the infection from its herd. Therefore, the study was designed to evaluate the effect of repeated skin testing and slaughter on subsequent incidences, and identify any barriers or limitations for its incompetence. It also assessed the impact of the existing control option on herd demographic change over a five-year period.

5.2. Materials and methods

5.2.1. Farm description

The dairy farm was established in 1977 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 crossbred 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). They were crossing 50% male with 50% female and 75% male with 75% female produced later generations. Besides herders, 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 primary 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 four 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 Weaned calves were then transferred to a group pen (calves housed in pens of 15- 20 calves) and kept indoors until six 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 comprised 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 (feces, blood, swabs, and tissues) were collected from both clinically sick and apparently 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.

5.2.2. Study design

A retrospective study was conducted to investigate the five-year (2014-2019) trajectories of bTB disease pattern 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 $\leq$ 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 was a total of four testing rounds on the herd in which all reactors were removed. The time interval between the two consecutive test rounds varied from 345 to 672 days.

In this study, the definitions of animal entries and animal exits were defined based on context to explain the herd demography. An animal entry is defined as the number of new animals entering the study herd and receiving their first SICCTT test at a given screening test round. Self-sourced replacement heifers and outsourced Boran heifers as damline are the main entry animals. Exit is defined as animals that had been tested by SICCTT in a penultimate test round but lost from the study herd at a given screening test round. Animals might exit from the herd either due to culling or death.

5.2.3. Single intradermal comparative cervical tuberculin test

The SICCTT was used as described in the WOAH Manual of Diagnostic Tests and Vaccines for Terrestrial Animals(WOAH, 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 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 in 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.

In the current study skin test is the dependent variable, that is being measured in response to changes in or association with the number of determinants called independent variables. Independent variable is defined as information regarding the conditions of skin testing, and results, that allow others to correctly interpret the results, such as previous tuberculin test results (negative or inconclusive), herd composition (e.g. new entry or re-tested herd), and breed type.

5.2.4. 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 univariable analysis, logistic regression model was used. In the final model, only the significant variables ($p < 0.05$) were included.

5.3. Results

5.3.1. The effect of repeated skin testing and slaughter on the incidence of bTB

The dairy farm conducted four rounds of SICCTT tests and removal of reactors during the monitoring period, irrespective of laboratory and molecular confirmation. Of 502 animals tested in the first-round test 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 incidences were examined. In this study, however, the incidence of bTB was not neither reduced steadily along successive test rounds, nor proven capable of making the incidence below 1% or nearly eliminate the disease from the herd. As the result, the incidence of bTB infection had declined to 8.4 % (n = 496) in the second test round. However, a resurgence of the bTB infection occurred in the third-round skin test, when 24.8 % (n = 503) incidence rate were reported. In a meantime, the incidence remarkably decreased to 5.4% in the fourth test round (Figure 5.1). In general, reactor animals were identified in all test rounds although the level of testing and the proportion of positive cattle varied along test rounds.

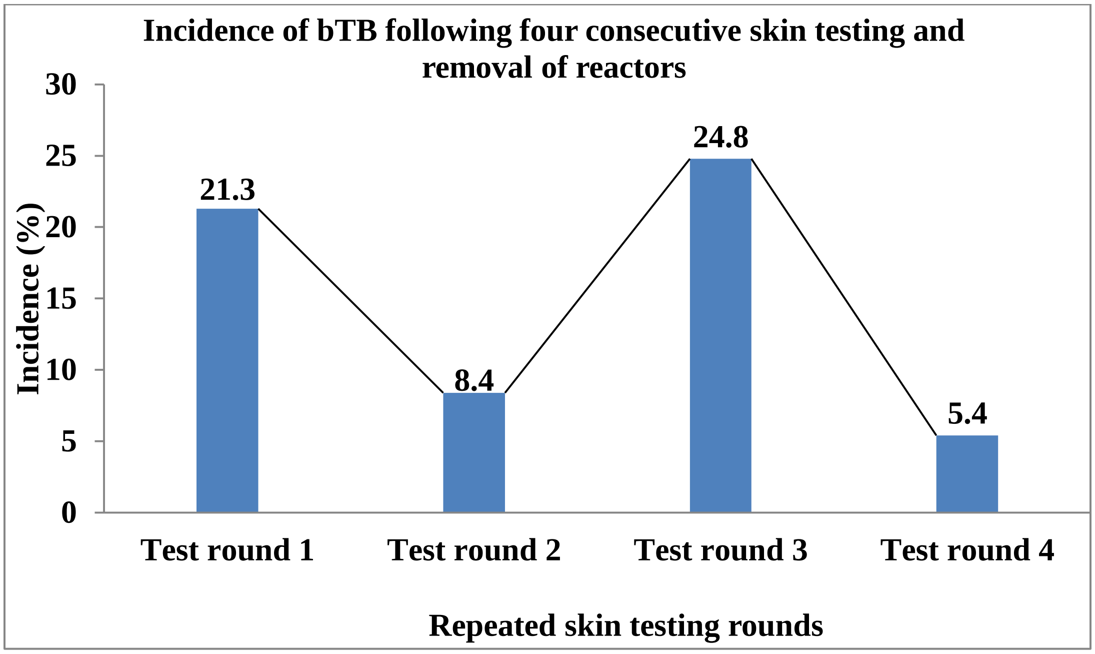


Figure 5. 1. Trajectories showing the effect of irregular skin testing and slaughter on the incidence of bovine tuberculosis in the study herd

The incidence, as evidenced by Figure 5.1, did not exhibit a direct or indirect proportionality with the repeated test and slaughter rounds.

5.3.2. Effects of repeated skin testing and slaughter on culling pattern, herd size and composition

As can be seen from the Figure 5.2, the herd size and its contents (herd composition) prior to the first-round test and slaughter intervention period was almost in uniform fashion with equivalent number of replacement heifers entering the herd for those animals leaving the herd. However, following bTB incidence, and the test-and-slaughter control measures taken, the culling pattern, the herd size, and the number of replacement heifers entered to the herd exhibited a fluctuating trend (Figure 5.2). Immediately after each test rounds, the herd size suddenly declines due to slaughtering of large number of reactors. Instead, replacement heifers started to join the herd gradually and then the herd size experienced resurgence till the next test and slaughter rounds.

Similarly, number of animals culled from the study herd largely 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, and the rest portions were new entry animals with no tuberculin test history. During each test rounds, more animals (15.8%) sourced from baseline herd (retested herd) were culled than others.

During the monitoring period, there were 810 (502(first round) +129 (second round), 52 (third round) and 127 (fourth round)) animals involved in the screening tests, of which 342 cows were culled, and 468 were still present when monitoring ceased. Regarding perspective test rounds, 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. Similarly, out of 129 newly introduced animals in the second test round, only 85 continued to participate until the fourth test round (Figure 5.2). The remaining animals left the herd via involuntary culling or death.

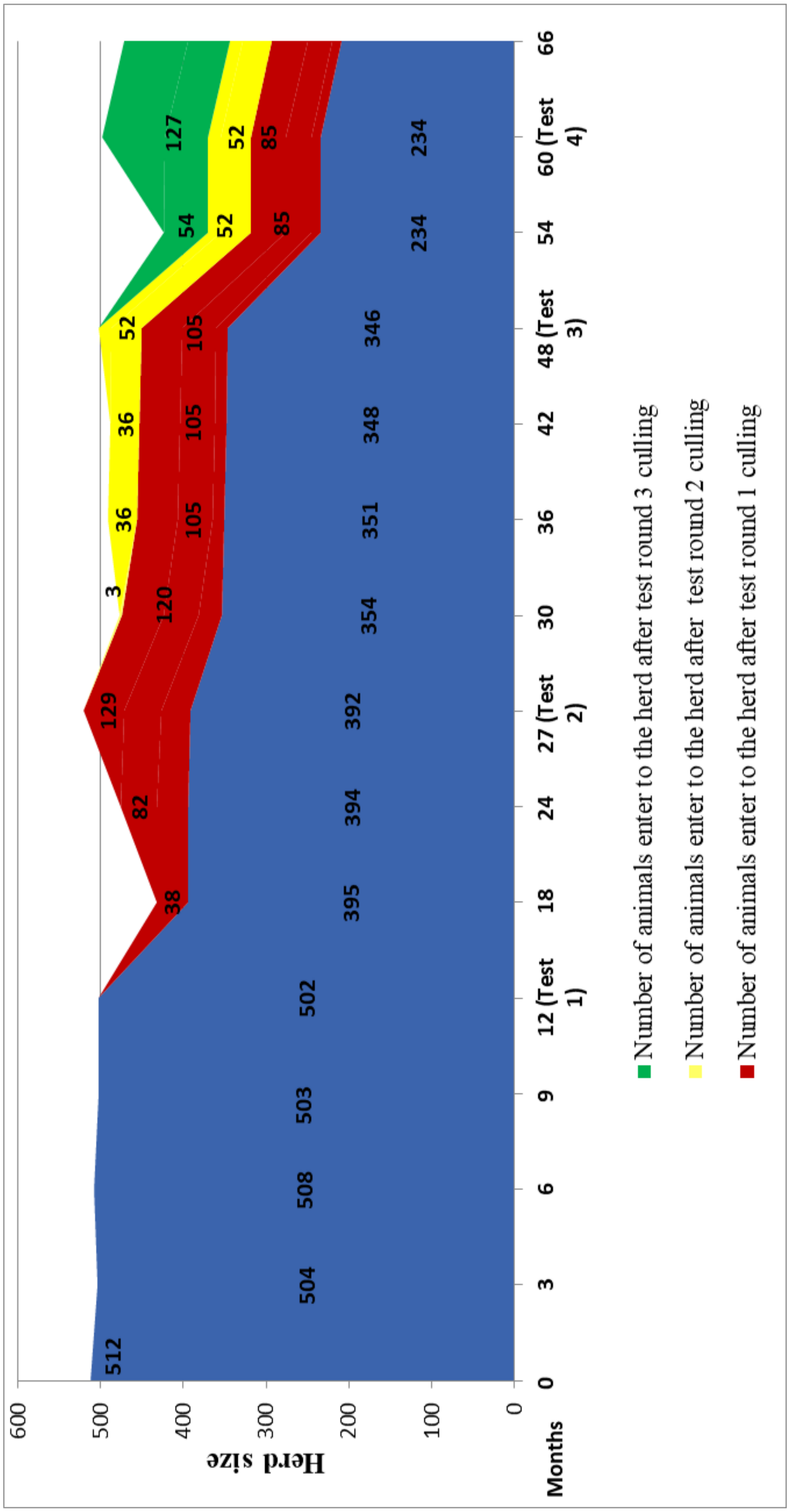

Herd size
600
500
400
300
200
100
0
Months
0
3
6
9
12 (Test 1)
18
24
27 (Test 2)
30
36
42
48 (Test 3)
54
60 (Test 4)
66
512
504
508
503
502
395
394
392
354
351
348
346
234
234
38
82
129
120
105
105
105
85
85
3
36
36
52
52
52
54
127
Number of animals enter to the herd after test round 3 culling
Number of animals enter to the herd after test round 2 culling
Number of animals enter to the herd after test round 1 culling

Figure 5. 2. Chart illustrating livestock entries and exits in successive screening tests

5.3.3. Influence of animal entries and exits

Following test and removal control measures, several cows (mean age = 8.7years) were culled from the herd, while heifers were introduced as replacement stock. The average ages of culled animals and new entry animals for successive test rounds were compared in Table 5.1. Around 94% of culled animals in the study herd were older than 3.19 years old age. These entries and exits played a crucial role in shaping the average herd age, parity and breed composition of the study herd. For example, 50% of the herd had less than or equal to 4.26 years old age before the first test and slaughter control intervention, however the median decline to 3.14 years after culling of reactor animals (Figure 5.3). The herd included animals ranging from 6 months to 17.5 years old.

Table 5. 1 Average herd age of new entry and culled animals at each test round

Test round	Mean age of new entry (Years)	Mean age of culled animals (Years)
Test 1	5.75	9.99
Test 2	2.10	8.73
Test 3	2.09	7.67
Test 4	2.09	6.86

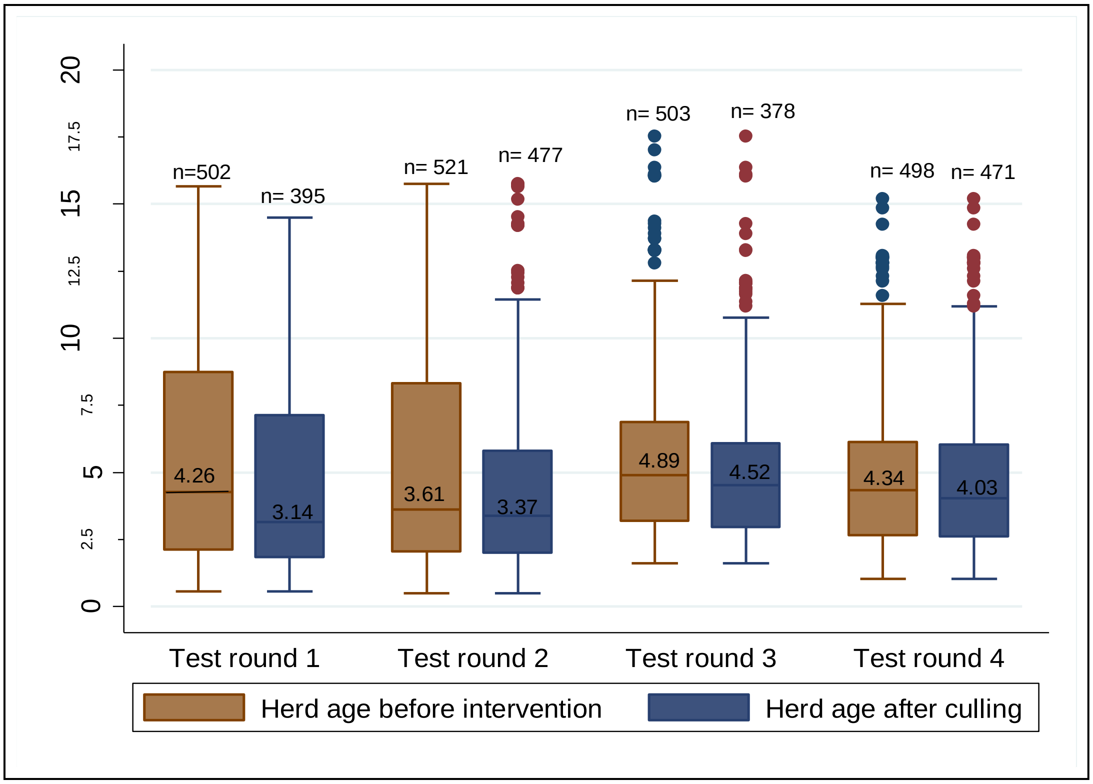
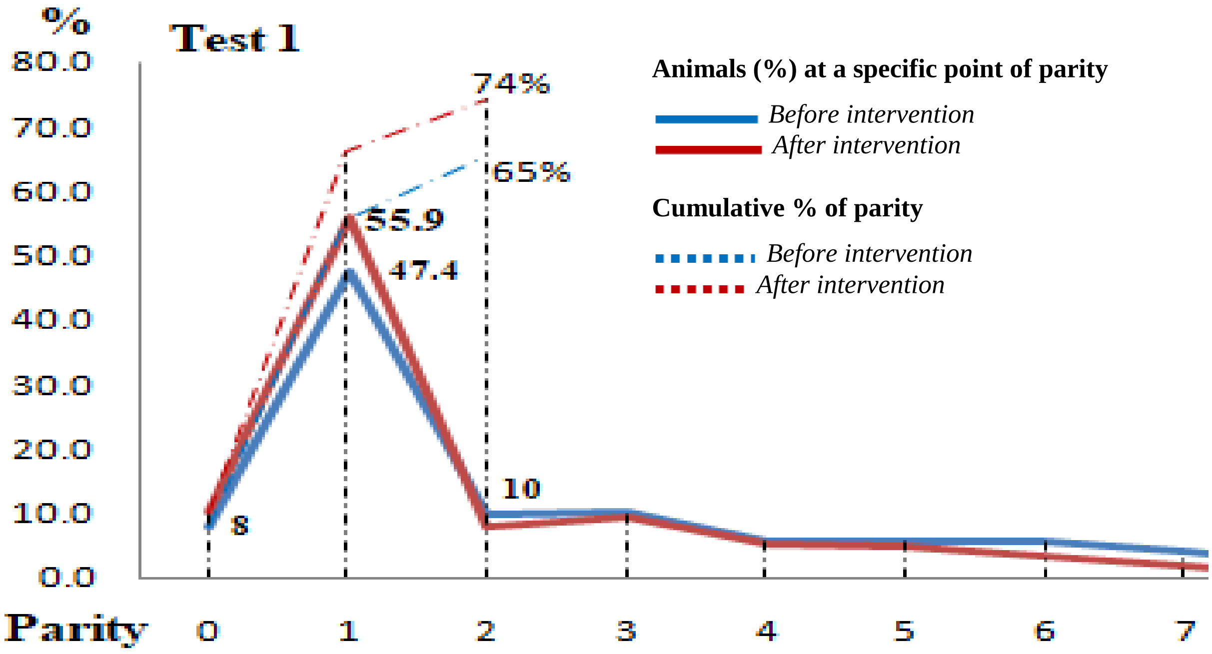


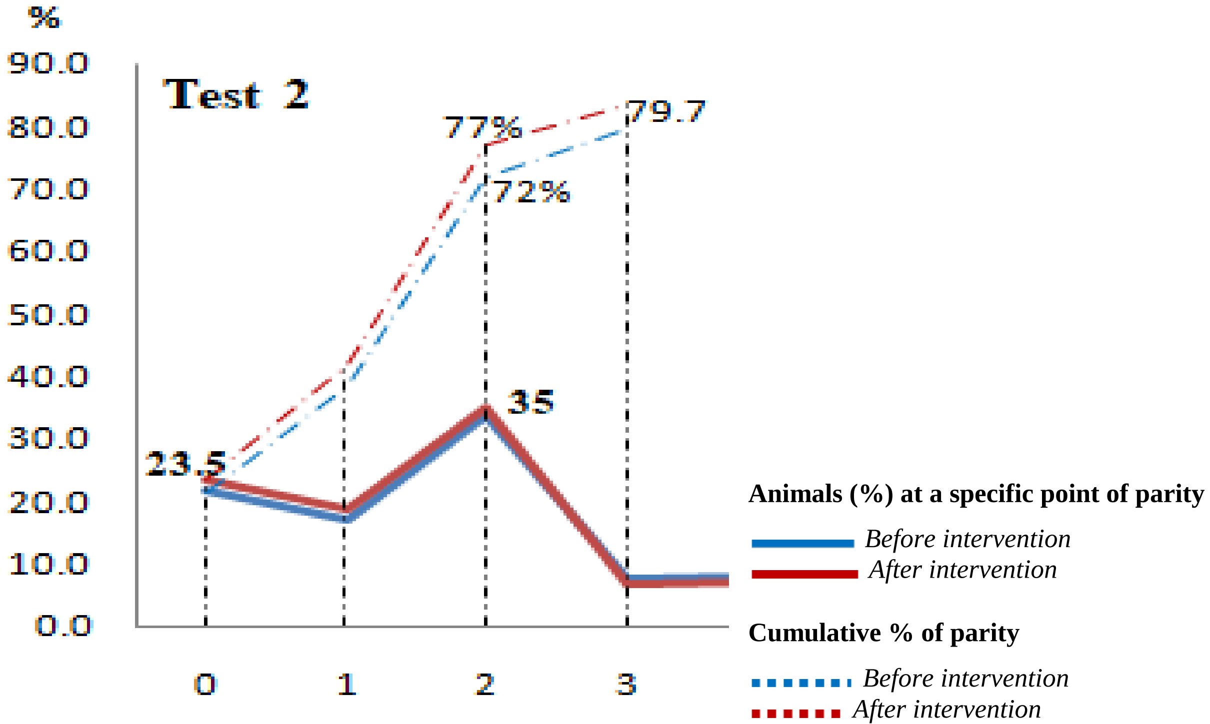
Figure 5. 3. The median age of the study herd before and after test and slaughter control measure

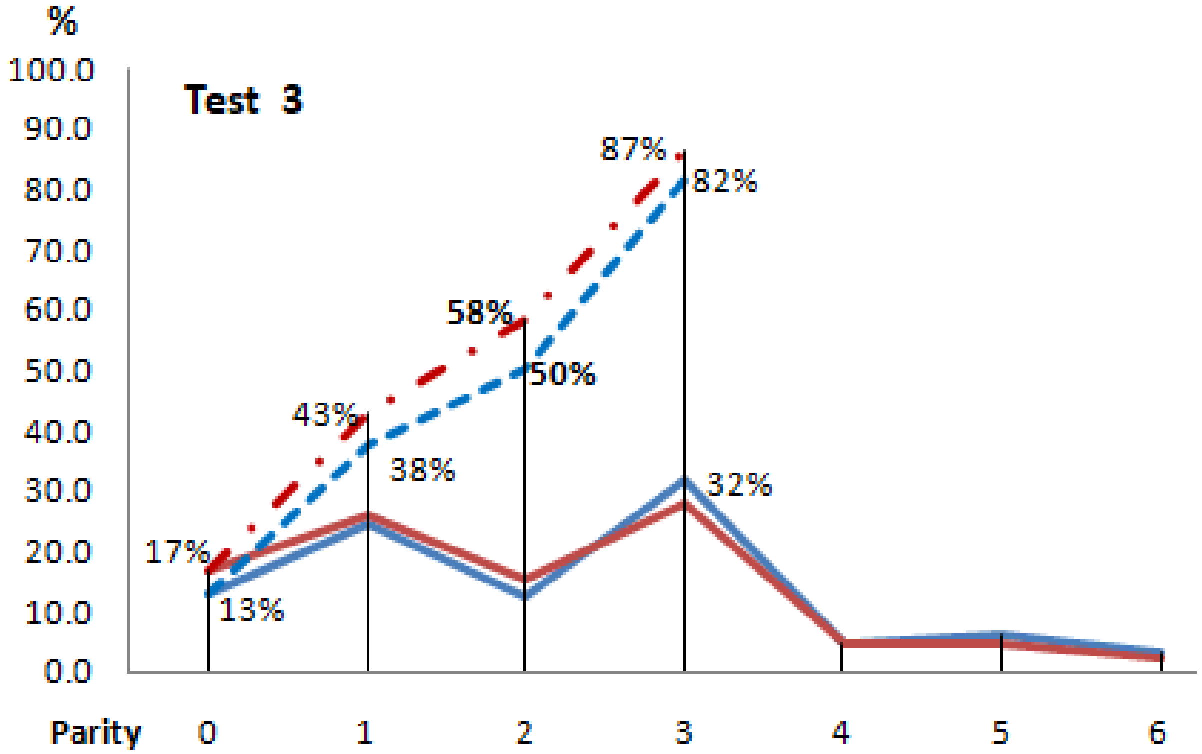
Assessing the impacts of the current test and slaughter interventions on parity, it made the herd to have more proportion of animals with ≤ 2 parity. Prior to the first test round, 65% of the study herd were animals with 2 or less parity, while the rest 35% were productive cows with greater than 2 parities. However, after test and slaughter control intervention, the proportion of these high milk yield multiparous cows declined to 26%. Similar trends were revealed in the rest test rounds as shown in figure 4. As cows go became multiparous, 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 impacted the total milk production of the herd.

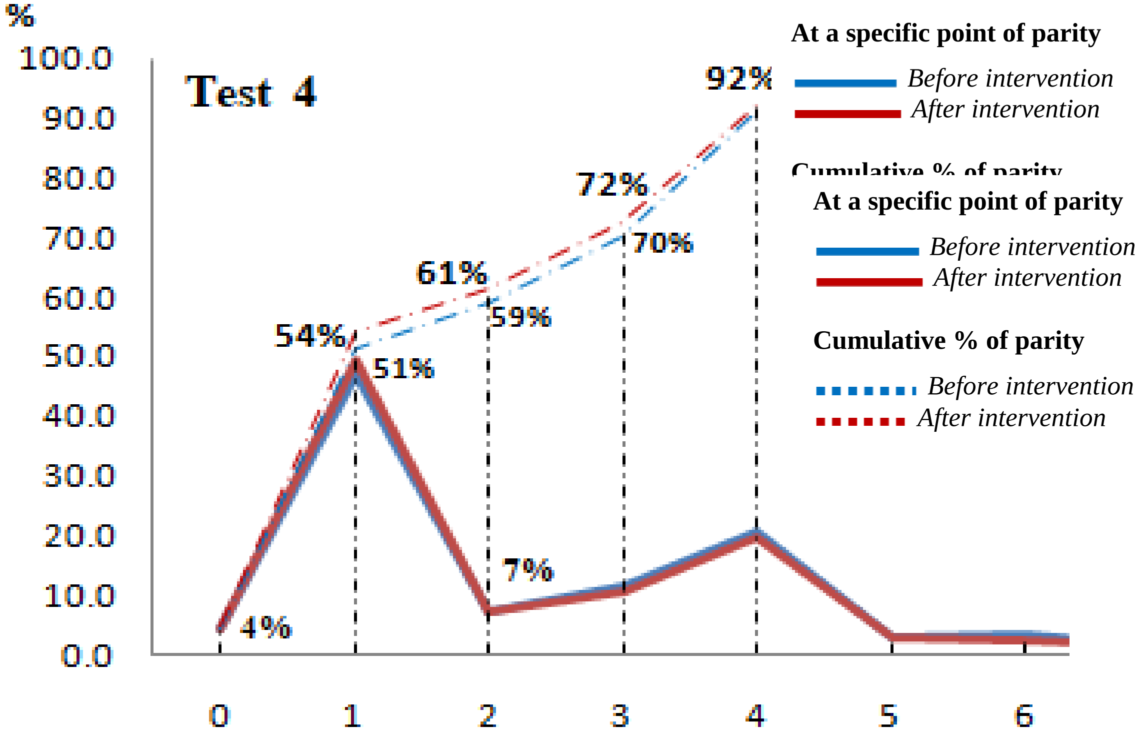


Note: Proportion of animals having ≤2 parity increased from 65% to 74% following test and slaughter intervention at test round 1

Proportion of animals with ≤2 parity increased from 72% to 77% following test and slaughter intervention at test round 2







Note: Parity refers to the number of times a cow has calved

▬▬▬ *animals at a specific point of parity (befortest and slaughter)*

▬▬▬ *Animals (%) at* X^{th} *parity/a specific point of parity (after test and slaughter)*

▪▪▪▪▪▪ *Cumulative % of animals having parity of ≤ x parity (before intervention)*

▪▪▪▪▪ *Cumulative % of animals having parity of ≤ x parity (after test and slaughter)*

Figure 5. 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 purebred Boran and different crossbred animals. The 50% crossbred animals consistently formed over 63% of the 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% of crossbred animals was inversely proportional to that of purebred Boran animals across subsequent herd test rounds (Figure 5. 5). Moreover, the incidence of bTB regarding breed composition for the respective test rounds is presented in Figure 5. 5. The incidence was higher in purebred Boran and 50% Boran-Friesian cross than high-grade animals (75% Friesian-Boran).

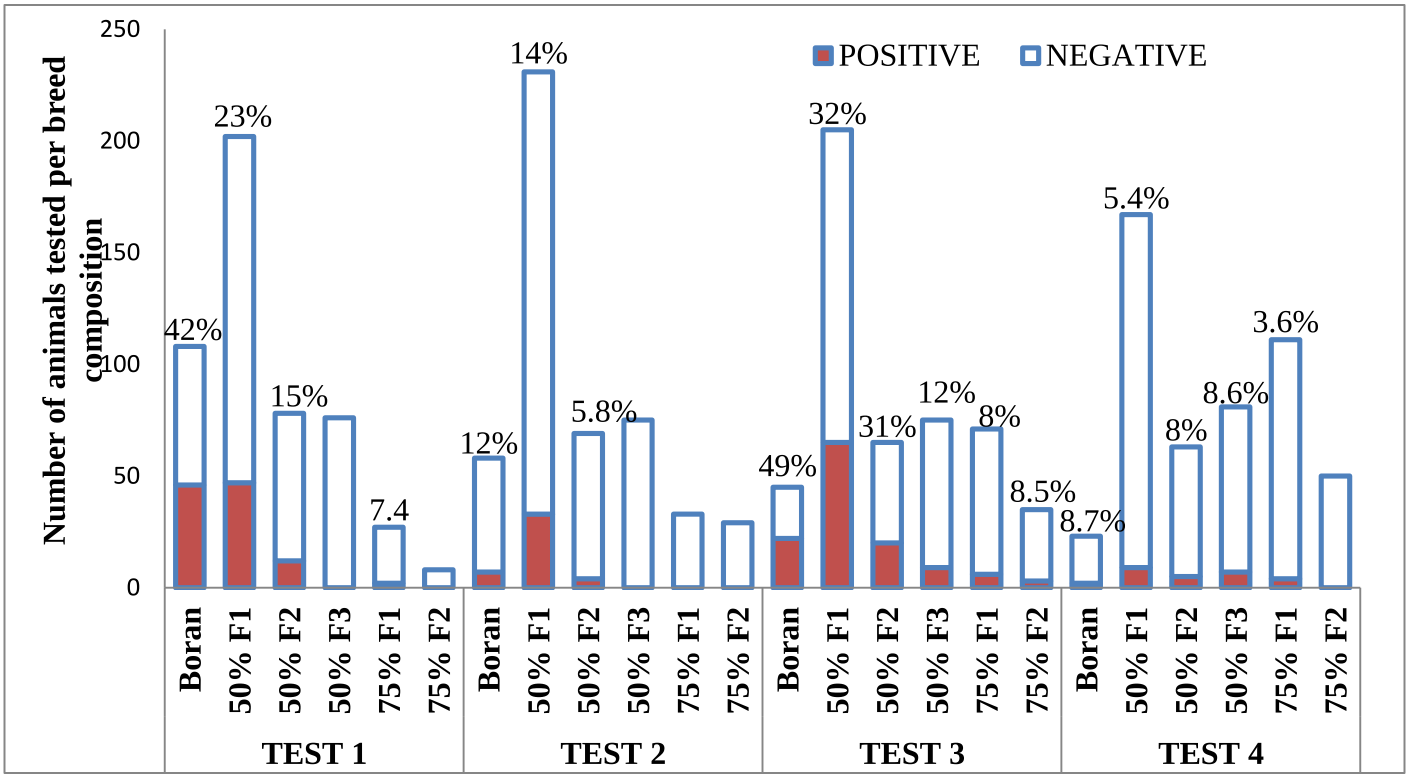


Figure 5. 5. bTB incidence over breed difference of the herd along successive screening test rounds

5.4. Discussion

Conventionally, SICCTT is 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 the disease from spreading beyond the herd. Only test-and-slaughter techniques have proven capable of eradicating tuberculosis 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 incidence (Ameni *et al.*, 2007; Shitaye *et al.*, 2007). The same authors also reported that test and slaughter resulted in a pronounced incidence 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 incidence of the disease 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 our study, 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 to 1.84 years. This prolonged inter-test interval would have allowed for continued transmission within the herd. However, the 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 incidence from 14% to 1% within a year. Similarly, according to the USDA protocol, to release quarantine and eliminate bovine tuberculosis (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).

Apart from the incompetence success story on incidence reduction, the study farm took decisive action by slaughtering numbers of infected animals (n=342)**,** thereby aiming to eliminate the infection from the herd. Normally, 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 the 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. However, in the current study bovine tuberculosis (bTB) contributed to culling. It devastated the farm, leading to the removal and slaughter of infected cattle as biological cull for which no possible productive future exists. Hence, repeated test and slaughter control was applied to reduce the cost due to bTB incidents. However, beyond substantial loss via test and slaughtering, not complying with the recommended strategies to apply the control intervention caused unintended consequence on the herd demography. Consequently, these demography 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 (Stewart, 1995), which was not favorable for herd demography maintenance. Furthermore, even if it could be nearly possible to maintain the herd size along the study period, there is huge gap in parity between the replacing heifer and culled cow. Culling is the act of, replacing the cow with another cow, often 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 multiple lactation cycles. This reduction in lactations per cow impacted overall milk production and reproductive efficiency. Unfortunately, the strategy used to apply the control intervention for bovine tuberculosis was not in compliance with the recommended strategy for maintaining stable herd demography.

In the current study, the proportion of infected animals was significantly higher in purebred Boran than in high-grade Holstein cross animals 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 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 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 taurine Ankole cattle, indicating that Zebu calves showed remarkable resistance compared to Ankole calves.

5.5.Conclusions

In this study, despite repeated irregular skin testing and removal of reactors, the incidence of bovine tuberculosis did not exhibit a significant reduction trend along the successive test rounds. The existing strategy of applying the control intervention measures was not satisfactory to reduce the incidence. These failures might be due to inconsistent and excessively prolonged test-and-slaughter schemes. Compliance to conventional test and slaughter procedural protocol is supposed to play an important role in succeeding disease control. These findings suggested the importance of time between consecutive SICCTT tests. Therefore, at least a minimum of 2-month a maximum of 6-month testing interval, and two consecutive negative whole herd testing should be carried out in order to declare a herd free of bTB.

The 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. It is also recommended that culling should be carried out with no or minimal significant impact on herd demography change.

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CHAPTER SIX: BAYESIAN MODELING OF BOVINE TUBERCULOSIS PREVALENCE ESTIMATES OF INTENSIVE DAIRY FARM

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Abstract

Emerging intensified and peri-urban animal husbandry for increased demand of livestock products under rapid human population growth and high rate of urbanization have corresponded with increased incidence of bovine tuberculosis (bTB). True disease states are uncertain in practice, because bTB tests have imperfect specificity and sensitivity. Bayesian methods present a natural way to propagate these uncertainties for use in prevalence analysis. The present study consists of two sections. A retrospective longitudinal study to generate information on apparent prevalence over four rounds of testing and removal of reactors was conducted in the first section, and then the true prevalence and the true characteristics of SICCTT (i.e. true sensitivity: Se; and true specificity: Sp) were estimated in the second section using Markov Chain Monte Carlo estimation with prior distributions. At any given test round, animal-level apparent prevalence of bTB based on the standard and severe interpretation of SICCTT ranged from 5.4-24.8%, and 9.8-28.2%, respectively. Median sensitivity estimates using standard and severe interpretations were 68.6% (BPI; 50.3-84.3%) and 78.1% (BPI; 62.5-90.9%), while the specificity median were 96.8% (BPI; 94.3-98.9%) and 94.5% (BPI; 91.5-97.1%), respectively. Furthermore, adjusted true prevalence estimates (median and 95 %; BPI) were produced for each testing round using the Rogan-Gladen estimator (RGE). Prevalence estimates were high in the third round of testing, then relatively low in the fourth round, regardless of standard and severe interpretations. Bayesian estimation with informative priors exhibited much wider credible intervals and strong coverage compared to uninformative priors and frequentist method (RGE). In conclusion, classic apparent prevalence estimates are overly precise when uncertainty around test performance is high. These Bayesian approaches provided a more accurate estimate of bTB prevalence in the study herd and provide a baseline data for the future true prevalence estimates using linked combined data.

Key word: Bovine tuberculosis; Bayesian modeling; Disease estimate; Apparent prevalence; True-prevalence.

6.1. Introduction

In Ethiopia, an intensive dairy sector is emerging 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, the combination of intensified animal husbandry and the development of peri-urban systems for livestock production have been associated with increased incidence of bTB (Vordermeier *et al*., 2012), which 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, the true disease state is rarely known in practice, because bTB tests are not accurate (Nuñez-Garcia *et al*., 2017). Thus, these tests may give either false a positive or a false negative or both results, with respective probabilities 1−specificity (Sp) and 1−sensitivity (Se) (Diggle, 2011).

The single intradermal comparative cervical tuberculin test (SICCTT) is the most common ante-mortem bTB diagnostic test and has been implemented for decades. Unluckily, it has 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*., 2016; Nuñez-Garcia *et al*., 2017; Lahuerta-Marin *et al*., 2018). The missed infected animals may be infectious, and thus play great role in the transmission of the disease.

Prevalence estimation is fundamental to many epidemiological studies and its estimation is usually based on the use of diagnostic tests to classify animals of unknown disease status with respect to the binary trait under investigation (Flor *et al*., 2020). In doing so, more parameters can be estimated in the absence of a gold standard and without external constraints by assuming constant sensitivity and specificity over populations and the tests are conditionally independent given the true disease status. However, these assumptions have been criticized as being unrealistic, and hence, some restrictions these estimates may need to be enforced (Dirk Berkvens *et al*., 2006). Estimating the true prevalence thus becomes a matter of adding constraints on the parameters. These constraints must come from external sources, for example from previous similar studies, expert opinion, and so on.

Hence, in epidemiological research, to obtain an accurate estimation of prevalence, misclassification and measurement errors should be considered as part of bias analysis (Lash *et al.*, 2014). Bayesian analysis is a useful tool to approach the problem of information bias caused by diagnostic misclassification due to imperfect sensitivity and specificity. Bayesian analysis can incorporate external information by specifying prior distributions on the parameters, such as those obtained from eliciting the opinion of experts, and thereby better estimate disease prevalence and the sensitivity and specificity of diagnostic tests when the true disease state is unknown. Most often, prior knowledge on sensitivity and specificity is incorporated, even though, in practice, experts often do not have a strong opinion on these test characteristics. For that reason, prior information on conditional probabilities is used (D Berkvens *et al.*, 2006).

In the present study, the true prevalence of the study herd being inferred from the apparent prevalence is not directly calculable due to these imperfect tests and unknown test characteristics. As a result, the true prevalence and diagnostic test sensitivity and the specificity must be estimated using the information from the apparent prevalence (Messam *et al.*, 2008). This then requires a Bayesian framework to jointly estimate these parameters while accounting for all relevant uncertainties. Therefore, the objective of this study was to estimate the true prevalence of bovine tuberculosis and test characteristics (sensitivity and specificity) of SICCTT in semi-intensive dairy farm over time.

6.2. Material and Methods

6.2.1. Herd management

The dairy farm in this study, owned by Holeta Agriculture Research Center (HARC), 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, were inseminated with semen from WWS (worldwide sire) to produce first and second generation cross-bred offspring. Besides herdsmen, a teaser bull is used for heat detection. 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. All cows had unrestricted access to fresh drinking water. Calves were allowed to suckle their dam for about four days to receive colostrum, and then moved to calf rearing pens and fed whole milk for 98 days through bucket feeding. Weaned calves were then transferred to a group pen and kept indoors until 6 months of age. Any information on livestock diseases and mortality as presented to and diagnosed at the clinic run by the animal health unit was recorded and available for research. The clinic conducted regular health monitoring and diagnosis and treatment of sick animals. All animals were dewormed orally prior to vaccination, and vaccinated against Anthrax, Black leg, Bovine Contagious Pleura Pneumonia (BCPP), Foot and Mouth Disease (FMD) and Lump Skin Disease (LSD).

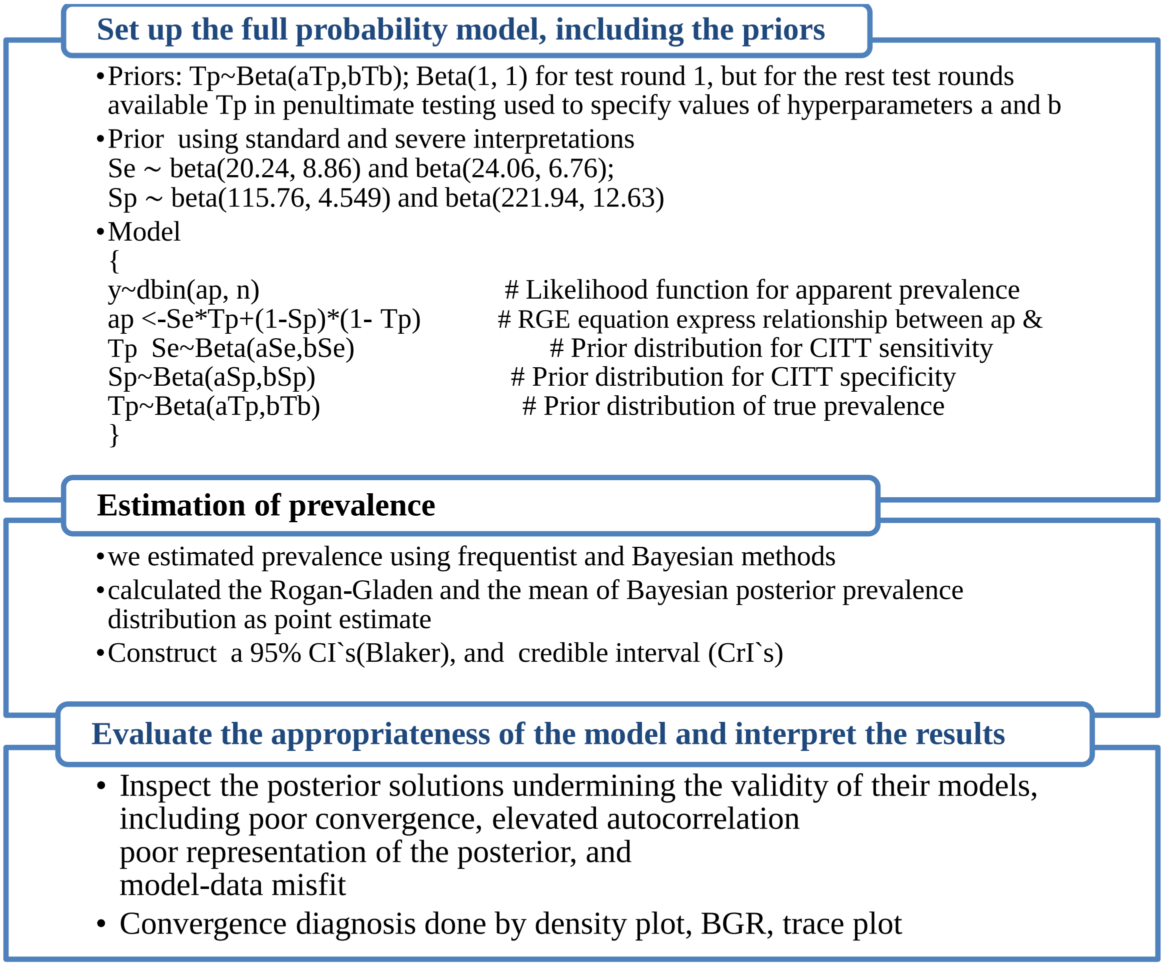
6.2.2. Data Collection

Data were collected from a retrospective longitudinal study to generate information on apparent prevalence over four rounds of testing and removal of reactors from the herd. SICCTT as described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2007) was carried out to screen bTB infection in the study herd. The time interval between consecutive test rounds varied from 0.95 years to 1.84 years (table 1). In each testing round, all animals in the herd (except the suckler/rearing calves and replacement heifer calves $\leq$ 6 months of age) were tested using SICCTT. Based on SICCTT results, cows were classified as reactors, doubtful/inconclusive, and negative. Reactors were culled, while doubtful and negative cows were maintained in the herd. Depending on the interpretation used, inconclusive animals were considered as positive (severe interpretation) or negative (standard interpretation) for data analysis. Test results were aggregated and analyzed independently for each testing round.

6.2.3. Bayesian prevalence estimation

Estimating the true prevalence (Tp) in the herd under study, and the true characteristics of the test (i.e. true sensitivity: Se; and true specificity: Sp), was the primary objective of this study. In this study, the definitions of prevalence, sensitivity, and specificity were based on earlier definitions given by Greiner and Gardner (Greiner and Gardner, 2000) and Thrusfield (Thrusfield, 2007). Apparent prevalence (Ap) is defined as the proportion of reactors to the SICCTT in a given test round, and true prevalence (Tp) is defined as the proportion of cows infected by *Mycobacteria bovis* in that herd.

In this study, a Bayesian framework was used to estimate the true prevalence of bTB infection (Taylor, 2019). In keeping with Gelman *et al*. (2014), analysis of this approach was organized around three steps (Figure 6.1). Briefly, the full probability model including priors was defined in the first step of the analysis, then posterior distributions were estimated in the second step, and the appropriateness of the model was evaluated in the third step. All computations and analyses were programmed using the 'rjags' package in the R statistical software version 4.1.2 (R Core Team, 2021), WinBUGS version 1.4.3 (Spiegelhalter, 2008), Betabuster (www.epi.ucdavis.edu/diagnostictests/ betabuster.html) and Bayesian Disease Freedom (BDFree) software, which was downloaded from www.epi.ucdavis.edu/diagnostictests. Supplementary information accompanies this paper; the R codes used as well as all analyzed data from this study are available in the supplemental material.



Set up the full probability model, including the priors

- Priors: Tp~Beta(aTp,bTb); Beta(1, 1) for test round 1, but for the rest test rounds available Tp in penultimate testing used to specify values of hyperparameters a and b
- Prior using standard and severe interpretations
  Se ~ beta(20.24, 8.86) and beta(24.06, 6.76);
  Sp ~ beta(115.76, 4.549) and beta(221.94, 12.63)
- Model

```
{
y~dbin(ap, n)                    # Likelihood function for apparent prevalence
ap <-Se*Tp+(1-Sp)*(1- Tp)        # RGE equation express relationship between ap &
Tp  Se~Beta(aSe,bSe)             # Prior distribution for CITT sensitivity
Sp~Beta(aSp,bSp)                 # Prior distribution for CITT specificity
Tp~Beta(aTp,bTb)                 # Prior distribution of true prevalence
}
```

Estimation of prevalence

- we estimated prevalence using frequentist and Bayesian methods
- calculated the Rogan-Gladen and the mean of Bayesian posterior prevalence distribution as point estimate
- Construct a 95% CI`s(Blaker), and credible interval (CrI`s)

Evaluate the appropriateness of the model and interpret the results

- Inspect the posterior solutions undermining the validity of their models, including poor convergence, elevated autocorrelation
  poor representation of the posterior, and
  model-data misfit
- Convergence diagnosis done by density plot, BGR, trace plot

Figure 6. 1 Procedural illustration of study methodology

6.2.3.1. *Setting priors*

The prior distributions of the true prevalence and test characteristics (sensitivity and specificity) were initially estimated based on previous studies and expert opinion.

Non-informative (uniform) Beta(1, 1) distributions were assigned as the prior of the true prevalence at first test rounds, according to common practice. However, in the next three successive test rounds, the estimated posterior of the prevalence of bTB infection in the previous test round was used to specify hyperparameter values a and b for a Beta(a, b) prior.

The sensitivity and specificity of SICCTT were modeled based on animal health researchers opinions in HARC conducting test- and – slaughter of the bTB control programs on prospective incidences in a previous study (Emeru *et al*., 2020). The mean of their response (expert opinion) values was solicited on SICCTT sensitivity and specificity in the form of a most likely estimate (mode) and a pessimistic estimate (minimum value). The researchers averaged the expected number of reactor animals per infected animal using standard and severe interpretation if SICCTT is applied to some animals of an infected herd. Their results provided 95% confidence that at least 55% and 65% of infected animals (using standard and severe interpretations, respectively) would have positive test results; similar values were reported for specificity. These values were used as inputs in the software Betabuster to parameterize and obtain the shape parameters for the Beta(alpha, beta) prior for sensitivity and specificity.

6.2.3.2. Model

We set up a model (figure 1) accounting for uncertainty in the values of Tp, Se and Sp. It includes the likelihood for each data point and a prior for every parameter of interest to be estimated. Our likelihood functions have three components in this model:

1) The deterministic component expressing relationship between apparent and true prevalence given by the Rogan–Gladen estimator equation by $ap = Tp*Se + (1-Tp)*(1-Sp)$

2) The component linking the response variable "y" to apparent prevalence (ap). i.e. under binomial sampling, the distribution of the number of animals testing positive is given by:

$$y|\mathrm{ap}\sim(n, \mathrm{ap}), \text{ and}$$

3) A component to track the priors (Se, Sp) by the model:

$$\mathrm{Tp}\sim\mathrm{Beta}(\mathrm{aTp}, \mathrm{bTp})$$

$$\mathrm{Se}\sim\mathrm{Beta}(\mathrm{aSe}, \mathrm{bSe}),\ \mathrm{Sp}\sim\mathrm{Beta}(\mathrm{aSp}, \mathrm{bSp})$$

where aTp, bTp , aSe, bSe , aSp, bSp are the parameters of the Beta prior distributions for Tp, Se and Sp, respectively.

Hence, using the above components, the Bayesian model to estimate true prevalence was mathematically constructed from the conditional distributions as shown in Figure 6.1. In order to customize the behavior of the model, we encoded our choices for our data model and priors to pass them to the fitting routines in JAGS.

6.2.3.3. *Prevalence estimation*

Here, the data are supplemented with prior information. We assume that Se = Sp=1 (i.e. ap=Tp). In test round 1, it is assumed that we have little known prior testing history, and no prior information on disease prevalence in the herd studied. Therefore, a diffuse prior beta(1, 1) for ap was used, which prescribes equal likelihood to every possible value of the prevalence. However, we incorporated posterior penultimate prevalence estimates into the Bayesian analysis to specify values for the hyperparameters a and b for a beta(a, b) prior of the consecutive test rounds. Finally, using a binomial distribution:

x~bin(n, ap), and
ap ~beta(a, b), prior for ap,

the apparent prevalence was estimated and, following the Rogen-Gladen formula as detailed above, the posterior distribution of the above model was analytically calculated through the use of Bayesian statistical programs (WinBugs, Jags, Stan) (Carpenter *et al.*, 2017; Lunn *et al.*, 2000).

Furthermore, posterior estimates using informative priors, uninformative priors, and with no prior distribution (RGE) were compared in order to explore the influence of the priors over the posteriors in this system. We started with the assumption that Se and Sp are known, fixed values, and then this assumption was relaxed. Our performance metrics to evaluate priors influence are the point estimates (median) and range of the 95% confidence interval (credible interval in the case of the Bayesian method).

6.2.3.4. *Evaluation of the appropriateness of the model*

Samples were generated by MCMC algorithm from the posterior distribution to assess convergence, autocorrelation, and representation of the posterior (Depaoli and Van de Schoot, 2017; Lee, 2007). The convergence of generated Markov chains was checked by density plot, Gelman-Rubin Statistics, and trace plot.

6.3. Result

6.3.1. SICCTT screening test results

The dairy farm conducted four consecutive SICCTT tests, resulting in 2024 screening tests, of which 15% and 20.2% were positive based on the standard and the severe interpretation, respectively. At any given screening test round, prevalence of bTB based on the standard and the severe interpretation ranged from 5.4-24.8%, and 9.8-28.2%, respectively. Comparing test rounds, higher prevalence was recorded in test round 3, while lower prevalence was obtained in test round 4 (Table 6.1).

Table 6. 1 Bovine tuberculosis screening test results characterized by test rounds

Test rounds	**Herd size**	**Test interval (days)**	**Prevalence (%)**		**Tuberculin test result**		
			Standard	**Severe**	**Negative**	**Doubtful**	**Reactors**
Test 1	502	-	21.3	27.5	364	31	107
Test 2	521	459	8.4	15.4	441	36	44
Test 3	503	672	24.8	28.2	361	17	125
Test 4	498	345	5.4	9.8	449	22	27

6.3.2. Bayesian analyses

The prior distribution of sensitivity of SICCTT using standard and severe interpretations were beta(20.24, 8.86) and beta(24.06, 6.76), while the prior distribution of specificity were beta(115.76, 4.549) and beta(221.94, 12.63), respectively (Table 6.2). The posterior estimates of Se and Sp are shown in Table 6.3 and Figure 6.2. Se estimate for SICCTT was 68.3% using standard interpretation, which were a little bit lower than the prior values (70.1%). However, posterior estimates of Sp using standard and severe interpretations, and Se estimate using severe interpretation were equivalent to that of the prior distributions.

Table 6. 2 Prior estimates of sensitivity, specificity, and disease prevalence (%) based on expert opinions on most likely prior (mode), and 95% confidence interval using betabuster analysis

Parameter	Test	Expert opinion		Beta prior	Prior estimate	
		mode	95% certain		median	95% PPI
Sensitivity	SICCTT [a]	0.71	> 0.55	(20.24, 8.86)	70.01	(52.01, 84.56)
	SICCTT [b]	0.80	> 0.65	(24.06, 6.76)	78.67	(62.13, 90.54)
Specificity	SICCTT [a]	0.97	> 0.93	(115.76, 4.55)	96.4	(92.17, 98.84)
	SICCTT [b]	0.95	>0.92	(221.9, 12.63)	94.74	(91.39, 97.12)
Prevalence	Test round 1			(1, 1)		
	Test round 2[a]	0.1	<0.276	(2.90, 18.14)	12.65	(2.98, 31.02)
	Test round 2[b]	0.1	<0.31	(2.44, 13.99)	13.43	(2.65, 35)
	Test round 3[a]	0.27	<0.41	(10.34, 26.26)	27.86	(15.11,43.65)
	Test round 3[b]	0.31	<0.42	(18.3, 39.48)	31.44	(20.42, 44.09)
	Test round 4[a]	0.05	<0.309	(1.488, 10.28)	10.54	(1.0,35.75)
	Test round 4[b]	0.05	<0.315	(1.47, 9.98)	10.71	(0.99, 36.44)

Note: [a] standard interpretation, [b] sever interpretation

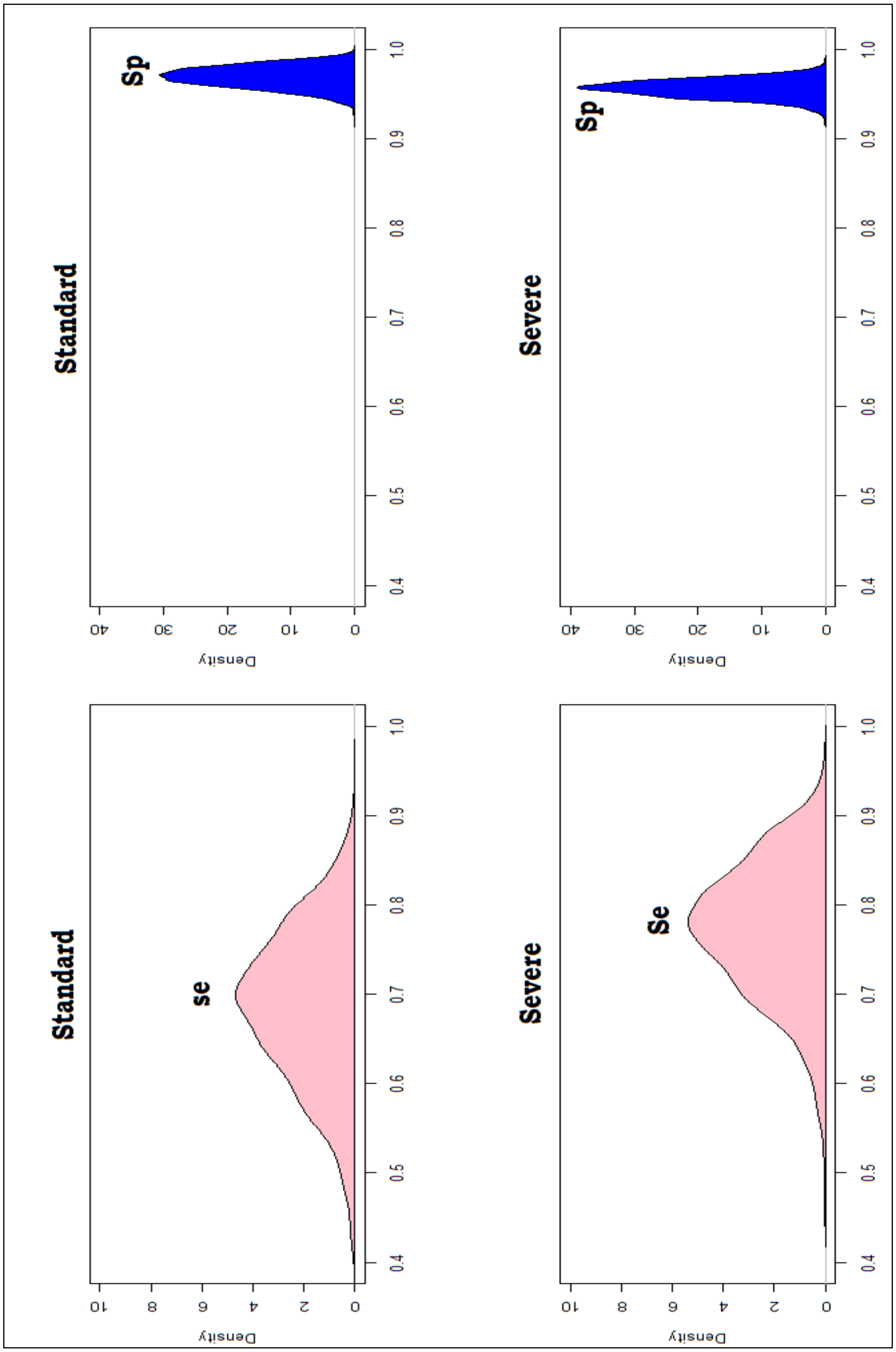


Figure 6. 2 Posterior distribution of sensitive and specificity of SICCTT using standard and severe interpretation

Estimates (median and 95 % Bayesian probability intervals; BPI) of sensitivity, specificity, and true prevalence for the four testing rounds from the model built are shown in Table 6.3. In test round 3 the estimated disease prevalence using standard interpretation criteria was higher than the prior estimate, while all prevalence estimates were lower than the prior estimates in test round 4 regardless of standard (4.1%) or severe interpretations (6.4%).

Table 6. 3 Bayesian estimates of disease prevalence (%), sensitivity, and specificity for each test rounds

Screening rounds	Parameter	Standard interpretation		Severe interpretation	
		Median	95% PPI	Median	95% PPI
Test 1	Prevalence	27.6	18.5- 40.6	31.0	23.1-41.7
Test 2	Prevalence	8.4	3.4-14.2	13.6	8-20
Test 3	Prevalence	30.91	23.3-47.5	31.5	25.1-39.1
Test 4	Prevalence	4.1	0.6-8.6	6.4	1.8-11.8
Overall	Sensitivity	68.3	50.4-83.7	78.1	62.5-90.9
	Specificity	96.8	94.3-98.9	94.5	91.5-97.1

Although true disease freedom is difficult to assess using the model above, we can infer the posterior probability of true prevalence less than 1%, which is the specified cut-off to define the herd free of bTB infection. For test rounds 1, 2 and 3, this probability was 0%, which suggested that there was no chance that the herd was free of *M.bovis* infection. However, in test round 4, there was a probability of 5.8% that the true prevalence was less than 1%.

6.3.3. Posterior estimates using informative prior, uninformative prior, and with no prior distribution (RGE)

Point estimates for the true prevalence using an estimator with informative priors was slightly higher than the median of both RGE and Bayesian estimators with uninformative priors in all test rounds, except test round 3 (Table 6.4). The Bayesian estimator with informative priors (unknown Se and Sp) exhibited larger predictive intervals than those with uninformative priors or a frequentist method (RGE). In test round 3, for instance, the 95% BPI for the posterior estimate assuming unknown but informative priors was (0.233,0.475), compared to (0.262, 0.372) when using fixed values of Se = 0.70, and Sp = 0.964. The estimate with fixed Se and Sp was much closer to the classical confidence interval (RGE), which was to be expected since both essentially used uninformative priors.

Table 6. 4 bTB prevalence estimates on subsequent single intradermal comparative cervical tuberculin test

Test Round	Rogan-Gladen point estimate	Bayesian estimate with fixed Se & Sp	Bayesian estimate with informative test prior
Test 1 (N = 502)	0.26 (0.215, 0.323)	0.268 (0.215,0.323)	0.276 (0.185-0.406)
Test 2 (N = 521)	0.073 (0.041, 0.114)	0.077 (0.046,0.114)	0.084 (0.034-0.142)
Test 3 (N=503)	0.319 (0.265, 0.379)	0.315 (0.262,0.372)	0.309 (0.233-0.475)
Test 4 (N = 498)	0.027 (0.002, 0.062)	0.033 (0.008, 0.065)	0.041 (0.007, 0.087)

Even though coverage is the common performance metric for a confidence (or credible) interval, the precision of these Bayesian estimates was also assessed using density plots. Posterior distributions of true prevalence estimate with unknown and known Se and Sp were compared (Figure 6.3). Bayesian estimates with fixed Se and Sp had high density posteriors, indicating higher precision than estimates with unknown test Se and Sp. The greater spread of Bayesian estimator posteriors with informative priors represents the loss of precision that results from not knowing those sensitivity and specificity of the test.

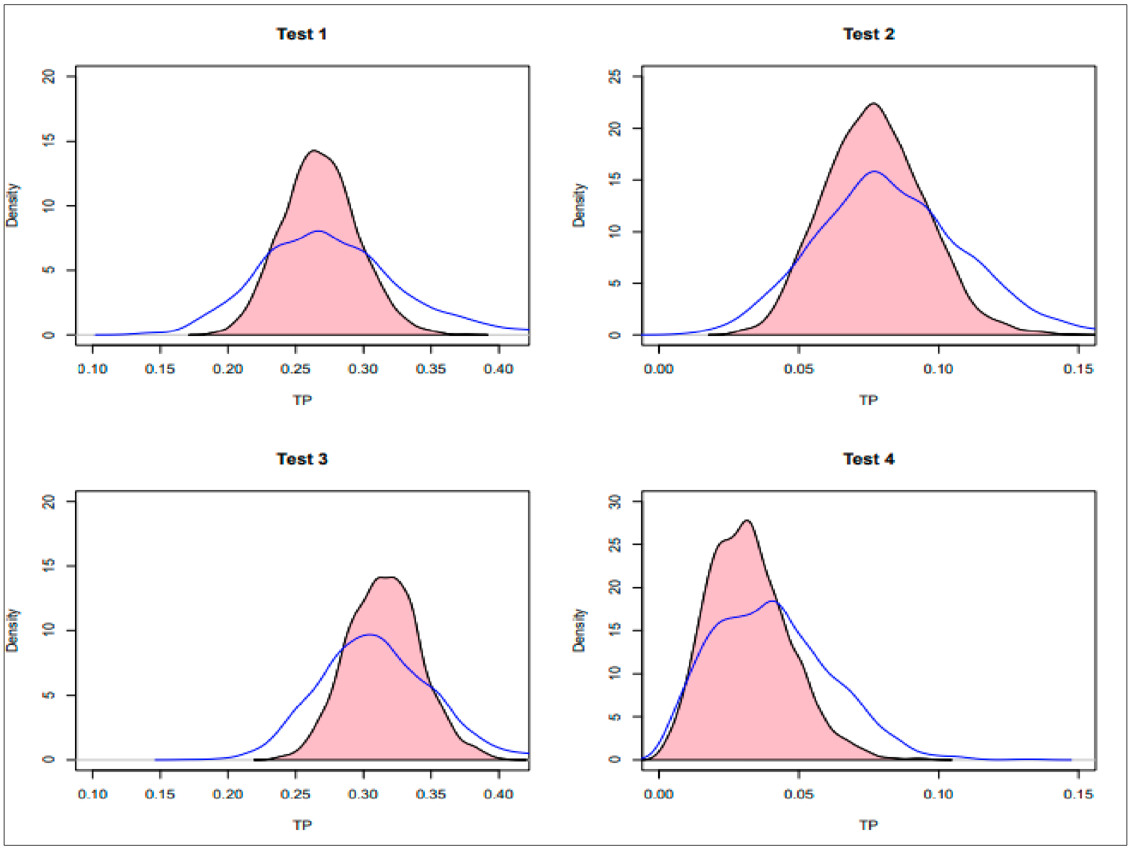


Figure 6. 3 Posterior prevalence distributions, using fixed test sensitivity and specificity (red shaded plot); and unknown sensitivity and specificity (blue line)

6.3.4. Convergence diagnostics for Markov Chain Monte Carlo

Convergence of generated Markov chains was verified by trace plot (Figure 6.4a), ACF plots (Figure 6.4b), and Gelman-Rubin Statistics (Figure 6.4c). Trace plots of true prevalence did not indicate non-convergence of the chains to the stationary distribution and demonstrated proper mixing of the two chains. Sample autocorrelation between the terms of the chains decreased rapidly as a function of their lag, indicating that the quality of these samples was sufficient to provide an accurate approximation of the target distribution. The Brooks Gelman-Rubin convergence diagnosis statistic for true prevalence estimates also indicated good convergence. Overall, there was no reason to suspect nonconvergence in the current study.

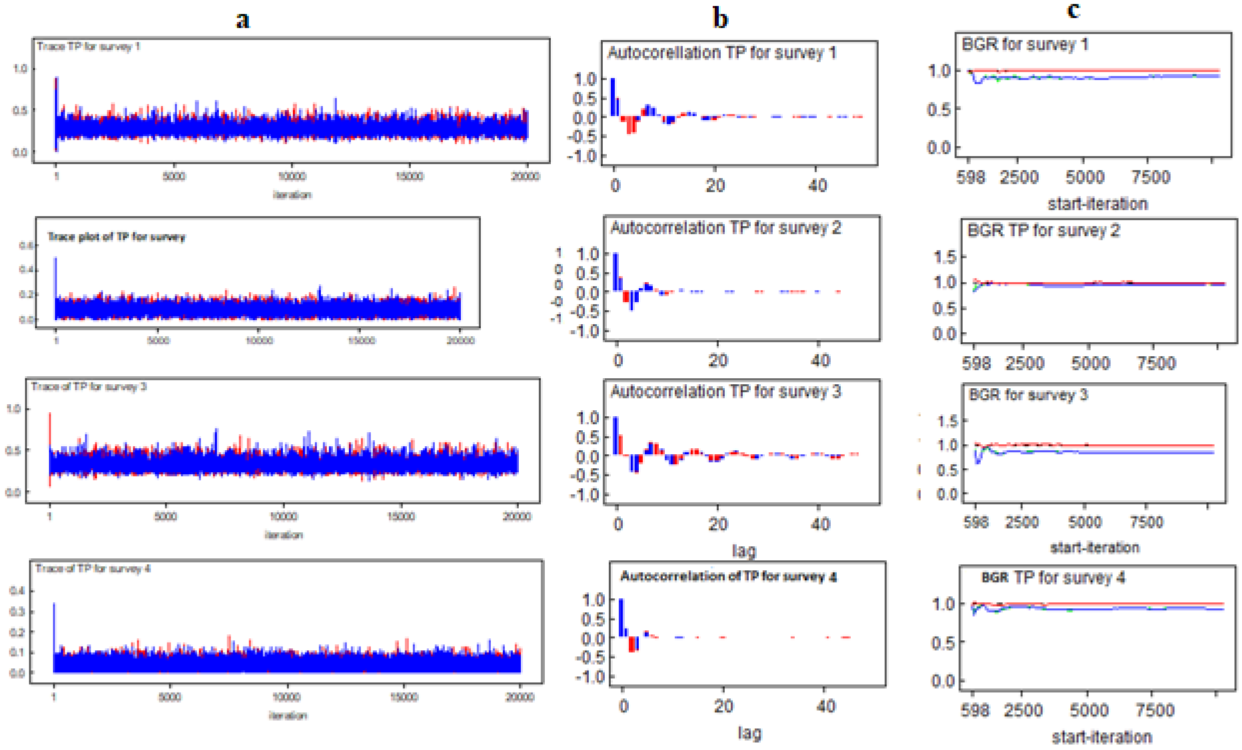


Figure 6. 4 MCMC diagnostic plots illustration

6.4. Discussion

A typical strategy for disease control involves regular field tests, quarantine of infected herds, and removal of positive animals. 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 cattle herds. 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) and a trend toward meaningful reduction of cattle-to-cattle transmission as soon as reactors and non-reactors were physically separated (PROUD, 2006).

Excessively prolonged and inconsistent tuberculin test intervals for herd retesting may be responsible for failures to reduce the number of new bTB cases. Besides these, lack of knowledge of, or disregard for test errors (false positives and negatives) can lead to inaccurate or misclassification of diseased states (Messam *et al*., 2008). All of these negatively impact disease surveillance, control and eradication programs, and consequently animal trade. One of the failures of “test and slaughter” approach might be due to invariably biased prevalence estimate (i.e apparent prevalence) among subsequent round screening tests using inaccurate tuberculin test. This is in line with the current study, in which “test and slaughter measures” failed to reduce the incidence. Conventionally, SICCTT is recommended as a diagnostic tool for reactor detection, for which diagnostic misclassification due to imperfect sensitivity and specificity is a typical source of (information) bias. For that reason Bayesian logic turns out to be useful tool as compared to traditionally adopted frequentist method (Staubach *et al*., 2002; Clough *et al*., 2003; Van Schaik *et al*., 2003). A Bayesian analysis was used to provide reliable information on the prevalence of bTB infection, and also to provide useful and relevant information on the diagnostic test commonly used for their detection in this population. In this study, employing the Bayesian approach, the test results of the four consecutive rounds of SICCTT from 2014 to 2018 were converted into the real probability events. Therefore, using the pre-established prior estimates of the test characteristics of SICCTT, the posterior true prevalence of bTB was estimated. Prevalence estimate in the third screening test round was relatively high, which might be due to an excessively prolonged test interval (672 days after test round 2).

Relatively low prevalence of the disease for test round 4 might be due to shortening the interval between test rounds (345 days). However, this test interval is still much longer than a previous study that recommended whole-herd test-and-removal be conducted with a 2–3 month testing interval (Smith *et al.*, 2014).

The positive predictive value of a test is very dependent on the prevalence of the disease in the study herd being tested. The higher the prevalence of disease is in the population being screened, the higher the positive predictive values. Low positive predictive value (PPV) in the second and fourth screenings may have resulted in needlessly culled animals in the test and slaughter scheme. Similarly, low NPV estimates in the high bTB prevalence rounds (test rounds 1 and 3) might indicate a number of false negatives, sustaining the disease in the herd and effectively making any test and cull policy ineffective (Rather *et al.*, 2020).

Our study demonstrated that the posterior distributions assuming known Se and Sp of SICCTT provide much narrower CI's than those assuming unknown Se and Sp and may result in significant under-coverage that could be considered unfit for prevalence estimation. The current findings are in line with previous studies (Diggle, 2011; Flor *et al.*, 2020), which have recommended against prevalence estimation under the assumption of known Se and Sp. In contrast, Bayesian estimation under moderately informative priors gives a reasonable assessment of certainty, and can be considered fit for use as it may provide coverage close to the level of true uncertainty (Bainter, 2017).

6.5. Conclusions

The current study anticipated that one of the likely responsible for the failures of “test and slaughter” measures in the study herd might be the invariably biased prevalence estimate (i.e apparent prevalence) among subsequent round screening tests using imperfect tuberculin test. Accurate animal-based estimates of disease prevalence in general and test characteristics (sensitivity and specificity) in particular are the cornerstone in bTB control and prevention strategy. Test and slaughter measures are expensive; therefore, estimating true disease status of the herd is very crucial. By using the estimates obtained by the Bayesian analysis it was possible to better estimate the true prevalence of bTB in the dairy herd under study while also providing a reliable estimate of the sensitivity and the specificity in this setting of the commonly used SICCTT for detection of bTB. This work provides a blueprint for future true prevalence estimates of bovine tuberculosis using linked combined data.

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CHAPTER SEVEN: SORI MODELING, PARAMETER ESTIMATION, AND THEIR APPLICATION FOR BOVINE TUBERCULOSIS EPIDEMIC PREDICTION IN ETHIOPIAN COMMERCIAL DAIRY FARM SETTING

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Abstract

Within the realm of compartmental models that provide a foundational mathematical framework for deciphering the intricate behavior of infectious diseases, the SIR model stands out as a significant tool for a multitude of infectious diseases. Our objective was to model disease dynamics at the herd level for commercial dairies in Ethiopia, considering herd demographics, bTB transmission, and alternative bTB control options. Here, we developed a compartmental stochastic model to simulate bTB within-herd transmission, fed it with epidemiological data from 9 herds and carried out parameter inference using Approximate Bayesian Computing methods. We aimed to assess the epidemiological effectiveness of some control intervention scenarios based on test-and-slaughter of positive animals, using mathematical modeling to infer bTB-within-herd dynamics. The estimated global median value of the transmission coefficient (β) was 0.33 newly infected animals per infectious individual per month (i.e. 4.03 per year). The median duration of the latent period was estimated 17.4 months. The global median value for the rate at which infected individuals become reactive to the skin test ($\gamma 2$) was 0.75 per month (equivalent to a latent period of 40 days). However, the results also evidenced a great variability in the estimates of those parameters (in particular β and γ) among the 9 dairy herds. Similarly, we estimated the number of days to detect a bTB-positive animal (in infectious class) after the infection entry to the herd. On average, it takes 630 days (21 months) to detect a bTB-infected animal ("I") after 1 infected animal is introduced into the herd. As the number of initial infected animals increases from 3, 5 and 10, the average duration decreases to 10, 7 and 4 months.

7.1. Introduction

Mathematical models and computer simulations are useful experimental tools for building and testing theories, assessing quantitative conjectures, determining sensitivities to changes in parameter values, and estimating key parameters from data. Typically, epidemic models are key tools to study the mechanisms by which diseases spread, to predict the future course of an outbreak, to design and evaluate detection and control strategies against diseases. epidemic (Dharmagadda, 2020; Kratzer, 2015).

Understanding disease transmission characteristics is crucial for developing and implementing more effective interventions and prevention strategies (Taghizadeh & Mohammad-Djafari, 2022).

In the field of animal health, a number of models have been useful tools for development of policy, design and evaluation of surveillance systems and the prediction of consequences due to introduction of new diseases and the expected impact of control strategies (Garner & Hamilton, 2011; Willeberg, Grubbe, *et al*., 2011; Willeberg, Paisley, *et al*., 2011); as for compartmental models, starting from the simple classical SIR model to more complicated proposals (Brauer *et al*., 2019). Within the realm of compartmental models that provide a foundational mathematical framework for deciphering the intricate behavior of infectious diseases, the Susceptible, Infectious, and Recovered (SIR) model stands out as a significant tool for many infectious diseases (Kratzer, 2015).

Regarding bovine tuberculosis (bTB), mathematical modeling has been employed to estimate the following:

- Within and between-herd transmission rates: these rates help us understand how bTB spreads both within individual herds and between different herds of cattle (Alvarez *et al*., 2012; Barlow *et al*., 1996; Griffin & Williams, 2000; Kao *et al*., 1997; Keeling & Rohani, 2008).
- Latency Period: The duration between infection and the detection or shedding of the bTB pathogen is commonly referred to as “latency.” Modeling provides insights into

this critical period (Conlan *et al*., 2012; Fischer *et al*., 2005; Kao *et al*., 1997; Perez *et al*., 2002; Smith *et al*., 2013).

- Reliability of bTB Diagnostic Techniques: Sensitivity and specificity of diagnostic methods play a crucial role in identifying infected animals. Modeling helps assess the reliability of these techniques (Conlan *et al*., 2012; Fischer *et al*., 2005; Smith *et al*., 2013).
- Disease Dynamics in wildlife: understanding how bTB behaves in wildlife populations is essential, especially when wildlife reservoirs contribute to disease incidence or persistence in livestock (Anderson *et al*., 2013; Delahay *et al*., 2013; Graham *et al*., 2013).
- Effectiveness of Control Measures: mathematical models evaluate the impact of various control strategies, both in cattle and wildlife reservoirs, to combat bTB (Fischer *et al*., 2005; Hardstaff *et al*., 2013; Smith *et al*., 2014).

Early diagnosis of bovine tuberculosis (bTB) allows us to understand disease dynamics and identify strategies for controlling transmission factors before it reaches an endemic stage. The single intradermal comparative cervical tuberculin test (SICCTT) is the most common ante-mortem bTB diagnostic test (Marassi *et al.,* 2013).

Despite repeated skin testing and slaughter efforts to eradicate bovine tuberculosis (bTB) as reported in the previous studies, the disease remains endemic in the studied dairy herd. The incompetence on incidence reduction is due to the existing control intervention that did not adequately consider the dynamics of bTB spread within herds. To address this, we developed a compartmental stochastic model for bTB within-herd transmission. We fed the model with epidemiological data (obtained from a previous study) and performed parameter estimation using Approximate Bayesian Computing methods. Finally, we use the proposed models and estimated parameters for the prediction of the transmission dynamics and spread of the infection in selected representative dairy herds.

7.2. Materials and Methods

The main methodological approach of this research can be summarized as follows:

1. Formulation of a compartmental stochastic model to understand within-herd transmission dynamics, accompanied by a detailed description of the model and its critical parameters.
2. Calibration and estimation of the model's parameters using collected data.
3. Utilization of the model for predicting the spread of infection once the parameters have been determined.
4. Demonstration of the method's effectiveness through application to both simulated and actual data sets.

The approach was organized in three steps (Figure 7.1). A compartmental stochastic SORI model operating in discrete time was developed and defined in the first step, then fed the model with epidemiological data from 9 dairy herds and carried out parameter inference using Approximate Bayesian Computing methods (i.e., parameterization) in the second step, and the dynamics of the infection was analyzed based on the schematic representation of the model given in Figure 7.2 on the third step.

Develop the full model

```
 • SIR model < function(beta, gamma,N){
      S<-N-1 # susceptible
       I<-1 # infected
       R<-0 # Recovered
        t <- 0 # Time
        while (I > 0) {
# Calculate rates of infection and transition
           Infection rate <- beta * I * S / N
           transition rate <- gamma * I
   # Sample the time until the next event from an
   exponential distribution
       dt <- rexp(1, rate = infection rate +
transition rate)
# Update the time
    t <- t + dt
# Sample the type of the next event (infection or
recovery) from a Bernoulli distribution
event <- rbinom(1, size = 1, prob = infection
rate / (infection rate + transition rate))
   # Update the state variables
     if (event == 1) {
# Infection occurs
           S <- S - 1
           I <- I + 1
        } else {
# Recovery occurs
          I <- I - 1
         R <- R + 1
          }
   }
# Return the summary statistics
      return(c(S = S, I = I, R = R, t = t))
}
```

Parameterization: estimation of parameter

```
 •  Define prior distribution for the parameters
prior <- function() {
  beta <- runif(1, min = 0, max = 1)
  gamma <- runif(1, min = 0, max = 1)
  N <- sample(10:500, size = 1) # Population size
  return(c(beta = beta, gamma = gamma, N = N))
}
 •  Generate simulated data from priors and the
    model
    # large number of simulations (e.g. 10000)
     nsim <- 10000 # Number of simulations
      param.sim <- t(replicate(nsim, prior()))
    # Matrix of simulated parameters
stat.sim <- t(apply(param.sim, 1, function(x)
SIR_model(x[1], x[2], x[3])))
View(stat.sim)
# Define the summarized observed data of S, I, R,
and t at some point of the epidemic
      stat.obs <- c(S = 50, I = 10, R = 40, t=10)
  •  ABC inference using the rejection method
   tol <- 0.1 # Tolerance level
   abc.rej <- abc(target = stat.obs, param =
param.sim, sumstat = stat.sim, tol = tol, method =
"rejection")
```

Analysis of the dynamics of the infection

Figure 7 1 Procedural illustration of study methodology

7.2.1. Development of the bTB transmission model

The transmission of bovine tuberculosis within a herd was modelled using a compartmental stochastic SORI model, which stands for Susceptible, Occult, Reactive, and Infectious (Chiu *et al.*, 2019; Conlan *et al.*, 2012), as shown in Figure 7.2. In this model:

- **Occult animals (O)** are those that have contracted the infection but remain undetected by the Single Intradermal Comparative Cervical Tuberculin Test (SICCTT) and are not yet capable of transmitting the disease.

- **Reactive/Exposed animals (E)** are those that have been infected and can be identified by SICCTT, yet they do not pose a risk of infection to others.

- **Infectious animals (I)** are those that not only have the infection and can be identified by SICCTT but are also capable of spreading the disease.

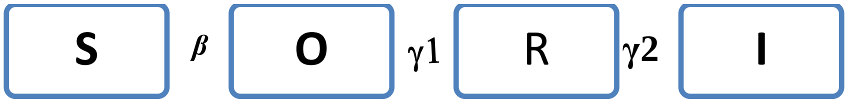

Figure 7 2 Flow diagram of the compartmental SORI (Susceptible, Occult, Exposed and Infectious) model representing the dynamics of the bTB spread within the herd

Animals that are susceptible to *M.bovis* infection become an occult state (O) at a rate represented by β, known as the transmission coefficient. These occult cattle then progress to reactive/exposed at a rate of γ_1, and then become an infectious state (I) at a rate γ_2. Once infectious, cattle can be identified as carriers of bTB, with the probability of detection determined by the sensitivity (Se) of the SICCTT. The model used for this process is homogeneous mixing, with transmission occurring at a frequency-dependent rate, which aligns with the true mass-action principle outlined in earlier research (Alvarez *et al*., 2012; Bekara *et al*., 2014; Fischer *et al*., 2005; Perez *et al*., 2002; Smith *et al*., 2013).

7.2.2. Dairy farm selection and herd data for parameter inference

7.2.2.1. Dairy farm selection

We used various data sources to estimate parameters related to bTB transmission in Ethiopian commercial dairy herds. Primary data was collected from previous KAP study. The study assessed the extent of farmers' knowledge and attitude associated with bovine tuberculosis (bTB), and identified some practices at farm level that are potentially relevant for bTB control and prevention. Therefore, 307 dairy farms were interviewed on their practices to bTB screening testing, and only 36 dairy farms had experienced bTB skin testing. Considering data collected from those 36 herds, we selected only those infected herds in which we had some certainty that the introduction of bTB into the herd had occurred through purchase of animals or new animal entry. To be more specific, first, we selected herds that had introduced or purchased animals between the two successive skin tests. i.e. negative herd result at the first test round and the detection of infection in the herd at the second test round. Likewise, at least one of the purchased animals reacted positive to the SICCTT at the time of detection (second round skin test).

Due to recall bias and lack of records some farmers could not recall a lot of information. Where possible, average or reasonable values that approximate the normal values were inputted for such farms and farms with too many missing data and information were discarded leaving a total of 9 positive farms used for the inference of bTB transmission parameters.

On those selected herds information to estimate bTB transmission (i.e. number of infected animals introduced, time for bTB transmission and final number of infected animals), was available. On the other hand, we developed a stochastic continuous-time compartmental model to allow us to simulate bTB within-herd transmission. And by feeding the data from these 9 herds to the transmission model, bTB transmission parameters in Ethiopian commercial dairy herds was inferred using a Markov Chain Monte Carlo (MCMC) algorithm within an Approximate Bayesian Computation (ABC) framework. Almost none of the study herds experienced skin testing before new entry animals to protect their herd from getting bTB infection.

7.2.2.2. Herd data

Data were collected from selected dairy herds that met the criteria in relation to introduction of bTB infection through purchase of animals. On those herds, data available included:

- Date of new animals entering into the study herd (x). i.e. the likely date of introduction of bTB into the herd.
- Date of bTB detection in the herd (y)

  Note: We assumed that the difference between both dates (y-x) represented the time available for the spread of bTB

- Number of animals in the herd on the date of bTB detection.

  We assumed a constant population size between infection of the herd and detection.
- Number of positives on the date of bTB detection.
- Number of positives among the purchased animals. As it is estimated at the time of detection, not at the time of purchase, it actually represents the maximum number of infected animals introduced into the herd (i.e. the number of occult animals introduced is modelled as a Uniform distribution between 1 and the number of positives among the purchased animals).

  Note: The difference between the number of infected among the purchased animals and the total infected animals in the herd on the date of bTB detection represented the spread of the infection within the herd since the introduction of bTB.

7.2.3. Parameterization

Even if building models that may explain our observations, or even feed some parameters to a model to simulate an artificial data set is possible, it is usually more difficult to carry out parameter inferencing that could have given rise to a given data set (Beaumont, 2010). Therefore, based on maximum-likelihood estimation some deterministic methods were developed for parameter estimation, despite they were constrained by the stochasticity, which is an inherent part of many biological systems (Hartig *et al.*, 2011; Toni *et al.*, 2009). Approximate Bayesian Computing (ABC) overcome those limitations (Beaumont, 2010). ABC methods are based on the calculation of summary statistics for a given configuration of the parameters obtained from the stochastic simulation model. Acceptance of that configuration is based on the comparison between observed and simulated data, and that comparison enables us to obtain an approximated posterior distribution of the model parameters (Hartig *et al.*, 2011). Therefore, we used ABC Markov chain Monte Carlo (MCMC) algorithm to generate the posterior distributions of the bTB transmission parameters (β, γ) within dairy herds. ABC algorithm may have the disadvantage that the rate of acceptance may be quite low when non-informative prior distributions are used (Toni *et al.*, 2009). To build the posterior chains, the algorithm drew candidate samples from a proposal distribution that was normally distributed, centred at the previous state of the chain, and with standard deviations set at 0.004 for β, 0.003 for γ_1, and 0.009 for γ_2.

The study-herds were analyzed individually by running MCMC chains with 100,000 steps, with the posterior distributions thinned to return 10,000 samples. Therefore, we obtained 9 posterior distributions for each of the parameters estimated. ABC-MCMC simulations were assessed using the "coda" package (Plummer *et al*., 2006). The estimated posterior distributions of the bTB transmission parameters (β, γ) within dairy herds are summarized with their mean and quantiles, and also displayed graphically as box-and-whiskers plots. For each of the transmission parameters we also calculated a global median value (i.e., aggregated value), obtained by binding together the posteriors distributions inferred from the 9 selected dairy herds, after determining that each of the individual posterior distributions were satisfactory. Algorithms were implemented within the R environment version 4.2.1 (R Core Team, 2021).

7.2.3.1. Setting prior distributions

The uncertainty of β, γ parameters was accounted for by the use of prior distributions. The prior distributions of the β and γ were initially estimated based on previous studies.

Table 7. 1 Prior distributions for the bTB within-herd transmission model parameters, their values and the sources from which those values were derived

Parameter	Description	Value (range)	Source
β	Transmission rate	0.01/year (0.004–0.028)	Barlow *et al*. (1997)
γ_1	Rate at which infected individuals become reactive to SICCTT	8.32/year (8.32–26.07)	Kao *et al*. (1997)
γ_2	Progression rate, reactor to infectious	0.347/year (0.347–4.06)	Kao *et al*. (1997)

7.2.4. Methodological approaches to assess bTB transmission dynamics without control intervention

We developed an agent-based simulation model for a 500-dairy cow in a closed herd, with the following assumptions. All cow replacements were produced within the herd. The dairy herd was parameterized to a HARC dairy herd. It was assumed that bTB was introduced in the herd at day 1 of the simulation from infected, but unreactive, heifers. Even in a closed herd, *Boran* heifers may, at times, be purchased from the market. In this analysis, we assumed that this occurred at the beginning of the simulation. After infection was introduced, no other outside animals were allowed into the herd. The results were summarized as the time taken by this model to reach at equivalent prevalence rate of the first, second, third and fourth test round of HARC herd, the number of days or months to detect a bTB-positive animal after the initial infection entry, and total number of infected animals found in the herd at different time tables.

7.2.4.1. Assessment of alternative strategies

The model was run without application of any control intervention until the apparent prevalence (the sum of the animals in compartments R and I) reached to that of the estimated apparent prevalence of the first test round in the previous bTB screening tests in HARC herd (i.e. P1 = 21.4%). Then, the approximate number of animals in each of the infected compartments (O-R-I) was used to seed each of the three models evaluating bTB-control strategies. Models with each control strategy were run for 10 years, or until no cattle categorized as infectious remained in the herd (i. e, herd clearance of *M.bovis*, termed fadeout), whichever occurred first. The number of infected cattle and the number of cattle culled because of a positive test result in each category were tracked, and the time at which fadeout occurred was recorded where applicable. Results were produced separately based on the first, second, third, and fourth skin test prevalence output (comprising the dynamics initiated by 21.4, 8.4, 24.8 and 5.4 % prevalence, respectively) to assess the effects of the procedural compliance on disease intervention.

7.3. Results

7.3.1. Estimate values of parameters

The median estimate for transmission coefficient (β) was 0.336 newly infected animals for a single infectious individual per month, which falls within the range of 0.268 to 0.705 (Figure 7.3). This estimated value is equivalent to approximately 4.03 newly infected animals per infectious individual annually (Table 7.2).

The median global value for γ, which represents the rate at which infected but non-reactive and non-shedding cattle (denoted as "O") become infectious (denoted as "I"), was 0.057 per month. Therefore, from estimated γ, the median value of the latent period was estimated as, $1/\gamma$, which was 17.46 months (i.e., the time between an animal's infection to infectious state). When considering individual herds, the inferred median value for γ ranges between 0.038 and 0.078, corresponding to latent periods of 12 to 26 months (Figure 7.4).

Table 7. 2 Summary of the value of parameters estimated

Summary	**β**	**γ_1**	**γ2**
Number of farms for inference	9	9	9
Mean	0.32	0.69	0.047
Median	0.336	0.75	0.062

The median value for $\boldsymbol{\gamma}_1$ (i.e. the rate at which animals at state "O" transferred to reactive state (R)) was 0.75 per month (Table 7.2). Thus, the median estimate of the occult stage, was 40 days.

Similarly, the median value for $\boldsymbol{\gamma_2}$**,** which represents the rate at which infected animals with reactive to the SICCTT but not infectious yet (denoted as "R") became infectious (denoted as "I"), was 0.062 per month. Therefore, the median estimate of the exposed stage, which represents the time between when an infected animal becomes detectable by SICCTT to the time it becomes infectious, is 16 months.

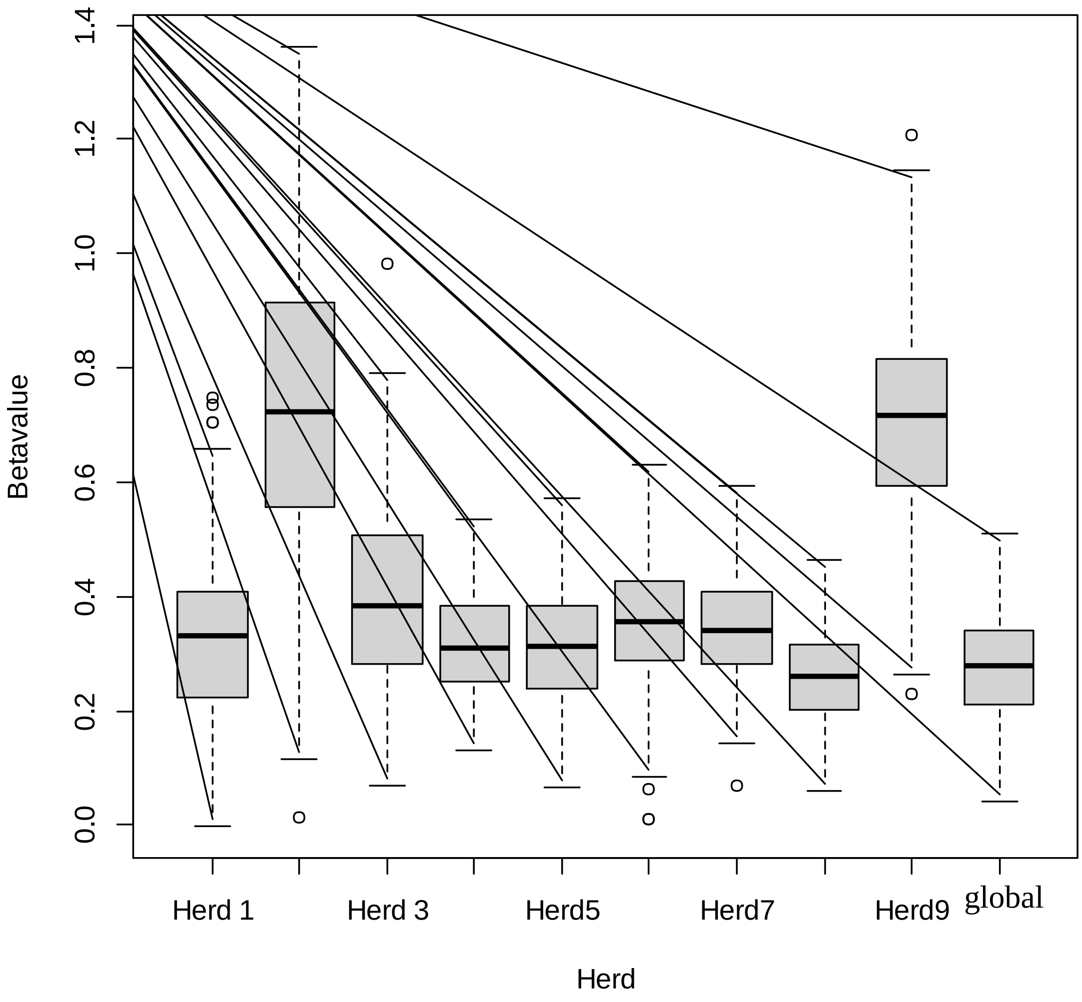

Figure 7 3 Estimated beta (β) values inferred from each of the 9 herds

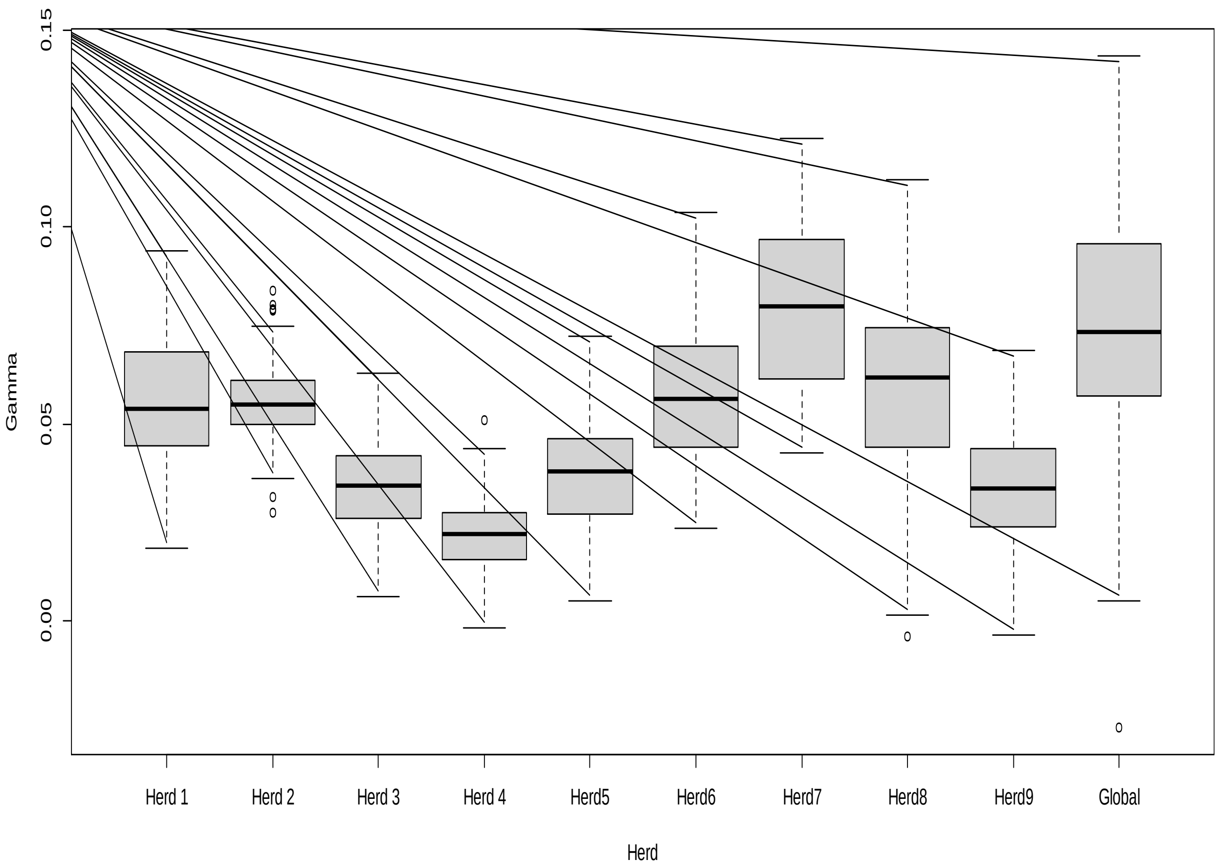

Figure 7 4 Estimated gamma (γ) values inferred from each of the 9 herds

7.3.2. Epidemics pattern of bovine tuberculosis with no control intervention

A SORI (Susceptible, Occult, Reactor, and Infectious) process, the most relevant model formulation for bovine tuberculosis, was used to easily capture the infection dynamics of the representative study farm (HARC). With the optimal parameter values obtained using the procedure given in the previous section ($\beta = 0.336$, $\gamma_1 = 0.75$, $\gamma_2 = 0.062$), we presented the results of the variation of S, O, R, and I listed through time. Without any control intervention, the characteristics of an epidemic following introduction of an infected animal is presented in Figure 7.5. The result showed that the number of susceptible animals decrease gradually along the infection courses after acquiring bTB when they come into contact with infected animals. Having a look on each compartment of Figure 7.5, revealed that the infection could not spread fast in the first several months despite it spread exponentially afterwards.

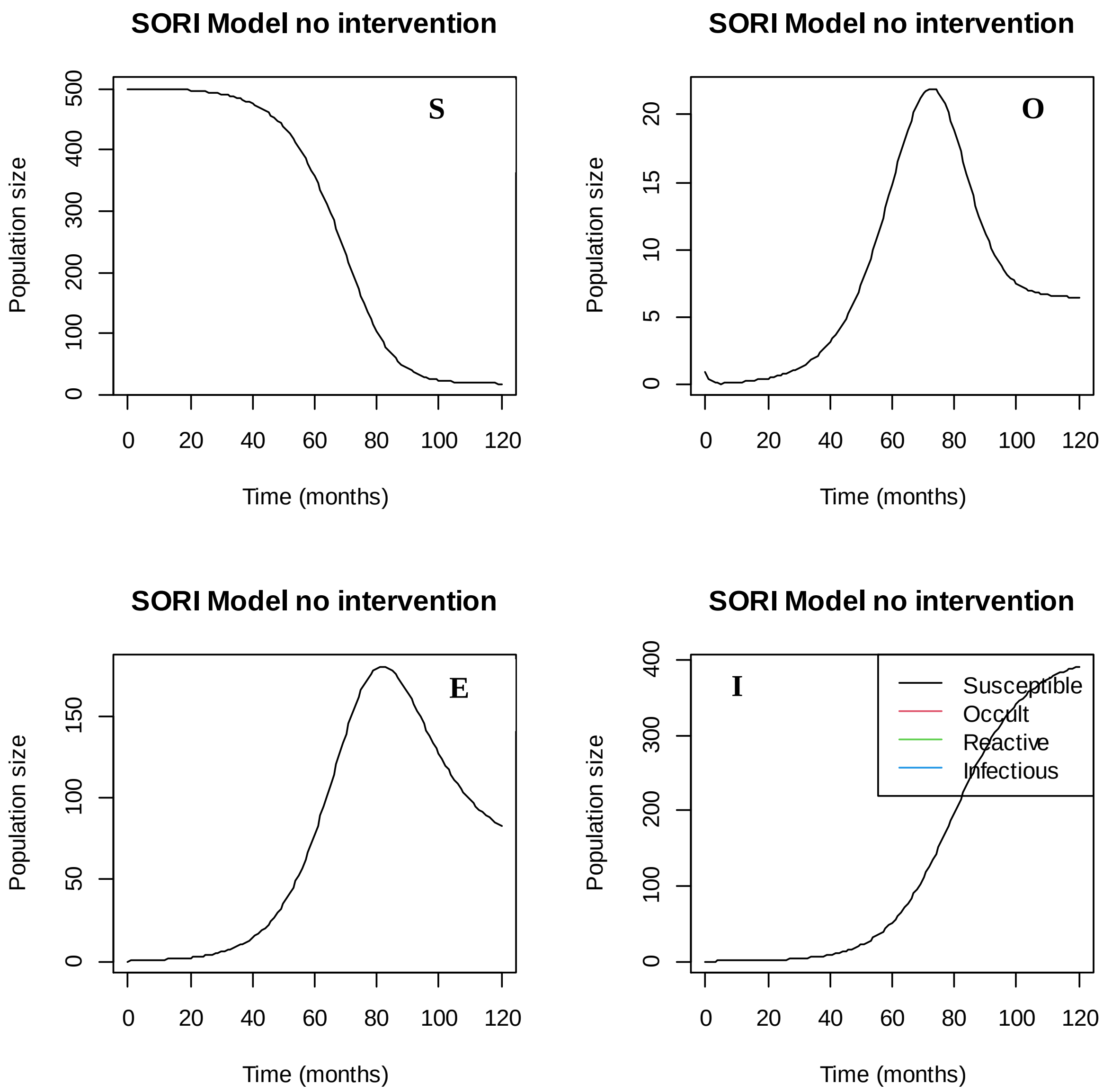

Figure 7 5 Trajectory of each compartment of the model over the time of simulation for 1 infected animal entry

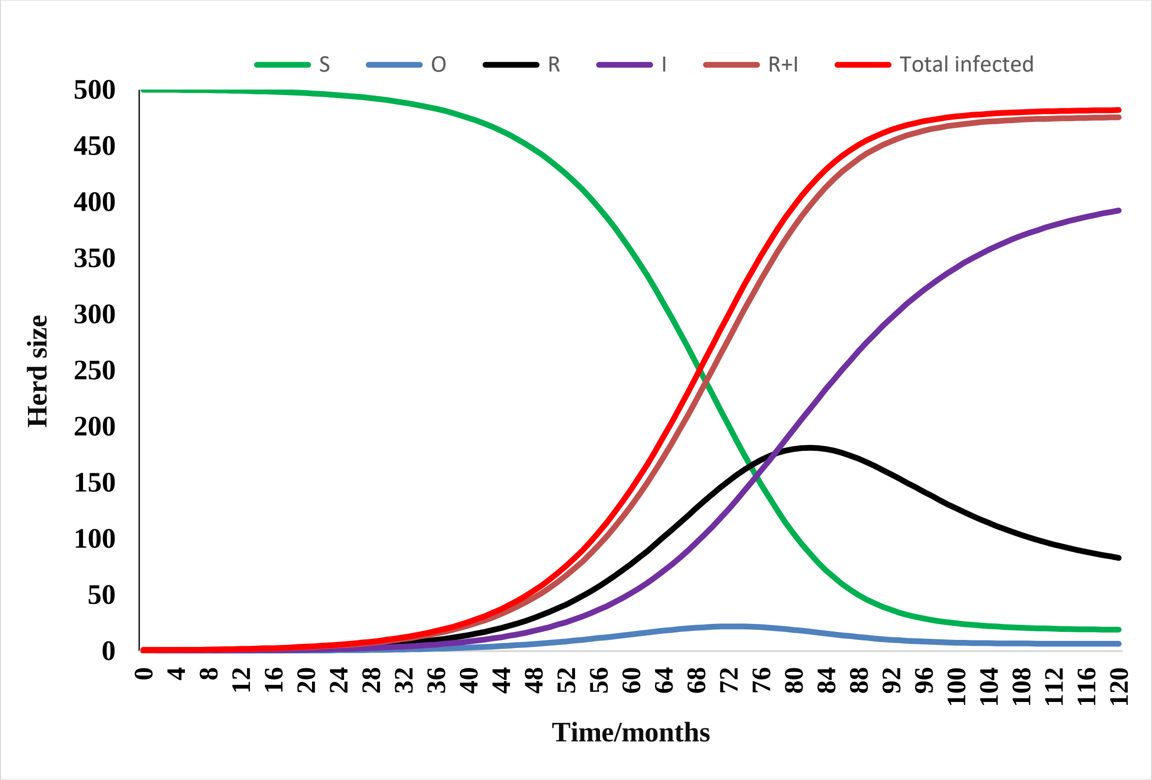

Figure 7 6 Infection transmission dynamics initiated by 1 infected animal entry to the herd along the time trend

7.3.2.1. Number of days or months for bTB detection

We estimated the duration in which it takes to detect a bTB-positive animal (in infectious class) after the infection is introduced in the herd, as well as the number of total infected animals found in the herd at different time tables. These results are shown in Table 7.3.

Table 7. 3 Time to detect infectious animals in the herd and total number of infected animals at different time tables

	Time	**Initial infected animals**		
		1 heifer	5 heifers	10 heifers
Time to "I" detection	(months)	18	5	3
Total number of infected animals				
	3 months later	1	5	10
	6 months later	1	6	11

	12 months later	2	9	18
	24 months later	6	28	54

On average, it takes 540 days (18 months) to detect a bTB-infected animal ("I") after one infected animal is introduced into the herd. As the number of initial infected animals increases, the average days to detection decreases. For instance, it takes, on average, 150 days (5 months) to detect an infectious animal if 5 bTB-infected animals are introduced in the herd, 90 days (3 months) to detect the infection if 10 bTB-infected animals are introduced in the herd; respectively.

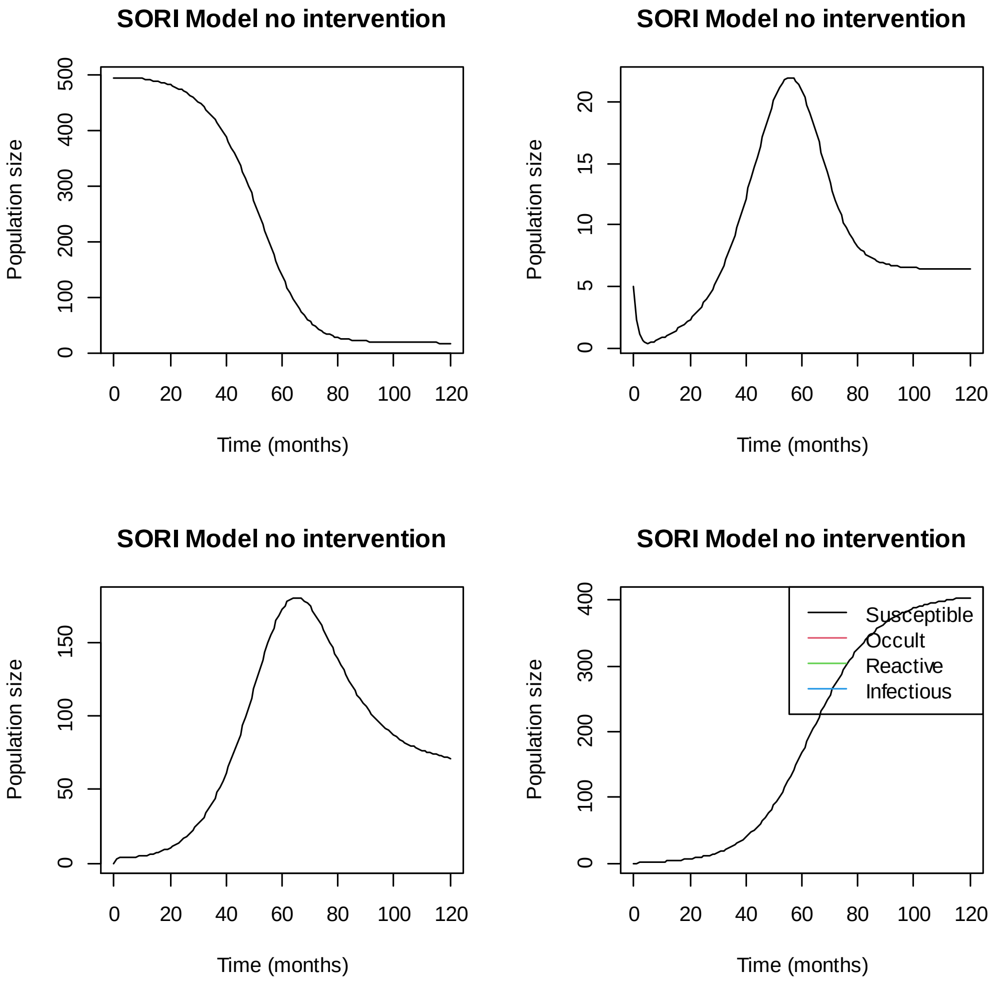

Figure 7 7 Infection transmission trend initiated by 5 infected animal entry to the herd along the time trend

Similarly, the transmission trend of the given model takes 58, 47, 60, and 42 months (Figure 7.8) to reach at the prevalence rate of Test round 1, 2, 3, and 4 of bTB screening round of HARC herd (for detail information described in the previous studies).

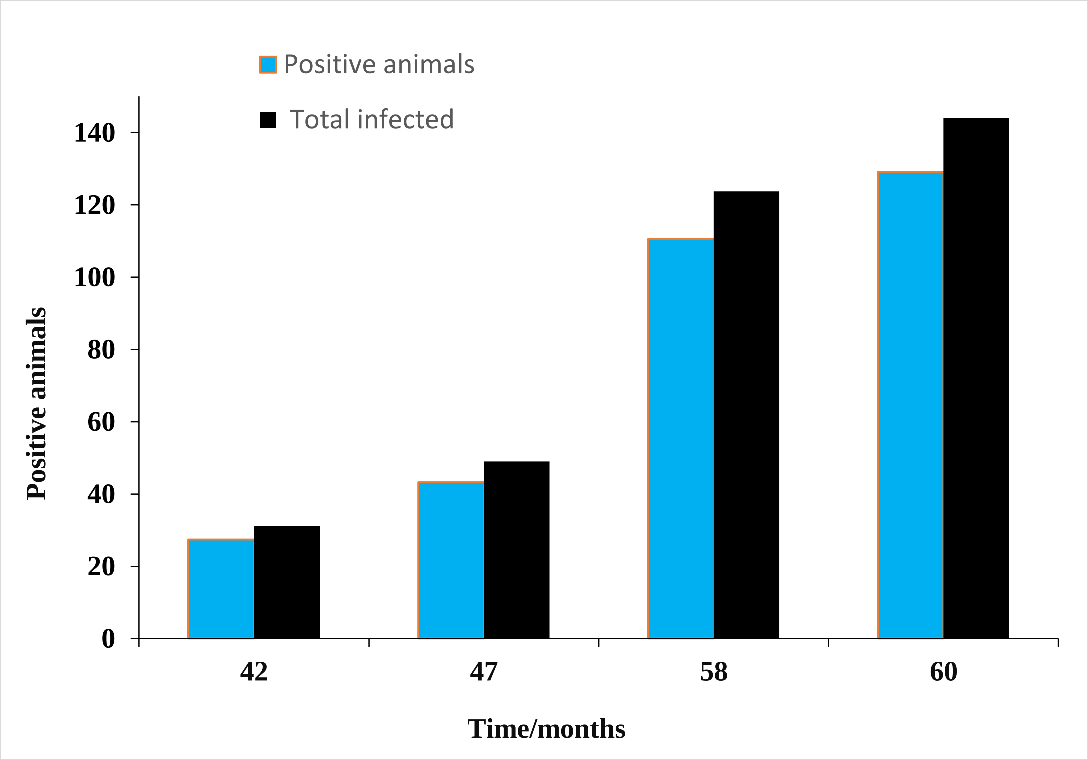

Figure 7 8 A time takes by simulation model to reach at equivalent prevalence of the previous test round of HARC herd

Note: Number of positive animals in this model at 42, 47, 58, and 60 months is equivalent to reactors at test round 4, round 2, round 1, and round 3, respectively

7.3.2.2. Latent state of the infection for 5 infected animal entry

Initially, susceptible animals took some period of time to be infected by the occult animals entered to the dairy farm. Since the fixed-parameter model could not give satisfactory results on real data, we presented the results of prediction of the variety of S, and I listed through time with a varying parameter model from a Poisson distribution with the purpose of incorporating stochasticity to the model. Therefore, the median time period at which no new infected animals are found was estimated 2.8 months (mean= 3.95; percentiles 25 and 75 of 1.13 and 5.62 months, respectively) (Figure 7.9).

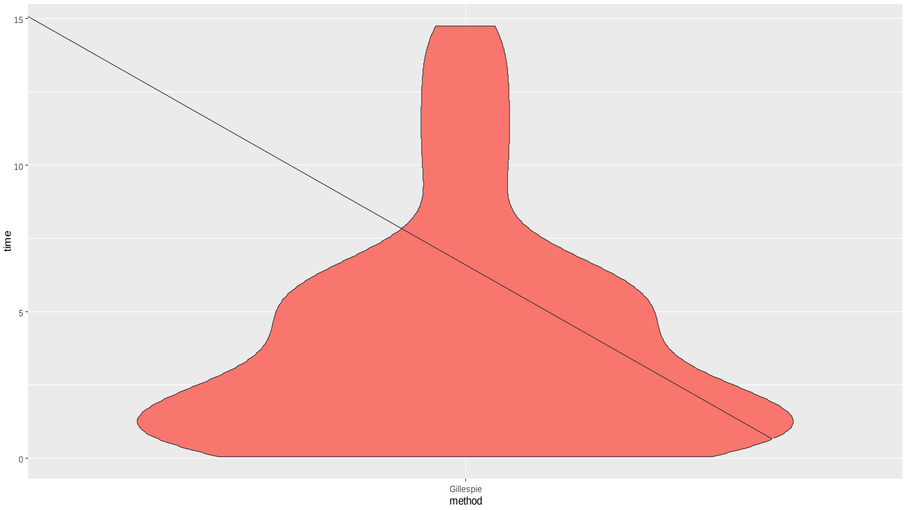

Figure 7 9 The latent state of bovine tuberculosis infection

7.3.3. Transmission dynamics with control intervention

We tried to show the transmission dynamic of bovine tuberculosis infection following three envisaged tuberculin test-based culling strategies. The first alternative strategy (Scenario 1: “closed herd”) placed the infected herd under quarantine with strict biosecurity measure including zero tolerance to add new animals neither to replace culled animals nor due to birth. Positive or culled animals are considered as removal. The second intervention approach (Scenario 2: “Between herd quarantine”) made animal entry and exit movement restriction between herds, but the model allowed within herd demography due to birth and natural death with similar rate. However, the scenario did not allow replacing culled animals. Culled animals are placed to removal compartment on the model. In the final control alternative (Scenario 3: “Constant replacement”), the infected herd are not placed under restriction, so that there is a constant replacement stock for culled animals, besides to the routine birth and natural death herd demography.

Three sets of results are presented in this analysis for each scenario: number of whole herd test (WHT) required to clear bovine tuberculosis (bTB) infected animals, number of days to infection clearance, number of culled animals till clearance.

7.3.3.1. Number of WHT required to herd clearance of bTB

The hypothesis whether the total number of WHT required to clear bTB infection in the herd differed depending on the envisaged tuberculin test-based culling strategies was tested. The total number of WHT needed to remove bTB from the herd for the three alternative strategies are shown in Figure 7.9. The number of total WHT needed to remove bTB was longer whenever there is a constant animal entry as replacement stock for those culled animal.

With a given 21.4% initial prevalence in 500 animals of dairy herd size, the bTB infection could be cleared from the herd after 3 consecutive WHTs with 3 months interval between WHT. That is, after the third WHT, the probability to get 1 infected animal in the study herd is approaching to 0% using scenario 1, and scenario 2 intervention strategies.

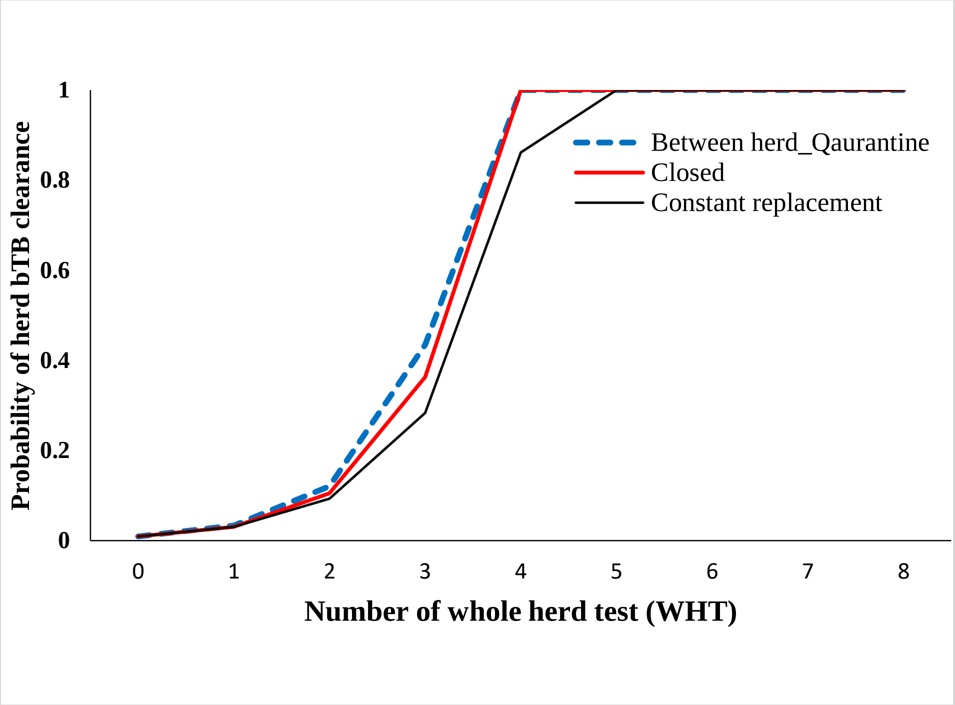

Figure 7 10 Empirical cumulative distribution of total WHT

Empirical cumulative distribution of the number of total whole-herd tests needed to clear bovine tuberculosis (bTB) infected animals using the three alternative test-based culling strategies are presented in figure 7.10

7.3.3.2. Time to clearance (month) and number of culled animals till infection clearance

Next, we estimated the number of months that it takes to clear the infection after the initial prevalence of 21.4%. On our alternative control strategies, it takes 12 to 15 months to remove bTB-infected animals for the given initial prevalence (Table 7.4). Our result revealed a longer time to remove all infected animals while constant replacement for culled animals was incorporated in the envisaged intervention model. Senario3 need additional 3 months to achieve herd clearance from bTB infection compare to 12 months requirement for herd clearance using scenario 1 and 2.

Table 7. 4 Table 7. 4 Time needed to remove all infected animals and number of culled animals until infection clearance

Tuberculin test-based culling strategies	Closed strategy	Quarantined	Replacing culled animals
Number of WHT till bTB clearance	4	4	5
Time to clearance (month)	12	12	15
Number of culled animals till clearance	179	127	
Final herd size ("S") at clearance	319	372	499

7.4. Discussion

In this study, bTB-transmission was simulated under very specific conditions (large herd size and high apparent prevalence) in Ethiopian dairy herd to assess the performance of current and potentially available control strategies, given that this is an emerging problem faced for which the effectiveness of the tools at hand has not been evaluated beyond adopting from other country, and not modeled to be adaptable to Ethiopian context extensively. The fixed size of the herd (500-animal) was considered large for the country. Furthermore, the starting apparent prevalence of 21.4% was high, most challenging bTB-infected herds (Abera *et al*., 2024; Lakew *et al*., 2022; Sibhat *et al*., 2017). In this context, the study assumed the whole herd would be subjected to bTB control intervention in every three months interval. The study considered three alternative control strategies and presented them for hypothesis testing. The scenarios took in account the common intervention tools applied for bTB control elsewhere and the existing control measures applied at HARC. By adopting SORI model with its compartments (S, O, R, and I), our analysis accounted for variations in the duration of the detection period (De la Rua-Domenech *et al*., 2006; Risso *et al*., 2020; Smith *et al*., 2013). Furthermore, high bTB-prevalence herds tend to have animals in various stages of the disease, including advanced stages, which can result in an improved Se for antibody-based diagnostics such as ELISA (De la Rua-Domenech *et al*., 2006; Waters *et al*., 2011). In the present study, there was no significant difference in the time to reach bTB clearance among the three alternatives. Our result was higher than Chiu *et al.* (2019) report of 202 days to eliminate bTB-infected animals from the herd. As the number of initial infected animals increases, the average days needed to eliminate a bTB outbreak from the herd increases (Chiu *et al*., 2019). However, our estimated time for infection clearance was so lower than previous study of bTB infection clearance with < 10 years of constant test–based culling (Smith *et al*., 2013). In most of the simulated scenarios, eradication was reached earlier in the adult category, since calves remain undetected until reaching the age to be tested (Risso *et al*., 2020). This explanation is in line with our findings in which the herd reaches too early (12-15 months) for bTB infection clearance. Undetected calves were responsible for sustaining bTB in the herd for longer periods in most simulations. We, therefore, need to consider this conclusion might not hold when simulating control strategies that include calfhood testing. Likewise, we observed a significant reduction in bTB-prevalence after the first 6 and 12 months with the use of any of the three alternative strategies, which was in-line with previous study exploring the effect of these control strategies in shorter periods (after 6, 12 and 24 months) (Risso *et al*., 2020). The model outputs suggest that alternative strategies can be selected as an initial strategy, and then could be followed by the use of other adopted or effective strategy for eradication. We recognize that a deeper understanding of the effect of different testing periods is needed. In order to identify optimal testing strategies, we balanced the epidemiologic effectiveness of disease control while minimizing the unnecessary culling of false reactors. While an initial useful approximation of the additional efforts imposed by each strategy, a next step is to provide an estimation of the economic cost, including costs of testing as well as unnecessary culling of false-positive cattle (Kao *et al*., 2018; Smith *et al*., 2013) and social acceptance (Ciaravino *et al*., 2017) both are essential before implementation.

7.5. Conclusions

In this report, we conclude that the assessed alternative strategies were able to improve the time to clear bTB infection clearance, reduce the number of culled animals compare to the cumulative number of culled animals in the previous four test rounds. Above all mentioned, the envisaged alternative control strategies were adaptable to the mission of the research center (HARC), and were in line with different mega projects on dairy breeding and nutrition. Results from this study contribute to the understanding of the necessity and implications of applying different adaptable and farmers demand-oriented intervention using simple and low resource cost approach in highly infected dairy herds in Ethiopia.

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CHAPTER EIGHT: GENERAL DISCUSSION

Deepening poverty; sluggish performance of the agricultural sector in general, livestock development in particular; accelerated resource depletion and emerging zoonotic disease in Ethiopia have always concerned policy makers and researchers and urged them to search for effective research and policy tools. The applicability of control and prevention of bTB in Ethiopia seems difficult to apply. This can be attributed to three important factors.

The first factor might be the farmer's knowledge gap about the disease: Knowledge on the potential source of infection, public health and economic burden of the disease, the transmission ways, and its control measures are critical for developing and implementing disease control and prevention strategies (Balkhy *et al.,* 2010; Brennan *et al.,* 2016). Non-biological factors such as farmers' knowledge, attitudes and practices, are a determining tools for disease control and prevention strategies (Zahedi *et al.*, 2014), which may remarkably impact the effectiveness of interventions against bovine tuberculosis (bTB)(Ciaravino *et al.*, 2017).

Secondly, the disease being least prioritized by dairy farmers: While bTB is a significant animal health and economic issue for many livestock industries, some farmers may not view it as their top concern, especially if the disease is not immediately impacting their herds (Reviriego Gordejo & Vermeersch, 2006). Farmers mainly perceived the control of bTB as an imposition rather than a necessary activity to protect their animals (Ciaravino *et al.,* 2017). Lack of visible disease outbreaks or perceived immediate threats can lead some farmers to deprioritize bTB relative to other pressing concerns like feed costs, labor shortages, or volatile market prices (Allen, 2017)

Lastly, the farmers and animal health technicians are not in compliance with the procedures followed to prevent and control bTB: A survey of UK farmers found that over a quarter did not always report suspected cases of bTB to authorities, citing reasons like financial impact and distrust of government policies (Enticott, 2012). In Ireland, researchers identified inconsistent farmer adherence to movement restrictions, animal testing, and biosecurity protocols as impediments to effective national bTB eradication efforts (More *et al*., 2015). In India, where bTB is endemic, farmers` poor compliance with test-and-slaughter programs and lack of traceability in informal livestock markets has undermined disease control strategies (Sharma *et al*., 2003). The present PhD research work; therefore, was aimed to reveal peculiarities of information on bTB transmission dynamics and control, relevant data for the implementation of a model-based disease control system and factors that might hamper the control of the disease in order to optimize resources.

In the first study, non-biological factors affecting bovine tuberculosis control and prevention in dairy herds was analyzed. This was very important to identify farmers knowledge ,attitude and practice on prevention and control strategies such as testing and slaughter. Bihon *et al* (2021) also analayzed the awareness level of dairy farmers in Ethiopia. Inaddtion, differrent factors that affect the KAP level among dairy farmers including on herd size, milk-shed, training availed, veterinary consultation and their previous farming experiences identified. The study found that 55% of participants were aware of bovine tuberculosis (bTB), which is relatively high compared to previous studies in Ethiopia. This could be attributed to that majority of the participants' proximity to Addis Ababa, inclusion of large and medium-scale intensive dairy farms, and participants' long years of dairy farming experience (Hailu *et al*., 2021; Mosalagae *et al*., 2011; Kidane *et al*., 2015; Bihon *et al*., 2021; Ameni and Erkihun, 2007; Kuma *et al*., 2013; Tigre *et al*., 2011). However, only 36.4% of participants had some basic knowledge about bTB, such as the potential sources of infection, public health and economic burden, transmission, and control measures. This highlights the need for more effective awareness and education campaigns (Balkhy *et al*., 2010; Brennan *et al*., 2016).

Majority of dairy farmers were not aware of the typical bTB control strategy involving regular testing and slaughter of infected herds. Only 12% were aware of the test and slaughter method, and 18% avoided buying cattle from potentially infected sources (Kao *et al*., 1997; Massó Sagüés *et al*., 2019; The ETHICOBOTS consortium *et al*., 2019). Farmers had a poor understanding of the zoonotic nature of bTB, perceiving it as a disease that only affects humans. This lack of awareness may be due to the belief that bTB control is mainly for commercial benefits, rather than also protecting public health (Grace *et al*., 2012; Liverani *et al*., 2013; Ciaravino *et al*., 2017). Among the farmers aware of bTB, only 1.6% considered it a priority animal health issue. This lack of interest in prevention and control actions could exacerbate the public health and economic burden of bTB (Ciaravino *et al*., 2017). Farmers expressed uncertainties about the reliability of the skin test, the common diagnostic tool for bTB. In the absence of a clear compensation policy for culled animals, almost none of the respondents thought the test and slaughter method was feasible (Ciaravino *et al*., 2017). Instead, farmers suggested educating the community about bTB and providing healthy heifers from reliable sources as key factors for successful bTB prevention and control (KAWSAR, 2022). These limitations may explain how bTB will continue to be a major threat. All these negatively impact disease surveillance and control intervention programs such as regular and repeated skin testing and slaughter strategy. Therefore, it was relevant to determine the magnitude or incidence of bTB in herds practicing irregular skin testing and slaughter strategy.

The second study assessed the incidence of bTB in a dairy herd with repeated irregular skin test and slaughter programme, and found that these incidences exhibited an oscillating pattern over subsequent time period. The study highlighted the importance of consistent testing and control measures to manage the risk of bTB in dairy herds. Penultimate test result, 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. Moreover, animals that had inconclusive results in previous tests were more likely to be bTB reactor, and high probable to have visible lesions at slaughter than those with a negative penultimate SICCTT result, emphasizing the need for careful monitoring and follow-up. It's crucial for dairy farms to implement regular and systematic skin testing and slaughter control programs to effectively control the spread of bTB. This not only protects the health of the herd but also ensures the safety of milk products for consumers.

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. Previous research 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, the USDA protocol requires the entire herd to have eight consecutive negative whole herd tests (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 third study, the impact of repeated test and slaughter for bovine tuberculosis control was examined. The effect of the intervention measures on resultant incidences, and its consequence on herd demographic changes were evaluated. Despite repeated testing and removal measures, the incidence did not exhibit a substantial reduction trend along the successive test and slaughter rounds. The time interval between successive SICCTT tests varied from 0.95 years to 1.84 years, which were excessively prolonged and inconsistent time intervals. Apart from the incompetence on incidence reduction, the study identified a vital knock-on effect on herd demography due to culling of a substantial number of cows (n=342) 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. Similarly, animal entries and exits also influenced the breed composition of the herd. The proportion of purebred Boran animals declined to 5%, while high-grade animals (75% Holstein blood) increased almost five-fold between the first and fourth test rounds. It is recommended that culling should be carried out with no or minimal significant impact on herd demography change. Compliance to conventional test and slaughter procedural protocol is supposed to play an important role in succeeding bTB control measures. Therefore, at least a minimum of 2-month a maximum of 6-month testing interval, and two consecutive negative whole herd testing should be carried out in order to declare a herd free of bTB.

Excessively prolonged and inconsistent tuberculin test intervals for herd retesting may be responsible for failures to reduce the number of new bTB cases. Besides these, lack of knowledge of, or disregard for test errors (false positives and negatives) can lead to inaccurate or misclassification of diseased states (Messam *et al.*, 2008). All of these negatively impact disease surveillance, control and eradication programs, and consequently animal trade. One of the failures of “test and slaughter” approach might be due to invariably biased prevalence estimate (i.e apparent prevalence) among subsequent round screening tests using inaccurate tuberculin test. This is in line with the current study, in which “test and slaughter measures” failed to reduce the incidence. Conventionally, SICCTT is recommended as a diagnostic tool for reactor detection, for which diagnostic misclassification due to imperfect sensitivity and specificity is a typical source of (information) bias. For that reason Bayesian logic turns out to be useful tool as compared to traditionally adopted frequentist method (Staubach *et al*., 2002; Clough *et al*., 2003; Van Schaik *et al*., 2003).

The fourth study in the present PhD thesis, “Bayesian modeling of bovine tuberculosis prevalence”, estimates the true prevalence and test characteristics (sensitivity and specificity) of SICCTT from the apparent prevalence in semi-intensive dairy farm over time. True disease states are uncertain in practice due to imperfect specificity and sensitivity, and so the true prevalence and characteristics of SICCTT were inferred using Markov Chain Monte Carlo estimation with prior distributions. Median sensitivity estimates using standard and severe interpretations were 68.6% (BPI; 50.3-84.3%) and 78.1% (BPI; 62.5-90.9%), while the specificity median were 96.8% (BPI; 94.3-98.9%) and 94.5% (BPI; 91.5-97.1%), respectively. Furthermore, adjusted true prevalence estimates (median and 95 %; BPI) were produced for each testing round using the Rogan-Gladen estimator (RGE). Bayesian estimation with informative priors exhibited much wider credible intervals and strong coverage compared to uninformative priors and frequentist method (RGE). Classic apparent prevalence estimates are overly precise when uncertainty around test performance is high. These Bayesian approaches provided a more accurate estimate of bTB prevalence in the study herd and provide a baseline data for the future true prevalence estimates using linked combined data. The current findings are in line with previous studies (Diggle, 2011; Flor *et al*., 2020), which have recommended against prevalence estimation under the assumption of known Se and Sp. In contrast, Bayesian estimation under moderately informative priors gives a reasonable assessment of certainty, and can be considered fit for use as it may provide coverage close to the level of true uncertainty (Bainter, 2017).

The fifth study in the present PhD thesis simulated bTB-transmission under very specific conditions (large herd and a high within herd apparent prevalence) in a dairy cattle herd in Ethiopia to try to assess the performance of current and potentially available control strategies, given that this is an emerging problem faced by animal health researchers in the country for which the effectiveness of the tools at hand has not been evaluated beyond adopting from other country, and not modeled to be adaptable to Ethiopian context extensively. The fixed size of the herd (500-animal) was considered large for the country. Furthermore, the starting apparent prevalence of 21.4% was high most challenging bTB-infected herds (Abera *et al*., 2024; Lakew *et al*., 2022; Sibhat *et al*., 2017). In this context, we assumed the populations in the herd would be subjected to every three months control strategies (three alternatives) considered alternatively. Alternative scenarios take in account the existing intervention tools applied for bTB control elsewhere and HARC. By adopting SORI model with its compartments (S, O, R, and I), our analysis accounted for variations in the duration of the detection period (De la Rua-Domenech *et al*., 2006; Risso *et al.*, 2020; Smith *et al*., 2013). Furthermore, high bTB-prevalence herds tend to have animals in various stages of the disease, including advanced stages, which can result in an improved Se for antibody-based diagnostics such as ELISA (De la Rua-Domenech *et al*., 2006; Waters *et al*., 2011). We found there was no significant difference in the time to reach bTB clearance among the three alternatives. In most of the simulated scenarios, eradication was reached earlier in the adult category, since calves remain undetected until reaching the age to be tested (Risso *et al*., 2020). This explanation is in line with our findings in which the herd reaches too early (12-15 months) for bTB infection clearance Undetected calves were responsible for sustaining bTB in the herd for longer periods in most simulations. Therefore, it needs to consider that this conclusion might not hold when simulating control strategies that include calf hood testing. Likewise, we observed a significant reduction in bTB-prevalence after the first 6 and 12 months with the use of any of the three alternative strategies, which was in-line with previous study exploring the effect of these control strategies in shorter periods (after 6, 12 and 24 months)(Risso *et al*., 2020). The model outputs suggest that alternative strategies can be selected as an initial strategy, and then could be followed by the use of other adopted or effective strategy for eradication. We recognize that a deeper understanding of the effect of different testing periods is needed. In order to identify optimal testing strategies, we balanced the epidemiologic effectiveness of disease control while minimizing the unnecessary culling of false reactors. While an initial useful approximation of the additional efforts imposed by each strategy, a next step is to provide an estimation of the economic cost, including costs of testing as well as unnecessary culling of false-positive cattle (Kao *et al*., 2018; Smith *et al*., 2013) and social acceptance (Ciaravino *et al*., 2017) both are essential before implementation.

8.1.Conclusions and recommendations

In conclusion, the first study underscores the critical role of farmers' knowledge, attitudes, and practices in controlling bovine tuberculosis (bTB). The present study determined that there is a gap on the KAP of dairy farmers in the study areas. While 55% of participants were aware of bTB, significant gaps remain in understanding its transmission, economic impact, and control measures. Many farmers perceive bTB control as a commercial rather than a public health necessity, with only 1.6% prioritizing it as a significant animal health issue. This highlights the need for improved educational campaigns and better networking with governmental and non-governmental organizations to enhance awareness and implement effective bTB control strategies. Addressing these gaps is essential to reduce the public health and economic burden of bTB.

The second study highlights the importance of consistent and timely tuberculin testing for effective bovine tuberculosis (bTB) control. Despite the recommended strategy of regular field tests and quarantine, our findings show variable bTB incidence due to prolonged and inconsistent testing intervals. Previous studies in Ethiopia and elsewhere demonstrate that more frequent and systematic testing significantly reduces bTB incidence. The study also confirms that animals with doubtful skin test results are more likely to become reactors in subsequent tests and exhibit visible lesions at slaughter, give emphasis to the need for vigilant monitoring and timely interventions. These animals should have been isolated from the negative tested animals until the next testing rounds. These insights emphasize the critical need for obeying to strict testing protocols to minimize bTB transmission and improve herd health.

Furthermore, despite repeated testing and removal measures, prolonged and inconsistent intervals between tests hindered the expected amount of incidence reduction. Adhering to recommended test-and-slaughter protocols with a 2 to 6-month interval and achieving two consecutive negative whole herd tests is essential for declaring a herd bTB-free. Additionally, managing culling practices to minimize impacts on herd demography is crucial for sustainable herd management. These findings highlight the need for stringent testing protocols to reduce bTB transmission and maintain herd health.

Excessively prolonged and inconsistent tuberculin testing intervals hinder effective reduction of bovine tuberculosis (bTB) cases. Inaccurate test results due to false positives and negatives further complicate disease control efforts. Our study showed that the "test and slaughter" approach often be unsuccessful due to biased prevalence estimates from imperfect tests like the Single Intradermal Comparative Cervical Tuberculin Test (SICCTT). Hence, employing a Bayesian approach provided more reliable prevalence estimates to effectively manage bTB. Accurate diagnostic methods and Bayesian estimation are crucial for improving bTB control and eradication programs.

The assessed alternative strategies were able to improve the time to clear bTB infection clearance, reduce the number of culled animals compare to the cumulative number of culled animals in the previous four test rounds. Above all mentioned, the envisaged alternative control strategies were adaptable to the mission of the research center (HARC), and were in line with different mega projects on dairy breeding and nutrition. Results from this study contribute to the understanding of the necessity and implications of applying different adaptable and farmers demand-oriented intervention using simple and low resource cost approach in highly infected dairy herds in Ethiopia.

From the above conclusions made, the following recommendations are forwarded:

- A customized training programme should be developed to raise the farmers' knowledge and encourage them to adopt bTB prevention practices
- To gain a more comprehensive and accurate insight into the KAPs of bTB, further all-inclusive studies should be studied
- Fair incentive from the government or stakeholder could motivate farmers to apply control intervention measures (example providing healthy heifers from known source, a recognition certification, etc)
- Avoid inconsistent and extremely prolonged skin testing schemes
- Consider the necessity of inconclusive animal contribution, which should have been isolated from the negative tested animals until the next testing rounds

- Comply to conventional test and slaughter procedural protocol. i.e. at least a minimum of 2-month a maximum of 6-month testing interval, and two consecutive negative whole herd testing should be carried out in order to make a herd free of bTB.
- Culling management of the dairy farm should be carried out with no or minimal significant impact on herd demography change.

8.2. Future research questions

The present PhD study provides insight into methodological tools (particularly bTB modelling) to study transmission parameters, dynamics, evaluate alternative intervention options for bTB, a chronic disease with very long and variable latent period, which makes field study difficult and complex. Therefore, the following research outlook have been identified for future study

- Apart from projecting the possible economic implications or enormous losses bTB could inflict on the society and country’s livestock economy based on high prevalence of the disease, the cost of bTB on economic impact on cattle productivity, bTB control programs, cattle value (social and breeding value) and other related economic effects of the disease should be enumerated (quantified), and documented, which would support for policy makers and other stakeholders to intervene.

- In order to achieve the earliest detection of infected herds and minimize the risk of continued infection after testing, it is crucial to design tailored screening-test policy that accounts for the accuracy variation of individual diagnostic testing. This policy should consider factors such as transmission timescales and testing frequency, with the ultimate goal of preventing disease spread to other farms.

- There is a need for a new approach to tackle bTB in humans and animals through cross-disciplinary integration of livestock and human health research. Some of the intervention measures, constraints and/or their severity varied across the husbandry systems, confirming the need for system-specific control strategies and interventions. The current status of the disease should be investigated on country level where such information has not yet been gathered. This will all support the future of a national bTB control program in Ethiopia.

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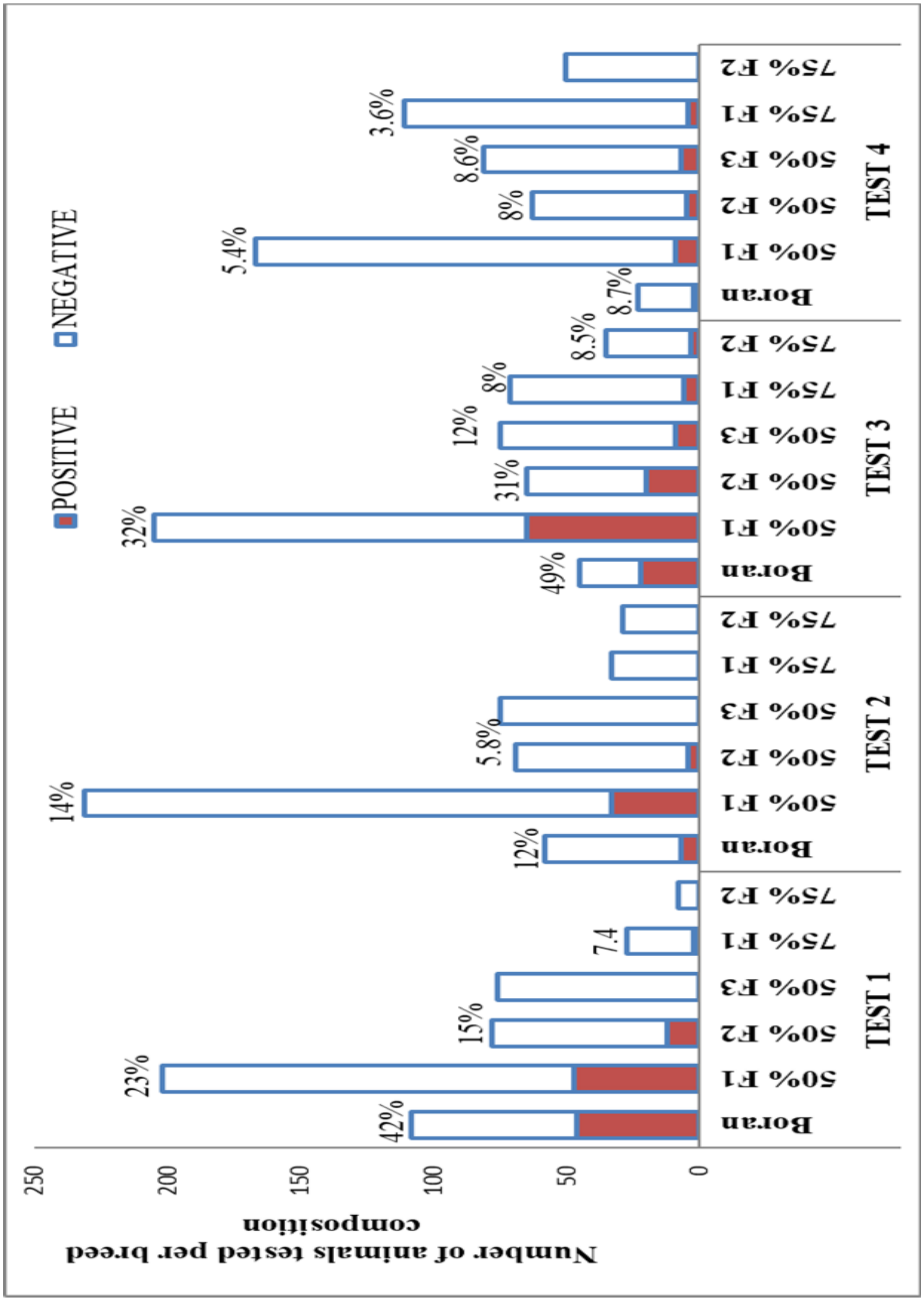
ANNEXES

1. HARC dairy herd practicing irregular test and slaughter control program

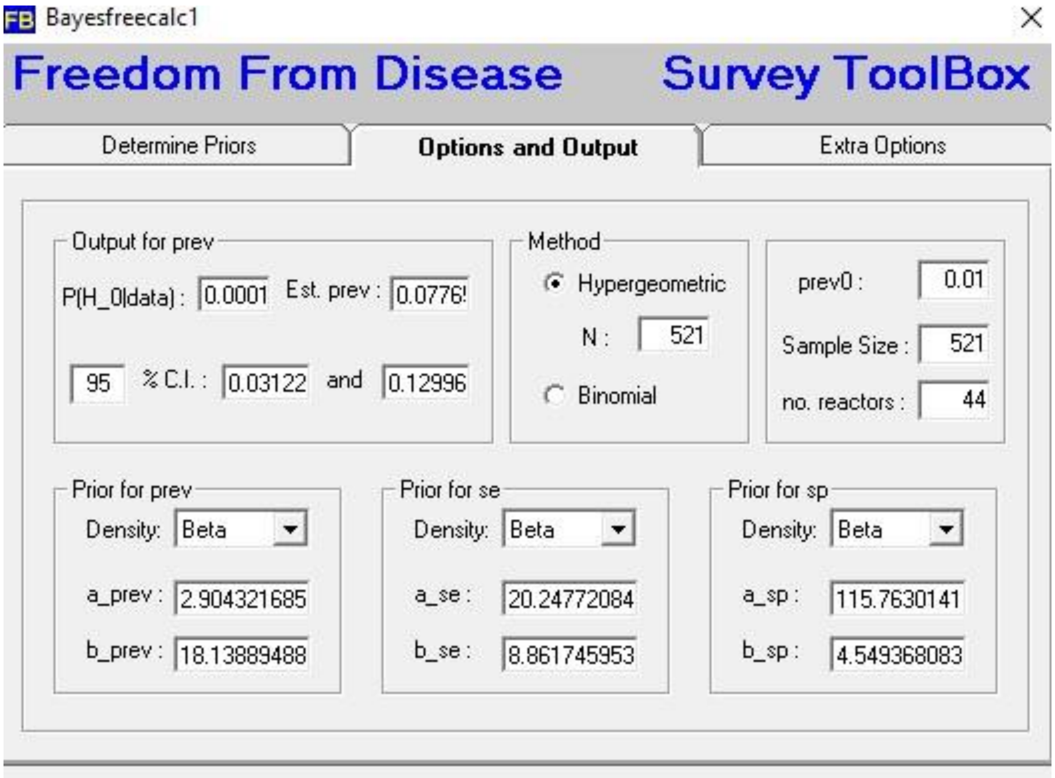
i. SICCTT

ii. Post mortem examination

iii. Breed composition of HARC



1. Disease freedom estimation using Bayesfreecalc1 (Bayesian modelling)



Output for prev

P(H_0|data) : 0.0001 Est. prev : 0.0776

95 % C.I. : 0.03122 and 0.12996

Method

● Hypergeometric

N : 521

○ Binomial

prev0 : 0.01

Sample Size : 521

no. reactors : 44

Prior for prev

Density: Beta

a_prev : 2.904321685

b_prev : 18.13889488

Prior for se

Density: Beta

a_se : 20.24772084

b_se : 8.861745953

Prior for sp

Density: Beta

a_sp : 115.7630141

b_sp : 4.549368083

2. SORI Modeling, parameter estimation, and their application for Bovine tuberculosis epidemic prediction in Ethiopian commercial dairy farm setting

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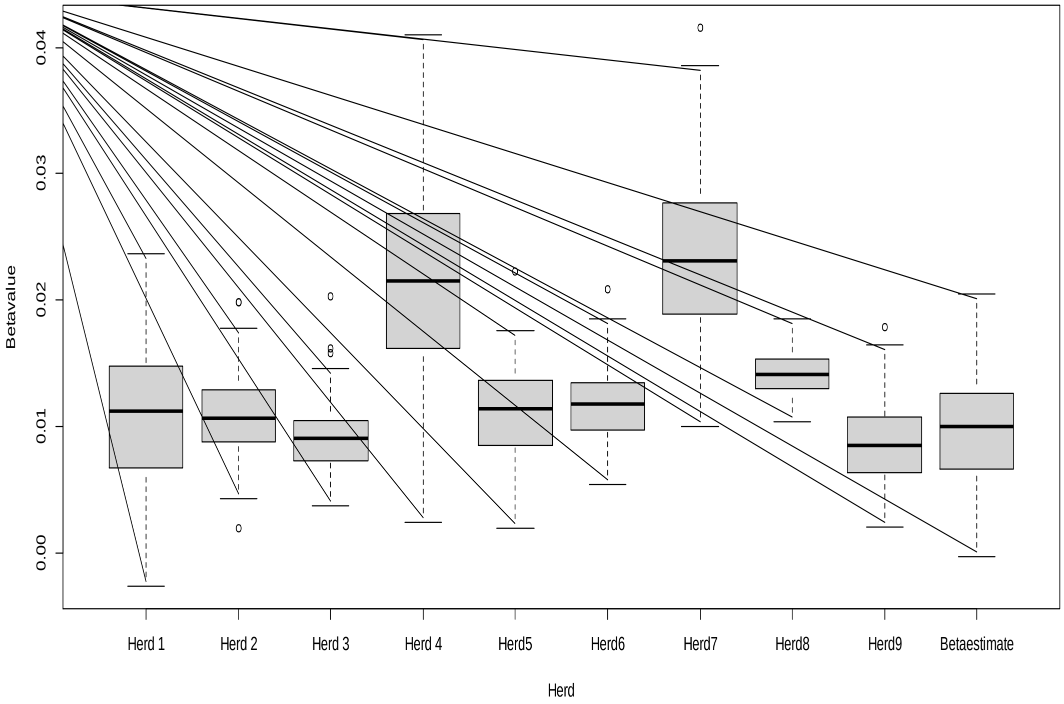
i. Beta estimation of 9 individual herds with global median estimate

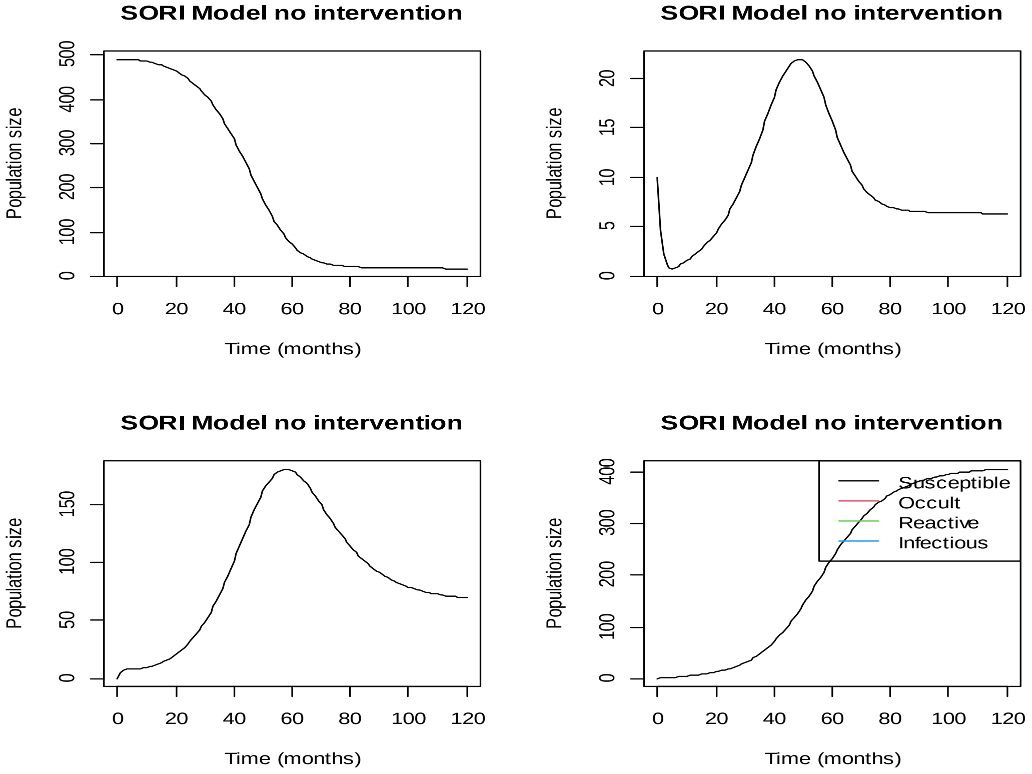


ii. R code for abc

```
library(abc)

# Define the SIR model
sir_model <- function(params, init, time) {
  require(deSolve)
  with(as.list(params), {
    N <- init[1]
    I <- init[2]
    S <- N - I
    beta <- beta0 * S / N
    dS <- -beta * I
    dI <- beta * I - gamma * I
    dR <- gamma * I
```

```
    return(list(c(dS, dI, dR)))
  })
}

# Define the summary statistics
sir_sumstats <- function(sim_data) {
  return(list(sum(sim_data[, "I"])))
}

# Define the prior distribution
sir_prior <- function(n) {
  return(data.frame(beta0 = runif(n, 0, 1), gamma = runif(n, 0, 1)))
}

# Load the data
data(sir_data)

# Define the observed summary statistics
obs_sumstats <- sir_sumstats(sir_data)

# Run the ABC algorithm
abc_out <- abc(sir_data, sir_model, sir_sumstats, sir_prior, method = "rejection", tol = 0.1, n
= 1000)

# Plot the posterior distribution
plot(abc_out, main = "Posterior distribution of SIR model parameters")
```

iii. Infection transmission dynamics initiated by 10 infected animal entry to the herd along the time trend

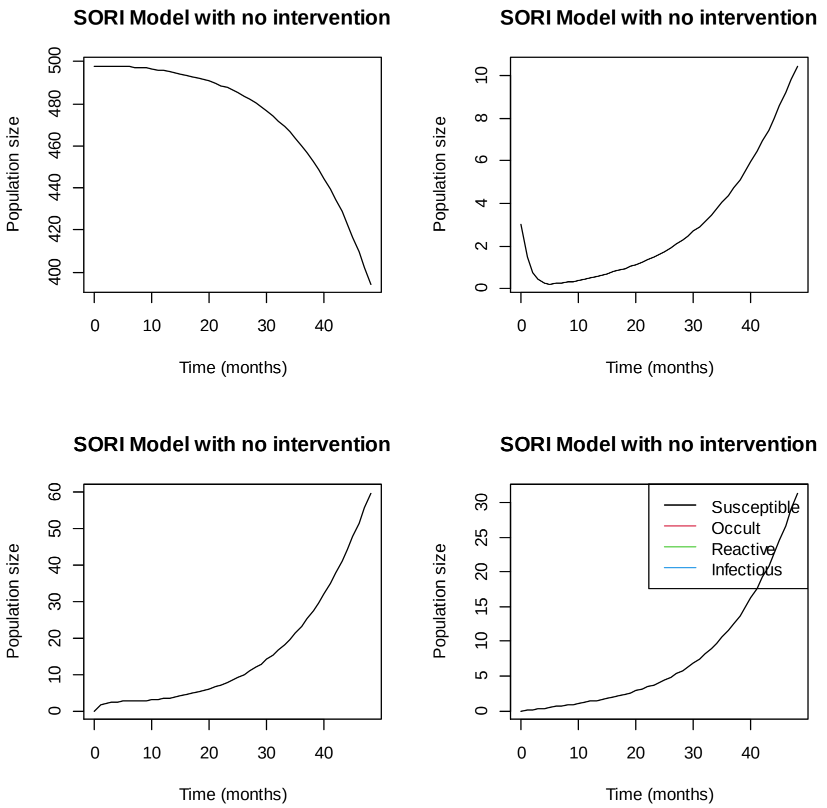


iv. Probability of the herd to be free from bovine tuberculosis using empirical cumulative distribution (ECD)

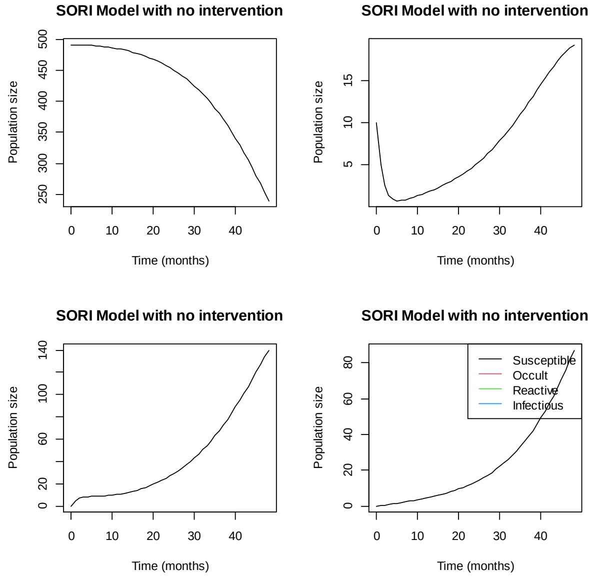
WHT (Whole herd test)	Between herd_Qaurantine	Closed	Replacing culled animals
0	0.009345794	0.009345794	0.009345794
1	0.033394549	0.030786261	0.03042185
2	0.120939761	0.105045728	0.09297645
3	0.434682239	0.362563904	0.283350516
4	1	1	0.861834501
5	1	1	1
6	1	1	1
7	1	1	1
8	1	1	1

v. Outbreak without control intervention

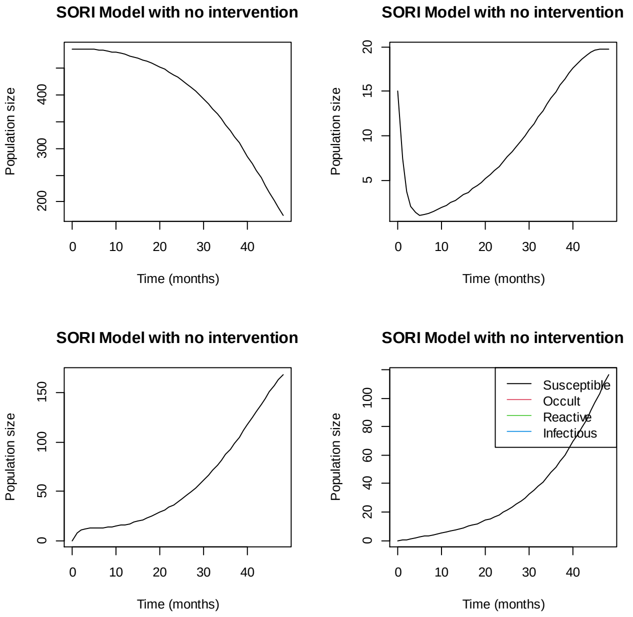
a) Initiated by 3 infected animal entry to the herd



b) Initiated by 10 infected animal entry to the herd

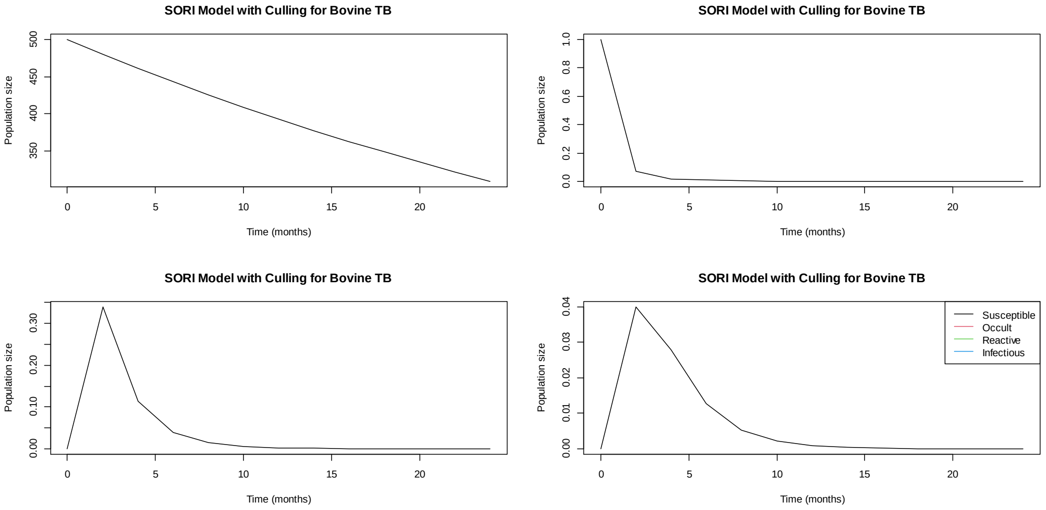


c) Initiated by 15 infected animal entry to the herd

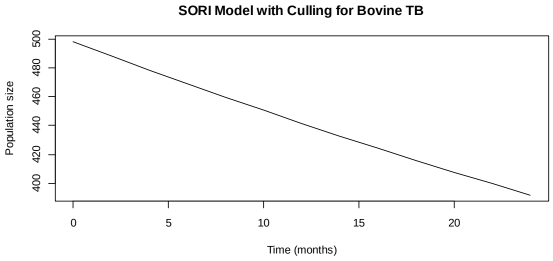


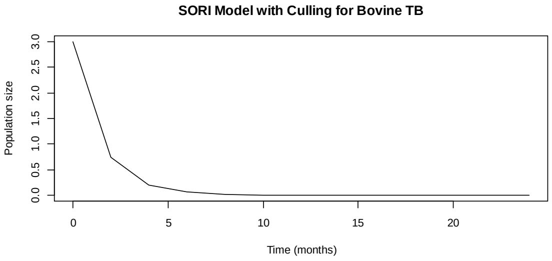
vi. Transmission dynamics with control intervention

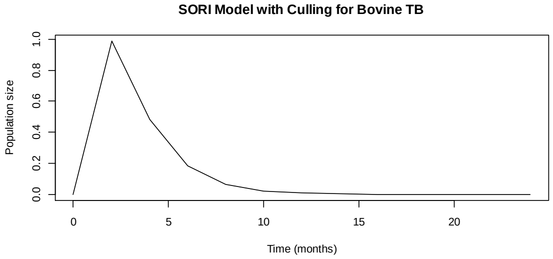
a) Outbreak due to 1 infected animal entry

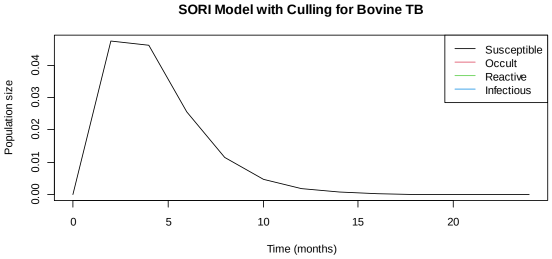


b) Outbreak due to 3 infected animal entry


SORI Model with Culling for Bovine TB
Population size
400
420
440
460
480
500
Time (months)
0
5
10
15
20


SORI Model with Culling for Bovine TB
Population size
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Time (months)
0
5
10
15
20


SORI Model with Culling for Bovine TB
Population size
0.0
0.2
0.4
0.6
0.8
1.0
Time (months)
0
5
10
15
20


SORI Model with Culling for Bovine TB
Population size
0.00
0.01
0.02
0.03
0.04
Time (months)
0
5
10
15
20
Susceptible
Occult
Reactive
Infectious