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BOVINE TUBERCULOSIS: PREVALENCE, RISK FACTORS, AND ASSESSMENT OF COMMUNITY AWARENESS IN WOLKITE TOWN, SOUTH CENTRAL ETHIOPIA

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Abstract: Bovine tuberculosis (bTB) is one of the most widespread chronic infectious diseases of zoonotic importance, causing significant economic losses in animal production. However, little epidemiological information is available on the disease in dairy cows in southern Ethiopia in general and the study area in particular. Therefore, a cross-sectional study was conducted between March 2022 and May 2023 to assess public awareness and examine the prevalence and associated risk factors of bTB in the city of Wolkite in southern Ethiopia. A total of 32 herds with 196 dairy cows were tested using the Single Intradermal Comparative Cervical Tuberculin (SICCT) test. To identify potential risk factors for bTB positivity in cattle, generalized linear mixed models (GLMM) and generalized linear models (GLM) were used to analyze the risk factors at the animal and herd level, respectively. An overall prevalence of 18.37% (95% CI: 13.57–24.38) and 46.87% (95% CI: 30.87–63.55) bTB was found at the animal and herd levels in the study areas. In this study, bTB positivity at the animal level was significantly associated with age (OR = 6.6, 95% CI: 2.19–37.02) and source of animal (OR = 3.1, 95% CI: 1.24–8.12). At the herd level, the analysis showed that management condition (OR = 4.7, 95% CI: 1.12–19.91), stocking density (OR = 6.3, 95% CI: 1.96-20.24), and new cattle introductions (OR = 2.8, 95% CI: 1.01–8.18) were significantly associated with bTB status. Of all dairy farm owners and workers surveyed, only 25.92% had information about bovine tuberculosis; of them, 32.14% were aware of the zoonotic importance of the disease. More than 69% of respondents said they prefer consuming raw milk. Therefore, animal owners and stakeholders should be made aware of the cause, the transmission routes and their effects on animals and humans.

***Index Terms* -** Bovine tuberculosis, Mycobacterium bovis, prevalence, Risk factors. Wolkite

1. INTRODUCTION

Ethiopia has the largest livestock population in Africa with 70.3 million cattle, 42.9 million sheep, 52.46 million goats, 8 million camels and 49 million chickens (CSA, 2021). Livestock is a significant source of animal protein, energy for growing crops, export goods, fertilizer for farmland and household energy, and for the accumulation of wealth. Nonetheless, animal diseases remain one of the biggest problems in sub-Saharan Africa, particularly in Ethiopia. Known zoonotic diseases include anthrax, rabies, brucellosis, bovine tuberculosis, hydatidiasis, cysticercosis, leptospirosis and Q fever (Tulu *et al*., 2021).

Bovine tuberculosis (bTB) is caused by *Mycobacterium bovis* (*M. bovis*). acid-fast, rod-shaped zoonotic bacterium from the Mycobacterium tuberculosis complex (MTBC) group. The MTCs are a group of genetically very closely related pathogens that can cause tuberculosis (TB) disease with similar pathology in a variety of mammalian species. It includes 12 closely related members of the genus Mycobacterium: M. tuberculosis, *M. bovis, M. africanum, M. microtii, M. canettii, M. caprae, M. pinnipedii, M. orygis, M. mungi*, and *M. suricattae* the Dassie and chimpanzee bacilli (Alexander *et al*., 2010). Cattle are believed to be the true hosts of *M. bovis*; the pathogen infects a wide range of hosts and can be easily transmitted to humans or a variety of domestic and wild animals (Fitzgerald and Kaneene, 2013).

BTB is a chronic debilitating respiratory disease that is difficult to diagnose clinically, although emaciation, anorexia, chronic cough and other signs of pneumonia can be symptoms that develop in cattle at relatively late stages of infection (Ayele *et al*., 2004). Especially in developing countries, the clinical forms of many other chronic, wasting diseases, such as African trypanosomiasis, chronic contagious bovine pleuropneumonia (CBPP) or chronic multiparasitism, are difficult to distinguish from bTB. bTB pathology is characterized by the formation of granulomatous lesions that regress or exhibit extensive necrosis, calcification, or liquefaction with subsequent disease progression (Fitzgerald and Kaneene, 2013).

If development slows, Africa and Asia will still experience rapid population growth and urbanization, leading to increased demand for food (UNDESA, 2022). According to the FAO Livestock Sustainability Analysis 2050 (2019) for Africa, demand for livestock products such as milk and meat will increase over the next 3040 years. As a result, there will be significant investment in dairy farming in urban and peri-urban areas and this will obviously have animal, public and environmental health implications. The WHO has published a global tuberculosis report every year since 1997. It is estimated that 10.6 million (95% CI: 9.911 million) people will develop tuberculosis worldwide in 2021. Among HIV-negative people, 1.6 million people died from tuberculosis.

The End-TB Strategy aims to reduce tuberculosis deaths by 90% and the tuberculosis incidence rate by 80% between 2015 and 2030 (WHO, 2017). Consistent with this global goal, it is important to know the true burden of zoonotic tuberculosis (TB), particularly in low- and middle-income countries, as these countries may have minimal or no TB control programs in place. The impact of zoonotic tuberculosis goes beyond human health. Bovine tuberculosis threatens the well-being of communities that depend on livestock for their livelihoods. The disease has a significant economic impact through reduced meat and milk production and the condemnation of carcasses or affected parts unfit for human consumption. Bovine tuberculosis also creates barriers to international trade in animals and animal products. When it becomes endemic in wildlife populations, it jeopardizes conservation efforts and can serve as a reservoir of infection for livestock and humans (OIE, 2013; Müller *et al*., 2013).

According to WHO's 2017 report, there were 147,000 new cases of zoonotic tuberculosis in humans and 12,500 deaths from the disease. However, estimates of the global burden of zoonotic tuberculosis are imprecise. This is due to the lack of routine surveillance data from human and animal populations in most countries (WHO, 2017). Studies have shown that bTB is still common in those developing countries that do not practice routine milk pasteurization and an estimated 1015% of human tuberculosis cases are due to *M. bovis* (Malama *et al.*, 2013).

Dairy intensification has the benefit of increasing milk production through larger herds, indoor housing and feeding, breeding technologies, and other inputs (Clay *et al*., 2020). However, this mode of production is becoming increasingly harmful to the environment and has been linked to rising diseases such as BTB, human health, environmental health and animal welfare, among others (Allen *et al*., 2021).

Among African countries in Ethiopia, tuberculosis is a common disease in the cattle population. Some study results performed with intradermal tuberculin skin tests show that the incidence of bTB varies between 0.878% in extensive versus intensive systems (Ameni *et al*., 2007). Intensive and semi-intensive milk production is an important emerging livestock farming system in several urban centers, especially regional cities, aiming to meet the demand for milk and dairy products in Ethiopia. The demand for dairy cattle in these areas is fulfilled by trade in areas where dairy farming is relatively well developed, both locally and in remote areas, but without prior knowledge as to the bTB status of the animals. Thus, the risk of spread of bTB and other production-related cattle diseases has become a constraining factor for the expansion of the industry, and the prevalence of uncontrolled *M. bovis* infection in cattle in this system is high (Shapiro *et al*., 2015). However, only a few studies have been attempted to assess the knowledge and practices of risk groups related to zTB and identify the main routes of transmission in an Ethiopian setting.

In Ethiopia, the prevalence of bTB in the livestock population is not well known and most studies have mainly focused on the central part of the country, mainly in and around Addis Ababa. The current status of the disease should be investigated in all parts of the country where such a study has not yet been conducted in order to be included in the national control program for bTB in the future. Wolkite is the administrative city of the Gurage Zone, known for its dairy cattle production. So far, the prevalence and associated risk factors of bTB in dairy cows in the city of Wolkite in southern Ethiopia are still unknown. Therefore, this study was conducted with the following objectives:

- To determine the prevalence of bTB at both herd and animal levels on dairy farms in the study area.
- To identify related risk factors for the prevalence of bovine tuberculosis and
- To assess the knowledge, attitude, and practices (KAP) of farm owners or workers on bTB and its zoonotic importance.

2. LITERATURE REVIEW

2.1. An overview of Bovine tuberculosis

Bovine tuberculosis (bTB) is a chronic bacterial disease of animals and humans caused by *Mycobacterium bovis*. In many countries it is a serious infectious disease affecting cattle, other domestic animals and certain wild animal populations (OIE, 2018). It is usually characterized by the formation of nodular granulomas called tubercles. Although generally defined as a chronic debilitating disease that is difficult to diagnose clinically, emaciation, anorexia, chronic cough and other signs of pneumonia can be symptoms that develop in cattle at relatively late stages of infection (Ayele *et al*., 2004).

The history of bTB is ancient, like human tuberculosis. In 1898, *M. bovis* was identified, 16 years after Kotch identified *M. tuberculosis*. Theobald Smith in 1898 isolated tubercle bacillus from tuberculous cattle and was the first to distinguish this organism from human tuberculosis (Karlson and Lessel, 1970). He did this by studying its culture and pathogencity properties where the bovine tubercle bacillus was slow-growing (dysgonic) and had great pathogenicity for rabbits. Later on, other cultural characteristics that helped identification were discovered, such as the production of niacin. The Judicial Commission of the International Committee on Nomenclature of Bacteria has accepted the name of the species *M. bovis* in the seventh edition of Bergey's Manual of Determinative Bacteriology (Karlson and Lessel, 1970). Throughout this document, "zoonotic TB" refers to disease caused by *M. bovis* infection in people, and "bovine TB" refers to disease caused by *M. bovis* infection in animals (OIE, 2017).

According to the 2017 WHO report, there were an estimated 147,000 new cases of zoonotic human tuberculosis and 12,500 deaths from zTB worldwide (WHO, 2017). The strategy to end tuberculosis cannot be achieved by only targeting tuberculosis mainly caused by *M. tuberculosis*, and human tuberculosis caused by M. bovis must also be addressed. Although insufficient attention has been paid to zTB, there have been some studies in high-income, middle- and low-income countries (Kock *et al*., 2021). Globally, ZTB burden varies widely across continents and countries due to a number of factors including predominant host species, sample size, geographic location and diagnostic tests (Ramos *et al*., 2020). According to a recent review of bTB by Ramos *et al*. (2020) they were 10.3%, 13.8%, 17.8%, 33.6% and 20.5% for Africa, Asia, Europe, North America and South America. Based on this research, Africa has a lower tuberculosis prevalence compared to other continents. This low figure could be due to a lack of resources in Africa for planned tuberculosis surveillance, and this information may only be gleaned from cross-sectional studies.

Although global population growth is slowing, Africa and Asia will still experience rapid population growth and urbanization, resulting in increasing demand for food (UNDESPD, 2022). According to the FAO Analysis of Livestock Sustainability 2050 for Africa, the demand for livestock products such as milk and meat will increase over the next 3040 years (FAO, 2019). As a result, there will be significant investment in dairy farming in urban and peri-urban areas and this will obviously have animal, public and environmental health implications. Dairy intensification has the benefit of increasing milk production through larger herds, indoor housing and feeding, breeding technologies, and other inputs (Clay et al., 2020). However, this mode of production is becoming increasingly environmentally unfriendly and has been linked to increasing diseases such as tuberculosis, human health, environmental health and animal welfare, among others (Allen et al., 2018; Clay et al., 2020). In Ethiopia, intensive and extensive milk production is an important emerging livestock system around several urban centers, and the prevalence of uncontrolled M. bovis infection in cattle in this system is high (Shapiro et al., 2015).

2.2. Bovine tuberculosis (bTB)

2.2.1. Etiology

2.2.1.1. Mycobacterium Tuberculosis-Complex (MTBC)

Mycobacterium (M) is an acid-fast, aerobic, non-motile, encapsulated, and non-spore-forming bacterium. The genus name Mycobacterium was originally proposed by Lehmann and Neumann in 1896 (ITIS, 2021). Currently, Mycobacterium consists of 170 different species (Forbes, 2017). In 1882, Robert Koch discovered the main causative agent of tuberculosis, MTB, which was named Koch's bacilli (Taylor *et al*., 2003). Over time, it became known that tuberculosis is caused by a group of bacteria called MTC, which are characterized by 99.9 percent similarity at the nucleotide level and identical 16S rRNA, but differ in phenotypes and host tropism (Brosch *et al*. , 2002).

The most important members of the *MTBC* are *M. bovis* and *M. tuberculosis*. In certain regions, *M. caprae* is also relevant (Mostowy *et al*., 2005). The host adaptation of the MTBC is not strict. All members have been found to cause disease in humans, and many have been isolated from mammals that are not considered to be the primary host (Fig. 1). This led to the concept of "maintenance" hosts, to which each species of the MTBC is adapted, and "spillover" hosts, in which the disease can be found but is not necessarily maintained (Smith *et al*., 2009).

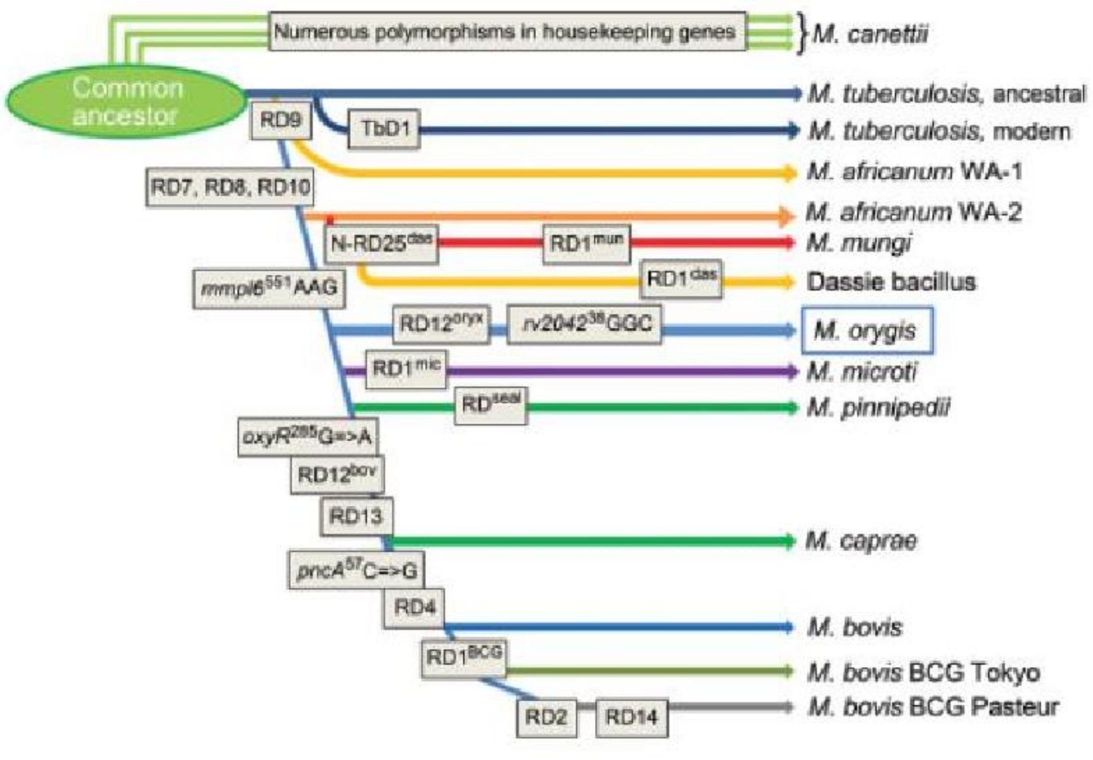

Figure 1: Phylogeny of the Mycobacterium tuberculosis complex (Van Ingen *et al*., 2012)

2.2.1.2. Mycobacterium bovis

Morphology and general characteristics

M. bovis is an acid-fast, immotile, nonspore-forming, facultatively intracellular, aerobic, encapsulated, and slow-growing bacterium of the MTBC group with a broad host range that causes tuberculosis in humans and animals (Carslake et al., 2011). Mycobacteria express unique mycolic acids in the cell envelope, which play a critical role in cell wall structure and function. The basic structure of the cell wall is typical for gram-positive bacteria (cytochemically), however, they do not take up the stains of the gram stain because the cell walls are rich in lipids, the majority of which are mycolic acid (Nataraj et al., 2015). This cell wall richness in lipids makes the surface hydrophobic and the mycobacteria resistant to many disinfectants and common laboratory stains. Once stained, the rods also cannot be decolorized with acid-alcohol solutions, hence the name acid-fast bacteria," a feature that can be exploited to identify mycobacteria via the Ziehl-Neelsen staining technique (Murray *et al*., 2020).

Cell wall lipids have strong biological activity and are believed to play a crucial role in pathogenesis (Nataraj *et al*., 2015). It is believed that mycosides, phospholipids and sulfolipids protect tubercle bacteria from phagocytosis. *M. bovis* is slow growing (generation time about 20 hours) and requires up to 8 weeks of incubation in egg-based Lownstein-Jensen (LJ) media. A pyruvate supplement (0.4%) promotes growth. *M. bovis* can be identified using culture and molecular testing (Nataraj et al., 2015). Work on optimizing the isolation of *M. bovis*, particularly from paucibacillary specimens such as milk and extrapulmonary specimens, is insufficient and needs further investigation.

Pathogenesis of Mycobacterium bovis

A comparative analysis of human, bovine, zebrafish, and non-human primate studies has contributed to a better understanding of granuloma formation in TB pathogenesis (Palmer, 2017). The development of overt disease after infection under field conditions may depend not only on the number of virulent organisms to which a susceptible host is exposed, but also on the frequency of exposure and the route of infection, as well as the general health and immunological status of the host animal (Domingo et al., 2014). Although tuberculous lesions are found in all parts of the body in cattle infected with M. bovis, they are most commonly found in the lungs and bronchomediastinal lymph nodes, most likely related to airborne infection. In contrast to human infection, the primary lung lesion in cattle rarely heals spontaneously but tends to spread locally via the natural cavities such as the bronchi or further via the lymphatic and hematogenous pathways. The result of infection is, with few exceptions, a chronic, debilitating disease of long duration (Sakamoto, 2012; Murray et al., 2020).

2.2.2. Clinical Signs

Bovine tuberculosis generally has a chronic, variable, and often subclinical course. Occasionally it can be acute and progress rapidly (Kanipe and Palmer, 2020). It usually takes many months or even years for clinical symptoms to develop. In most infected cattle, the disease is unknowable and detectable only by tuberculin testing. When clinical symptoms are manifested, their nature depends on the organ system or organ systems involved and the severity of the infection. It is usually characterized by the formation of nodular granulomas called tubercles. Any body tissue can be affected, but lesions are most commonly seen in the lymph nodes (especially in the head and chest), lungs, intestines, liver, spleen, pleura and peritoneum (OIE, 2018).

In countries where control is active and aggressive, the disease is mainly respiratory in nature and the extent of the lesions is limited. In countries where the disease is not actively controlled, advanced disease and generalization are common. Common symptoms in the late stages of the disease include progressive emaciation, slightly fluctuating fever, weakness and loss of appetite. Animals with lung involvement usually have a wet cough that gets worse in the morning, in cold weather, or with exercise, and may have dyspnea or tachypnea (Palmer, 2017). In the final stage, the animals can become extremely emaciated and suffer from acute shortness of breath. In some animals, the retropharyngeal or other lymph nodes enlarge, rupture, and drain. Severely enlarged lymph nodes can also block blood vessels, airways, or the digestive tract (Murray et al., 2020). When the digestive tract is affected, intermittent diarrhea and constipation may occur. In some animals, abscesses of unknown origin in isolated lymph nodes may be the only symptom, and symptoms may not develop for several years. In other cases, the disease can spread with a rapid, fulminant course (Druszczy et al., 2022).

2.2.3. Diagnosis of Bovine Tuberculosis

Clinical signs of bTB are not only specific to bTB, but are also present in other diseases or syndromes and therefore do not allow for a definitive diagnosis based on clinical symptoms alone. Various laboratory tests are currently used to confirm bTB, but none can confirm it with certainty (none with high sensitivity and specificity). Delayed Hypersensitivity Test: The tuberculin skin test (TST) is one of the standard diagnostic methods for detecting *M. bovis* infection in cattle accepted by the OIE for bTB international trade (export testing), surveillance and eradication purposes. the other is the IFN assay (OIE, 2018). This includes measuring skin thickness, injecting bovine tuberculin intradermally into the measured area, and measuring any subsequent swelling at the injection site 72 hours later. The TST can be performed at various body sites, but the OIE recommends the caudal fold test (CFT), the (mid)cervical intradermal test (CIT), or the single intradermal comparative cervical test (SICCT) (Schiller *et al.*, 2010). Compared to the TST, the IFN assay has better sensitivity but lower specificity (de la Rua-Domenech *et al*., 2006).

Classic mycobacterial culture remains the routine method for confirming infection (OIE, 2018). There have been various research results using amplification methods (RD-4 deletion typing by PCR) and real-time PCR to directly detect various samples from humans and animals. Several molecular technologies are currently used by clinical diagnostic laboratories to identify isolates from culture, including nucleic acid hybridization probes, line probe hybridization assays, matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF-MS), and DNA sequencing (Caulfield and Wengenack, 2016). The epidemiological molecular typing method most commonly used in M. bovis is spolygotyping (Singleton, 2010).

2.2.3.1. Tuberculin Skin Test

The tuberculin skin test, based on a delayed type of hypersensitivity to mycobacterial tuberculous protein, is the standard antemortem test both in humans and cattle. It is a convenient and cost-effective method for assessing cell-mediated responses to a variety of antigens, and it is the "gold standard" for diagnostic screening for the detection of new or asymptomatic MTC infection (Katial *et al*., 2001). In cattle, the reaction is usually detectable 30 - 50 days after infection. The tuberculin is produced from cultures of MTB or M. bovis grown on synthetic media. The tuberculin test is usually performed between the centers of the neck, but the test can also be performed in the caudal crease of the tail (Lee and Holzman, 2002; Radostits et al., 2007). The skin of the neck is more sensitive to tuberculin than the skin of the tail fold. To compensate for this difference, higher doses of tuberculin can be used in the caudal fold of the tail. Bovine tuberculin is more effective and specific; The potency of tuberculin must be estimated using biological methods based on a comparison with standard tuberculin. Efficacy is expressed in International Units (IU).

In several countries, bTB is considered to be of acceptable efficacy if the estimated efficacy per bovine dose ensures at least 2000 IU in cattle. A higher dose of bovine tuberculin is required in cattle with reduced allergic sensitivity, with the volume of each injection dose not exceeding 0.2 ml. Cell-mediated hypersensitivity acquired through infection can be systematically demonstrated by fever, ophthalmically by conjunctivitis, or dermally by local swelling when the tuberculin test or its purified protein derivative (PPD) is administered subcutaneously, conjunctivally, or intradermally (Radostits *et al*., 2007).

SINGLE INTRADERMAL TEST (SIDT)

Administration is by intradermal injection of 0.1 ml of bTB PPD into a skin fold at the base of the tail or into the crease of the neck and subsequent detection of swelling secondary to delayed hypersensitivity. The response is read between 48 and 96 hours after injection, with 48 - 72 hours being preferred for maximum sensitivity and 96 hours for maximum specificity. The positive reaction represents a diffuse swelling at the injection site. The main disadvantage of the SID test is its lack of specificity and the number of reactor lesions that occur. Mammalian tuberculin is not specific enough to distinguish between reactions due to *M. bovis* infection and *M. avium*, *M. tuberculosis* and *M. paratuberculosis* infection, including vaccination or Nocardia farcinicus (Radostits, 2007). Other disadvantages of the SID test include the failure to detect cases of minimal sensitivity in old cows and cows that have just calved, as well as early infection in some cattle in an unresponsive state, called anergy, which is due to of the antigen, excess or immunosuppression occurs, which in turn is caused by non-specific factors such as malnutrition and stress (Hirsh and Zee, 2000; Andrews, 2003; Quinn *et al*., 2003).

Single Intradermal Cervical Comparative Tuberculin Test (SICCTT)

Two mid-neck sites 10-12 cm apart are shaved and thickness is measured in millimeters with calipers prior to tuberculin injection. In SICCTT, 0.1 ml of PPD from Mycobacterium avium (PPD-A) and 0.1 ml of PPD from M. bovis (PPD-B) are injected intradermally into separate, clipped sites on the side of the neck. Care must be taken when placing the injection as it varies from place to place in the skin.
After 72 hours, the skin thickness at the sites is measured again (Quinn et al., 2003; Ameni et al., 2010). If the change in skin thickness at the PPD-A injection site is greater, the result is considered negative for bTB. As the change in skin thickness increased at both sites, the difference between the two changes was taken into account. Thus, if the increase in skin thickness at the injection site in cattle (B) is greater than the increase in skin thickness at the injection site in birds (A) and (B-A) is less than 1 mm, they are between 1 and 4 mm or 4 mm and more, the result is classified as negative, equivocal, or positive for bTB, respectively, and the animal showing signs of infection is designated a reactor. The comparative test is used to discriminate between M. bovis infected animals and animals reactive to bovine tuberculin due to exposure to other mycobacteria. This sensitization can be attributed to antigenic cross-reactivity between mycobacterial species and related genera (OIE, 2018).

2.2.4. Transmission of bTB

M. bovis can be transmitted by inhaling aerosols, by ingestion, or through skin breaks. The importance of these routes varies depending on the host species (Dibaba *et al*., 2019). Cattle shed *M. bovis* in respiratory secretions, faeces, and milk, and sometimes in urine, vaginal fluid, or semen. The risk of infection from infected cattle depends on their excretion of the pathogen. The bacteria are regularly shed shortly after the initial infection; At a later stage, shedding is sporadic and large numbers of organisms may be shedding in the late stages of infection. Asymptomatic and anergic carriers exist (Palmer *et al*., 2019; Druszczy *et al*., 2022). In most cases, *M. bovis* is transmitted in aerosols during close contact between cattle. Some animals become infected when they ingest the organism; this pathway can be particularly important for calves nursing from infected cows. Skin, genital, and congenital infections have been observed but are rare. Not all infected cattle may transmit the disease (Drewe *et al*., 2014). In addition to the respiratory tract, there are several other transmission routes for *M. bovis* (Table 1).

Table 1: Summary of the main routes of transmission of *Mycobacterium bovis*

Route	Description	Infective dose	Evidence
Inhalation (most common)	Aerosols generated by coughing, sneezing	Very low: single bacillus	Experimentally demonstrated in a variety of species
	Direct inhalation of aerosols		Involvement of lymph nodes associated with the respiratory tract
Ingestion (common)	Feed, water contaminated with mucous, nasal secretions, feces, urine	High: several million bacilli	Lesions in mesentric lymph nodes of lymph nodes of naturally infected animals
	Milk from infected dam		Experimentally demonstrated Epidemiological evidence
Transcutaneous (uncommon)	Contamination of existing skin abrasions	Unknown	Humans handling infected carcasses, e.g., butcher's wart
	Bite wounds		Bite wounding in ferrets, badgers
Pseudovertical (rare)	Consumption of milk from infected mothers	Unknown	Epidemiological evidence in cattle, badgers, brushtail possums, and white-tailed deer
	Close contact between mother and offspring		
Vertical (very rare)	Intrauterine from infected dam to offspring	Unknown	Lesions in liver and portal system in calves from infected dams

Source: (Drewe *et al*., 2014)

2.3. Epidemiology of Bovine Tuberculosis.

Bovine tuberculosis is usually found in cattle herds, but some other species can also become reservoir hosts (Devi *et al*., 2021). Known conservation hosts include possums (and possibly ferrets) in New Zealand, badgers in the United Kingdom and Ireland, bison and elk in Canada, and kudu and African buffalo in southern Africa. Most species are considered spillover hosts. Populations of spillover hosts do not sustain *M. bovis* indefinitely without maintenance hosts, but can transmit the infection between their members (or to other species) for a time (Ciambrone *et al*., 2020). Some spillover hosts can become conservation hosts when their population densities are high. Species reported as spillover hosts include sheep, goats, horses, pigs, dogs, cats, ferrets, camels, llamas and many species of wild ruminants including deer and elk; elephant, rhino, fox, coyote, mink, primate, possum, otter, seal, sea lion, rabbit, raccoon, bear, warthog; Big cats (including lions, tigers, leopards, cheetahs and lynxes) and various species of rodents. Most mammals are potentially susceptible (Malama *et al*., 2014).

M. Tuberculosis was shown to be avirulent in cattle as early as the 19th century. Localized lesions may develop, but infection does not result in progressive disease. More recently, Whelan *et al*. (2010) confirmed this with the strain M. tuberculosis H37Rv. They suggested that the animal's immune status or the genotype of the infecting bacillus could have a significant impact on a strain's virulence for cattle. There is currently no evidence of animal-to-animal transmission of *M. tuberculosis* or *M. africanum* in cattle (Gagneux, 2012), but they can cause reactions in cattle tested for tuberculin. Goats are very susceptible to *M. bovis* and *M. caprae*. Goat TB has long been underestimated, although it causes economic losses in endemic areas and goats in contact with cattle could act as a bTB reservoir. Interestingly, goat TB is not an OIE detectable disease (Vordermeier *et al*., 2014).

2.3.1. Distribution

Although bovine tuberculosis was once prevalent worldwide, control programs have significantly reduced or nearly eradicated the disease in livestock in many industrialized countries. However, bTB is still widespread in Africa, parts of Asia and some Middle Eastern countries (EFSA, 2012). From January 2017 to June 2018, out of the 188 countries and territories that reported their bTB situation to the OIE, 82 countries (44%) were affected, indicating a wide spread of the disease (Fig. 2). Of the 82 affected countries, 29 (35.4%) reported the occurrence of bTB in both livestock and wild animals. Two (2.4%) countries reported that bTB was only found in wild animals, compared to 51 (62.2%) that reported only farmed animals were affected (WHO, FAO, OIE, 2017). In addition, out of these 82 affected countries, 66 (80.5%) provided quantitative data on outbreaks via WAHIS, showing relatively good coverage of the global situation of this disease. The persistence of infection in wildlife poses a disease control challenge in some countries (Palmer, 2013), as wildlife as reservoirs and overflow hosts can have significant impacts.

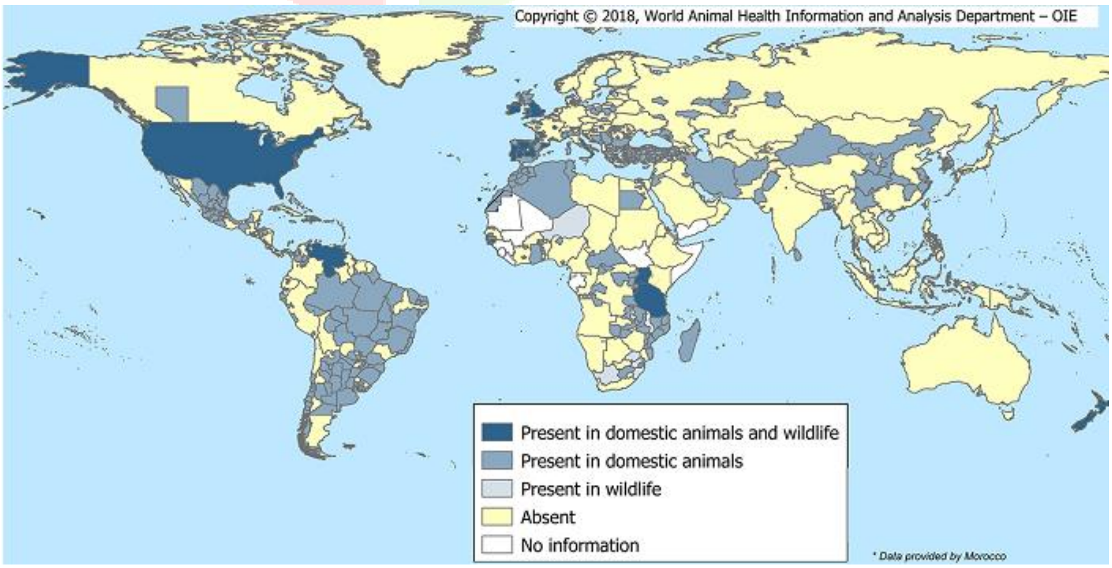

Figure 2: Global distribution of bovine tuberculosis in 2017 and the first semester of 2018 (OIE, 2018)

2.3.2. Risk Factors of Bovine Tuberculosis

Humblet and colleagues (2009) classified bTB risk factors in cattle as animal-level risk factors (age, sex, breed, body condition, immune status, genetic resistance and susceptibility to bTB, vertical and pseudo-vertical transmissions, autocontamination). and risk factors at herd level (history of bTB outbreak in the herd and human history of household tuberculosis, herd size, type of cattle industry or farm, management, intensity of farming system and cattle husbandry, manure, feeding, supplementary feeding and forage storage, transmission from bovine to bovine via the faeco-oral route). However, the contribution of each risk factor to bTB infection may vary from farm to farm, region to region, or country to country due to differences in management, national flock types, etc. Therefore, their relevance varies (Skuce *et al*., 2012). Some are identified consistently across settings, such as age, herd size and bTB history.

2.3.2.1. Age

Several studies have identified age as an important risk factor for bTB here in Ethiopia or elsewhere where bTB is endemic (Firdessa *et al*., 2012; McKinley *et al*., 2018; Dejene *et al*., 2016). McKinley *et al*. (2018) analyzed bTB detection in UK slaughterhouses from 1998 to 2013 and found a strong influence of age: animals slaughtered at > 60 months of age had a 5.3-fold likelihood of detection compared to animals slaughtered between 0 and 18 months of age Age. In Ethiopia, Mamo *et al*. (2013) examined 1087 cattle using SICCT in Afar, and the age-specific proportion of reactors was 4.9% for less than 2 years, 5.9% for 2 to 5 years, 13.1% for more than 5 to 9 years and 15.9% patients older than 9 years old with bTB infection gain weight with age. However, there is still controversy as to whether the association between age and tuberculosis infection is linear or resembles an epidemic curve (i.e. less early age and old age, i.e. allergies, and high middle age).

The relationship between age and bTB infection could be linear as suggested by Broughan et al. (2016) or epidemic curvilinear as seen in age-specific reactor rate modelling. It is a complex subject and probable explanations have been proposed for each scenario. longevity and a higher likelihood of contact or exposure over time; A higher likelihood of responsiveness or non-responsiveness (anergy) to skin tests at a given age, which in turn may be related to age-related resistance, is commonly mentioned when explaining the association between age and BTB. One of the problems with studying age as a risk factor is how accurate and reliable the age estimate was. Here in Ethiopia there is no national cattle registration and tracing system - a system where each cattle has an ID, their date of birth and other parameters are recorded and their whereabouts can be traced and cattle movements can also be regulated. In reality, however, age estimation sometimes depends on how well the owner can remember, as few farms tend to keep records. It is therefore important that data for risk factor analysis are obtained from commercial dairy farms where records are normally kept, or at least an attempt is made to best estimate the age of each animal using a combination of parameters such as teeth.

2.3.2.2. Breed

According to the Central Statistics Agency of Ethiopia (2021), there are 70.3 million cattle in Ethiopia, ranking the country first in Africa and fifth in the world, after Brazil, India, China and the United States in that order (FAOSTAT, 2021). Proportionally, the local Zebu breeds make up the lion's share of Ethiopia's national livestock (97.71%), followed by crossbreeds (1.91%), and exotics (pure) make up just 0.32%. Vordermeier and colleagues (2012) conducted a well-designed field-level experimental study in which a total of 5,424 cattle of different breeds (925 Holsteins, 1921 crossbreeds and 2578 zebus) were selected from the same housing system of Selalle (Northwest Shoa). were compared for their susceptibility to M. bovis infection. These cattle were assessed using tuberculin skin tests (which compare response magnitudes), postmortem (severity and lesion distribution assessed using a quantitative pathology scoring system) and in vitro whole blood culture immune responses measured using interferon gamma (IFN-).

In all of these parameters, Zebus were found to be less susceptible to M. bovis compared to Holsteins and their crossbreeds.

In an observational study by Firdessa et al. (2012) 1837 crossbred cattle were tested with the SICCT test and 62.8% were reactor-eligible, whereas local zebu estimated that only small numbers were included in the study, resulting in a low prevalence (1.5%).

2.3.2.3. Herd size

Numerous studies in developed and underdeveloped countries identified herd size as one of the most important herd-level bTB risk factors (Ameni *et al*., 2003; Pascual-Linaza *et al*., 2017; Islam *et al*., 2021). As the number of animals on a farm increases, the likelihood of contact between cattle increases, leading to the spread of tuberculosis infection. According to a recent study in Bangladesh (Islam *et al*., 2021), herds with more than four cows were 3.9 times more likely to have bTB than herds with four cows. Ameni *et al*. (2003) in central

Ethiopia and Kemal *et al*. (2019) found a significant association between herd size and bTB prevalence in Dire Dawa. Mamo *et al*. (2013) and Dejene *et al*. (2016) in Afar, a region known for its pastoral production system, identified herd size as a major risk factor for bTB prevalence.

In a similar study by Gumi et al. (2012), herd size was not a major risk factor among Somali and Guji pastoralists in south-eastern Ethiopia. Gummi et al. (2012) discussed their finding that in a grazing system where animals live continuously outdoors and under extended conditions, bTB is rare regardless of herd size. In agreement with Gumi et al. (2012), but in the rural livestock systems of the highlands of Ethiopia, Tschopp et al. (2009) found no significant association between herd size and bTB prevalence in their cross-sectional study conducted in three regional zones of three regional states (Oromia, Amhara, and Southern Nations, Nationalities, and People Region (SNNPR)). Whether herd size is a confounder of dairy farming system type or a risk variable in its own right requires a properly designed study, particularly in Africa where we see different types of farming systems.

2.3.2.4. Dairy farming system

Bovine tuberculosis is strongly associated with different types of farming systems. bTB thrives on intensification. The farming system determines contact between cattle and cattle, between cattle and wild animals, and between cattle and a contaminated environment. In the pastoral livestock system, there is frequent movement of cattle and other animals, covering vast areas in search of water and pasture, with close contact for only brief periods during gathering at watering holes. Ghebremariam *et al*. (2018) in Eritrea examined the association of water points with bTB prevalence and found no significant association. While the urban and peri-urban areas are characterized by the keeping of a large number of improved breeds (commercial farming), particularly in Addis Ababa, this leads to overcrowding due to the scarcity of land and the constant stabling of cows. And because bTB is an intensifying, high-density disease, more surveys were conducted in urban and suburban dairy production systems than in mixed agricultural and pastoral production systems.

Ameni et al. (2006) compared the effects of zero grazing to free grazing in 54 Holstein and 37 Zebu cattle and reported that the severity of bTB was significantly higher in cattle housed indoors with a higher population (with significantly higher interferon-gamma values and more severe lesions) density than grazing cattle. In addition to close contact, stress due to overcrowding or nutritional differences between stabled and grazed animals have also been cited as causes for the spread of the disease. A similar observation was made in Bangladesh, where bTB risk was 3.3 times higher in non-grazing cows than in grazing cows (Islam et al., 2021).

2.3.2.5. BTB history

It has been consistently found that an area or flock with a history of bTB is at significantly higher risk of future bTB outbreaks than other areas or flocks (White and Benhin, 2004). A detailed review of 4255 articles by Broughan *et al*. (2016) concluded about these studies that despite differences in study design and location, some risk factors are consistently identified, e.g. herd size, bTB history, presence of infected wild animals, while the evidence for others is less consistent and coherent, e.g. nutrition, local livestock movements. Persistent infection may be due to the performance of the test (false negative results leading to an incorrect decision by not taking such animals), the stage of the disease at the time of the test, or an allergy.

In conclusion, previous studies on risk factor analysis of bTB in Ethiopia have identified significant risk factors that play a role in bTB transmission and are helpful for any future intervention strategy. However, most of these studies, with the exception of a few (Tschopp *et al*., 2009; Dejene *et al*., 2016), have problems with study design and the use of appropriate statistical tests for data analysis; these two are important in any epidemiological risk factor analysis study. How sample size was determined and how herds or individual animals were selected was not properly accounted for. For a correct representation it is important how the cattle herds were selected in relation to the size of the cattle population. Also the way in which clustering effects (pen, herd, area, etc.) were treated in the data analysis is considered another area of limitation. Therefore, proper identification of potential risk factors requires proper study design and rigorous statistical analysis.

2.4. Status of Bovine Tuberculosis in Ethiopia

BTB is a common disease in Ethiopia's cattle population. Some survey results using intradermal tuberculin skin testing in Ethiopia show that the incidence of BTB varies between 0.8-78% in rural free-range systems where zebu cattle are predominantly reared and intensive systems where exotic and crossbred cattle are reared (Cosivi, 1998; Ameni *et al*., 2007; Ameni *et al*., 2008). The prevalence of bTB at the human-livestock-wildlife interface in Hamer Woreda, southwestern Ethiopia, revealed individual bTB prevalence in cattle of 0.8% with the 4 mm cut-off and 3.4% with the 2 mm cut-off. Limit. Berg *et al*. (2011) provided a comprehensive survey of bovine tuberculosis in Ethiopia and showed widespread prevalence of the disease with an average prevalence of about 5%. The spoligotype pattern of 17 strains of *M. bovis* isolated from a herd with a high prevalence of bovine tuberculosis was identical for all animals and was recently published (Firdessa et al., 2013). BTB studies in Ethiopia to date have not addressed all of the differences in geographic areas, breed and host species, husbandry practices, and other area-specific risk factors that could be potential future areas of research. Bovine tuberculosis is one of the neglected diseases in southern Ethiopia, especially in the city of Wolkite. No research has been conducted about the disease's dynamics. Studies reported in the region are summarized in Table 2.

Table 2: Bovine tuberculosis studies reported in the southern Ethiopia

Study area	Sample size	CIDT skin testing (%)	PM lesion score in cattle (%)	Culture isolation Positive (%)	m-PCR positive (%)	Source
Wolaita Sodo	780	**14.2**	ND	ND	ND	(Ameni and Regasa 2001)
Hossana,	751	ND	**4.5**	**11.8**	ND	(Teklu *et al*., 2004)
Hamer (South Omo)	499	**0.8**	ND	ND	ND	(Tschopp *et al*., 2010)
Hawassa,	413	**11.6**	**1.1**	ND	ND	(Regassa *et al*., 2010)
Meskan, Gurage zone	1214	**6.8**	ND	**25.9**	**27.3**	(Tschopp *et al*., 2011)
Butajira	446	ND	**9**	**13**	**8**	(Biratu *et al*., 2014)
Dilla	440	4.3	ND	ND	ND	(Gebremedhin *et al*., 2014)
Gamo	221	8.2	ND	ND	ND	(Tora *et al*., 2022)

2.5. Economic Importance of Bovine Tuberculosis

In addition to being an animal health and public health concern, bTB has significant direct and indirect economic impacts worldwide related to direct animal health and productivity impacts, surveillance, control and eradication programs, market losses and animal movement restrictions . Condemning organs and carcasses for TB-like visible lesions has cost implications at the slaughterhouse level (Ejeh et al., 2014; Abebe et al., 2021). However, studies have generally focused on TB-visible lesions during meat inspection and rarely performed laboratory diagnostics to confirm M. bovis (Jemalo et al., 2018; Adelakun et al., 2019).

Productivity analyzes in cattle have rarely been performed. Such analyzes require large numbers of animals, longitudinal studies or very well-kept detailed records of productivity parameters over years. Studies on the scale of the Meisinger study have never been repeated in recent times. In Africa, only very few longitudinal studies are conducted to estimate the fertility and mortality of local zebu cattle under traditional husbandry management (Tschopp et al., 2014). bTB is endemic in Ethiopia (Tschopp and Aseffa, 2016; Sibhat et al., 2017). The costs of bTB are mainly related to losses in animal production, including increased mortality and reduced milk and meat production. Estimates of such losses have been made for countries with large livestock populations, such as Ethiopia (Azami & Zinsstag, 2018). In most cases, cost assessments mainly focus on animal production losses. Comprehensive studies are needed to estimate the global burden of this disease, including its overall cost to society (Caminiti et al., 2016).

2.6. Prevention and Control

Mycobacterium species are resistant to pyrazinamide, which is widely used in the treatment of infections caused by MTBC in humans. Cattle should not be treated at all and therefore livestock infected with tuberculosis must be slaughtered (culled). This is because the risk of shedding the organisms, hazards to humans and the possibility of drug resistance make treatment controversial (Nwanta *et al*., 2010). Pasteurizing milk from infected animals to a temperature sufficient to kill the bacteria has prevented the disease from spreading to humans. Treatment of infected animals is rarely attempted because of the high cost, length of time, and greater goal of eliminating the disease.

Vaccination is practiced in human medicine but is not commonly used as a preventive measure in animals: the effectiveness of existing animal vaccines varies and interferes with tests to eradicate the disease. A number of new vaccine candidates are currently being tested (OIE, 2018). In high-income countries, the public health risk and economic losses associated with M. bovis have been significantly reduced or eliminated through the adoption of rigorous testing, slaughter, and meat inspection protocols for cattle, milk pasteurization, financial compensation for farmers, and public education (Schiller *et al* ., 2011; Sheferaw and Abdu, 2017). However, in most low- and middle-income countries where bTB is endemic, such as Ethiopia, such action is hampered by financial constraints, particularly in compensating farmers, and inadequate veterinary services (Zinsstag *et al*., 2007). Currently, there are several ongoing zoonotic tuberculosis control efforts to end the global tuberculosis epidemic worldwide by 2030 (WHO/FAO/OIE, 2017). However, there are no policies and implementation measures in Ethiopia that are consistent with this global effort to fight bTB. Conventional disease prevention and control interventions can have significant environmental, social and economic impacts (Aenishaenslin *et al*., 2013). For example, testing and slaughter policies are effective in controlling bTB (OIE, 2019).

However, it has several effects such as: the culling of large numbers of animals that tested positive, the raising of animal welfare concerns, and the cost of testing and compensating livestock farmers, making implementation economically difficult, particularly in resource-constrained countries. bTB was reported in 55% of herds and 32.3% of cattle on urban and suburban dairy farms in central Ethiopia (Firdessa *et al*., 2012). The national bTB prevalence estimate was 5.8% in individual cattle, with a prevalence of 21.6% in exotic breeds and their crossbreeds and 16.6% in herds kept in intensive and semi-intensive production systems in urban and peri-urban areas % (Sibhat *et al*., 2017). Therefore, it is a particular problem for intensive dairy systems that raise improved breed dairy cattle. For example, in 2005–2011, the maximum production loss due to bTB in urban animal production systems in Ethiopia was estimated at US$4.9 million (Tschopp *et al*., 2012). There are currently no national policies and strategies to control bTB, although the disease ranks among the top three most common diseases in urban and peri-urban dairy producing areas of the country in terms of prevalence and household impact (LMP, 2015). Because of the public health importance of bTB and concerns about the spread of the disease through the dairy trade from the high-prevalence urban system to low-prevalence sedentary rural areas, researchers have recommended the introduction of control measures on intensive and semi-intensive dairy farms.

2.7. One Health Concept

A comprehensive description of One Health is provided by Destoumieux-Garzón *et al*. (2018) as "The One Health Concept: A Holistic, Transdisciplinary, and Multisectoral Approach to Health. Over the last decades, zoonotic infectious agents such as SARS-CoV-2 (COVID-19) have become a health, social, and economic challenge on a national and global scale, which calls for closer cooperation between animal and public health experts and institutions (Latif and Mukaratirwa, 2020).

In Africa, the interaction of animals and humans is common and hence needs a collaborative approach in early detection, diagnosis, and data sharing to tackle zoonotic diseases. As new findings emerge, a new definition for zoonotic tuberculosis (human infection with *M. bovis* of animal origin) has been suggested, as other members of the MTBC, such as *Mycobacterium orygis,* which causes tuberculosis in antelopes and possibly in other animals, have been reported as the cause of tuberculosis in humans, especially in patients from India (Duffy *et al*., 2020). This study further emphasized that the burden of zoonotic tuberculosis might be underestimated by surveillance studies restricted to *M. bovis.*

Given that over 60% of emerging infectious disease events are caused by the transmission of an infectious agent from animals (zoonoses), with 75% of these originating from wildlife, employing a systematic One Health approach has great potential for reducing threats to global health from infectious diseases. The One A health approach should advance health care for the twenty-first century and beyond by accelerating biomedical research, enhancing public health efficacy, expeditiously expanding the scientific knowledge base and improving medical education and clinical care (Atlas *et al*. 2010).

3. MATERIAL AND METHODS

3.1. Study Area Description

This study was conducted in Wolkite town, administrative center of the Gurage Zone, south-central Ethiopia, which is found 158 km away from Addis Ababa in the southwest direction along the main road from Addis Ababa to Jimma (Fig. 1). The town is geographically located at 8° 16′ 50″ north latitude and 37° 46′ 40″ east longitude and situated at an elevation between 1910 and 1935 meters above sea level (CSA, 2017). There are about 3,792 dairy cattle in Wolkite town and its suburbs. These animals were mainly cross-breed (Zebu X Holstein-Freisian) and kept under semi-intensive to intensive management systems (WCAFD, 2016).

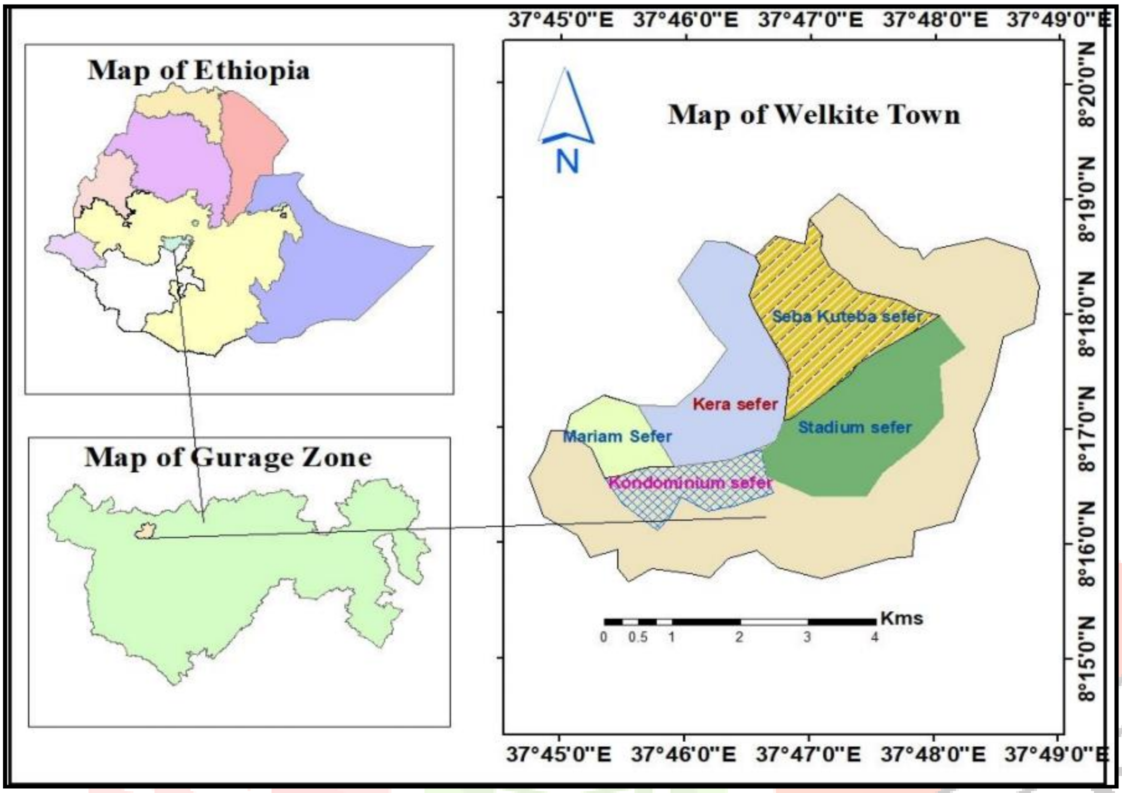

Figure 3: The map of the Wolkite town, January, 2022.

3.2. Study design and Period

A cross-sectional study was conducted throughout the course of the study in Wolkite town, south-central Ethiopia. The study was carried out between March 2022 and May 2023.

3.3. Study Populations

The study population was cattle managed on the selected intensive or semi-intensive dairy farm in the study area.

3.4. Study Variables

3.4.1. Dependent /Outcome Variable/

The occurrence of bovine tuberculosis among cattle and the awareness of dairy farm owners and workers on the routes of transmission of zoonotic tuberculosis were the outcome variables in this study.

3.4.2. Independent /Exposure Variables/

Potential risk factors for bTB at the animal level were age, sex, breed, physiology (pregnancy, stages of lactation, body condition), and source of animals. Similarly, risk factors for bTB at the herd level were herd structure, farm antecedents, farm management and husbandry, housing and ventilation, animal health (veterinary services), and animal bio-security. In addition, the age, sex, educational status, duration of employment, consumption of raw animal products, and sleeping on the farm as part of professional obligations for dairy farm owners and workers were used as independent variables.

3.4. General Inclusion and Exclusion Criteria

On the sampling frame, in the randomly selected herds, all eligible animals were sampled; some of these are justifiable in that animals lose sensitivity to tuberculin shortly before and after calving. Dairy farm owners and workers who were permanently living in the town for at least two months prior to the study, aged five years and above at the time of the study, as well as those who consented and were willing to participate in the study, are included. Whereas individuals who are not willing to participate, are aged below 18 years, or are seriously ill are excluded from participating in the study. As well, those less than 6 months of age, cows in the last month of pregnancy, and clinically sick cattle with disease not suggestive of bTB were excluded from the test. These exclusion criteria were set to avoid possible interference with the action of the tuberculin test (De la Rua-Domenech *et al*., 2006).

3.5. Sampling Method and Sample Size Determination

A multistage sampling procedure with a random selection of sampling units at each stage was employed. The study town was selected purposefully. Sub-city, kebelle, and study units were selected by simple random sampling techniques. Besides, herds with a number of animals greater than 10 were selected without any prerequisite in all selected study sites as they were few in number, while herds with fewer than 10 animals were recruited using the random selection method among the list of dairy herds obtained from local agricultural agents.

The sample size was determined according to Thrusfield and Christley, (2018) by considering a 6.8% expected prevalence based on a previous report from Meskan district, Gurage zone (Tschopp *et al*., 2011), 5% absolute precision, and a 95% confidence interval.

$$\text{Hence, } n = \frac{(z)^2\, p(1-p)}{d^2}$$

Where n = is the required sample size, p = is the expected prevalence, z = the multiplier of the 95% confidence interval, and d = the desired absolute precision. Based on the formula, the minimum sample size required to estimate the prevalence of bTB was 98 dairy cattle. However, considering the small sample size, the design effect (to account for clustering of cases in a herd), and the resources available to carry out the study, the sample size can be recalculated using the formula from Dohoo *et al*. (2003).

$$n' = n(1 + p(m - 1))$$

Where:
n' = sample size adjusted for clustering
n = original sample size assuming simple random sampling
ρ = intra-cluster or intra-class correlation coefficient
m = average number of animals per aggregate (e.g., herd), Assuming an intra-cluster (or intra-class) correlation coefficient (rho) of 0.2 and an average sample size within clusters of 6, the calculated design effect is 2.0. An adjusted sample size required from the selected households or farms was 196 cattle, which were subjected to a single intradermal cervical comparative tuberculin test.

3.6. Study Methodology

3.6.1. Single Intradermal Cervical Comparative Tuberculin Test (SICCTT)

The Single Intradermal Comparative Cervical Tuberculin (SICCT) test method was used to differentiate between animals infected with *M. bovis* and those sensitized to tuberculin due to exposure to other mycobacteria or related genera. For larger cattle, two sites at the middle of the neck were shaved and cleaned 12–15 cm apart on the same side of the neck parallel to the shoulder, while for calves, the opposite sides of the neck were used because of the limited space. The skin of the neck and nearby lymph nodes were checked for any visible lesions or swelling before measuring the skin fold thickness at the two sites with a digital caliper. The skin folds at the two sites are measured by a caliper and recorded. Animals were then injected with 0.1 ml (2500 IU/ml) of avian PPD and 0.1 ml (3000 IU/ml) of bovine PPD (Lelystad B.V., the Netherlands) intradermally using insulin syringes at the respective sites according to a published protocol (OIE, 2016). The injection sites were examined, and the skin thickness was measured 72 hours post-injection. The difference in the increase in skin thickness measurements at the bovine and avian sites before and after inoculation was considered for interpretation.

The results were interpreted in accordance with the recommendations of the OIE (OIE, 2018). Briefly, when the skin thickness is increased by 4 mm or more at the bovine PPD injection site regardless of the increase at the avian site, the animal is considered positive for bTB, and when the skin thickness is increased at sites, the difference between the increase at the bovine (B) and the increase at the avian (A) sites is considered. A reaction was considered positive when B-A was more than 4 mm. If the increase in thickness was from 1 to 4 mm or less than 1 mm, the reaction was interpreted as suspect, doubtful, or negative, respectively. A severe cut-off value of > 2 mm was also applied to re-estimate the prevalence and compare it with that of the standard cut-off (Ameni *et al*., 2008; Downs *et al*., 2013; Goodchild *et al*., 2015). A herd was considered positive if it had at least one tuberculin-reactive animal.

3.6.2. Questionnaire Survey

Dairy farm owners and workers were interviewed according to their willingness to participate and after verbal consent on the same day that their cattle were tested for bTB. Interviews were conducted on all sites in the local languages (using Amharic and Guragegna), depending on the preference of the respondent at the time the SICCTT was done. Questionnaires included closed and open questions on livestock husbandry and management and household characteristics, such as herd size and structure, presence of other livestock, vaccination and de-worming of cattle, mixing of cattle and other livestock, watering and grazing systems, reproduction, cattle contact with other cattle herds, and purchasing of animals. Furthermore, questions related to human consumption habits, contact between humans and cattle, knowledge of TB, and known TB status in the household were also asked. Annex I shows the questionnaire that was presented to cattle owners.

3.7. Data Collection

Data regarding all the possible potential risk factor for the occurrence of bTB were collected using a structured and pretested questionnaire designed for this purpose from the cattle owners' while testing the cattle and the study follow up (Annex I).

3.8. Data Analysis

The SICCTT test and other questionnaire data obtained from tuberculin skin testing were logged in the format prepared for the purpose (Annex I) and then entered into a Microsoft Excel spreadsheet. STATA version 17.0 was used for the analysis of the data. The overall prevalence was calculated as the total number of positive samples for bTB divided by the total number of samples tested multiplied by 100. Herd-level prevalence was computed by dividing the number of positive herds with at least one infected animal by the total number of herds sampled. Herd and animal prevalence were calculated both at standard (4 mm cut-off) and severe interpretations (2 mm cut-off). The strength of association between herd-level risk factors and bTB status (binary response: positive or negative) was analyzed by Generalized Linear Models (GLM, binomial family with logit link). Animal-level risk factor analysis was conducted using a Generalized Linear Mixed Model approach (GLMM) using maximum likelihood (adaptive Gauss-Hermite quadrature) with the logit link of the binomial family. In this study, farm code nested in the study site was considered a group-level random effect to account for clustering.

In both the herd and animal-level risk factor analyses, variable selection for the multivariable analysis was made based on the p-value in the univariate regression, i.e., variables with a p-value less than 0.20 were considered in the multivariable regression (Sperandei, 2014). Confidence intervals for prevalence were calculated using 'EpiTools epidemiological calculators' with Wilson methods (Sergeant, 2019). In all cases, a 95% confidence level and a significance level of 5% were used to determine statistical significance.

4. RESULTS

4.1. Over all Animal Level and Herd Level prevalence

The animal and herd prevalence was 18.37% (95% CI: 13.57–24.38) and 46.87% (95% CI: 30.87–63.55), respectively. This prevalence increased to 23.98% (95% CI: 18.54–30.42) and 53.13% (95% CI: 36.45–69.13), respectively, when a > 2 mm cut-off (severe interpretation) was used (Table 3). Gubre subcity had a higher prevalence of 21.5% (95% CI: 13.3–33) at animal level and 63.6% (95% CI: 35.4–84.8) at herd level than Bekur, with animal and herd prevalences of 16.8% (95% CI: 11.4–24.1) and 38.1% (95% CI: 20.7–59.1), respectively (Table 4).

Table 3: Herd and animal level prevalence of bTB at the standard and severe interpretations

	Animal Level			Herd Level		
Interpretation	Cattle tested	Positives	Prevalence (%) (95% CI)	Herd tested	Positives	Prevalence (%) (95% CI)
Severe > 2 mm cut-off	196	47	23.98(18.54-30.42)	32	17	53.13 (36.45-69.13)
Standard > 4 mm cut-off	196	36	18.37(13.57-24.38)	32	15	46.9 (30.87-63.55)

Table 4: Animal and herd level prevalence in study site at standard interpretation.

Level		Gubure sub city	Bekur sub city	Total
Animal level	Cattle tested	65	131	196
	Positives	14	22	36
	Prev %.(95%CI)	21.59 (13.29-32.97)	16.79 (11.36-24.12)	18.37(13.57-24.38)
Herd level	Herd tested	11	21	32
	Positives	7	8	15
	Prev %. (95%CI)	63.64(35.38-84.83)	38.1(20.75-59.12)	46.9(30.87-63.55)

4.2. Animal Level Potential Risk Factors of BTB

The animal-level prevalence of bTB was found to be very high among cattle older than 9 years (65.52%) when compared to those aged 3–8 and 1-3 years, which were 12.84% and 7.89%, respectively. Purchased dairy cattle were known to have a higher prevalence of bTB (31.82%) than farm-bred cattle (11.54%). The bTB positivity was significantly associated ($P<0.05$) with age and source of animals in both GLMM univariate and multivariable analyses with farm code as a random effect for risk factors. However, breed, sex, BCS, and lactation were not significantly associated ($P > 0.05$) with animal-level bTB positivity (Table 5).

Table 5: GLMM analysis for animal level risk factors of bTB positivity based on standard interpretations

Risk factors	Level	Proportion % (positives/total examined)	Univariate analysis Crude OR (95% CI)	P value	Multivariable final model Adjusted OR (95% CI)	P value
Breed	Local (Zebu)	11.1 (1/9)	ref			
	Cross	18.7 (35/187)	1.9 (0.19-19.21)	0.579	-	-
Sex	Male	5.3 (1/19)	ref			
	Female	19.8 (35/177)	4.1(0.49-35.39)	0.190	6.3 (0.56-73.30)	0.136
Age	1-3 year	7.89 (3/38)	ref			
	3≤8year	12.84 (14/109)	2.1 (0.51-8.47)	0.317	0.8 (0.15-4.59)	0.832
	> 9 year	38.77 (19/49)	8.2 (2.94-34.47)	0.004	6.6 (2.19-37.02)	0.031
BCS	Good	15.9 (19/119)	ref			
	Medium	19.1(13/68)	1.1(.44-2.75)	0.827	1.7 (0.61-4.95)	0.296
	Poor	44.4 (4/9)	3.1(0.57-16.92)	0.188	0.4 (0.06-2.90)	0.385
Animal source	On farm bred	11.54 (15/130)	ref			
	purchased	31.82 (21/66)	4.0 (1.72-9.38)	0.001	3.1 (1.24-8.12)	0.016
Lactation	Non lactating	8.2 (5/61)	ref			
	Lactating	22.9 (31/135)	4.4 (1.42- 13.67)	0.010	2.5 (0.75-8.75)	0.134

4.3. Herd Level Potential Risk Factors of bTB

The GLM point estimates of the herd univariable analysis showed that the prevalence of bTB among herds was statistically significant (P<0.05) for management condition, stocking density, biosecurity measure, age of farm establishment, and animal health care, but not significant (P > 0.05) for herd size, new cattle introduction, and ventilation (Table 6).

Table 6: Herd univariate analysis of risk factors using GLM models for bTB positivity

Risk factors	Level	% positive	Univariate analysis	
			Crude OR (95% CI)	P value
Herd size	Small	10.26	ref	
	Medium	17.05	1.7 (0.55-5.81)	0.327
	Large	24.64	2.8 (0.88-9.22)	0.078
Management condition	Good	9.51	ref	
	Medium	14.56	1.5 (0.49-5.09)	0.430
	Poor	26.53	3.4 (1.21-9.78)	0.020
Ventilation	Good	8.49	ref	
	Satisfactory	11.30	0.4 (0.13-1.22)	0.108
	Poor	27.11	1.5 (0.60-4.18)	0.352
Stocking density	Less	10.81	ref	
	Satisfactory	10.78	1.1 (0.41-3.35)	0.754
	High	33.31	4.0 (1.63-10.08)	0.003
Biosecurity measures	Present	10.89	ref	
	Absent	23.81	2.5 (1.14-5.83)	0.023
Age of farm established	<5 years	7.12	ref	
	5-10 years	16.08	2.4 (0.66-9.26)	0.175
	>10 years	27.40	4.9 (1.36- 17.67)	0.015
New cattle introduction	No	13.49	ref	
	Yes	23.01	2.0 (0.94 - 4.23)	0.068
Animal health care	Regularly	10.18	ref	
	When ill	23.02	2.7 (1.19- 6.50)	0.017

The final herd-level multivariable generalized linear model (binary family) revealed that bTB positivity was significantly associated (P<0.05) with management condition, stocking density, and new cattle introduction (marginally significant). However, herd size, biosecurity measures, age of farm establishment, and animal health care were not significantly associated (P > 0.05) with herd-level bTB prevalence (Table 7).

Table 7: Herd level GLM multivariable analysis of potential risk factors of bTB positivity

Risk factors	Level	% positive	Multivariable final model	
			Adjusted OR (95% CI)	P value
Herd size	Small	16.6	ref	
	Medium	17.1	1.4 (0.30 - 7.11)	0.624
	Large	24.6	1.2 (0.21- 6.81)	0.837
Management condition	Good	9.6	ref	
	Medium	14.5	2.3 (0.39-13.69)	0.353
	Poor	26.5	4.7 (1.11- 19.92)	0.034
Stocking density	Less	10.9	ref	
	Satisfactory	10.7	2.2 (0.57 - 8.58)	0.244
	High	33.3	6.3 (1.96 - 20.24)	0.002
Biosecurity measures	Present	10.8	ref	
	Absent	23.9	2.2 (0.35 - 13.89)	0.390
Age of farm established	<5 years	7.1	ref	
	5-10 years	16.1	2.3(0.35-15.05)	0.378
	>10 years	27.4	2.4 (0.41-14.31)	0.328
New cattle introduction	No	13.5	ref	-
	Yes	23	2.8 (1.01 - 8.18)	0.047
Animal health care	Good	10.1	ref	
	Poor	23.9	0.5 (0.1- 3.34)	0.537

4.4. Questionnaire Results

4.4.1. Socio Demographic Characteristics of the Participants

A total of 108 respondents participated in the study, with a response rate of 100%. All owners and/or attendants were enrolled in this study. From the total participants, males accounted for 64 (59.3%) and females for 44 (40.1%), the average age of respondents was 29.61. The highest proportion of educational level of the respondents was illiterate at 28 (25.9%). These sociodemographic characteristics of the respondents were explained in Table 8.

Table 8: Socio demographic characteristics of the respondents

Characteristic	Category	Frequency (N)	Percent (%)
Gender	Male	64	59.26
	Female	44	40.73
Age	29.61 mean in year		
Marital status	Married	52	62.96
	Single	56	37.04
Time in farm	≤ 1year	18	16.67
	1−3years	47	43.52
	≥3years	43	39.81
Education	Illiterate	28	25.93
	Basic writing and reading	24	22.22
	< Grade 8	36	33.33
	Secondary school	14	12.96
	Degree and above	6	5.56
Current occupation	Farm owner	28	25.93
	Animal attendant(workers)	68	62.96
	Other	12	11.11

4.4.2. Assessment of respondents awareness on zoonocity and means of transmission bovine TB

From the total participants, 74.08% had never heard about bovine TB, while the rest (24.92%) had information on bovine TB. Among the respondents who had information about bTB, less than 25% of participants stated that raw milk and meat were the sources of bovine tuberculosis. However, 67.86% of respondents think bTB affects animals only. 75 (69.4%) of the respondents drank raw milk, and 76 (70.4%) of them had a habit of eating raw meat. On the other hand, 45 (41.6%) of the study subjects confirmed that they shared a common house with their animals (Table 9).

Table 9: Overall knowledge, attitude and practices of the Participants towards bTB in the study area

Indicative variable	Frequency / Percentage	
	Yes	No
Knowledge		
Heard of the diseases	28 (25.92%)	80 (74.08%)
Know that cattle transmit bTB to humans or vice versa	9 (32.14%)	19 (67.86%)
Know that bTB is transmitted through raw milk	7 (25%)	21(75%)
Know that bTB is transmitted through raw meat	6 (21.43%)	22 (78.57%)
Know that bTB is transmitted through coughing	8 (28.57%)	20 (71.43%)
Know that share the same house with animals is a source of disease.	58 (53.71%)	50 (46.29%)
Had noticed respiratory problems in their cattle	43 (39.81%)	65 (60.19%)
Attitude		
Do you think the consumption of raw animal product exposes to TB?	32 (29.6%)	76 (71.4%)
Do you think that bTB affects only animals with poor BCS?	26 (24.1%)	82 (75.9%)
Practice		
Habit of drinking raw milk.	75 (69.44%)	33 (30.56%)
Habit of raw meat consumption	76 (70.37%)	32 (29.63%)
Sharing of a house with animal	45 (41.67%)	63 (58.43%)
Isolation of diseased animals	52 (48.15%)	56 (51.85%)

4.4.4. Factors Associated with KAP Level of the Respondent towards bovine TB.

The participant's education status was found to have a statistically significant association with knowledge level in both univariable and multivariable analyses. However, other respondent characteristics such as gender, age, time on the farm, marital status, and current occupation were not significantly associated with overall awareness about bTB (Table 9).

Table 10: Factor associated with respondents KAP towards bovine TB

Variables	Knowledge		OR(95% CI)	AOR (95% CI)	P- value
	Good	Poor			
Gender			.07 (0.03-0.20)	-	-
Male	18	46			
Female	7	37	.07 (0.03-0.20)		
Age (mean)	29.61 year		.9 (0.91-0.99)	-	-
Time in farm			.9 (0.56-1.61)	-	-
≥3years	6	1	1.08 (.35-3.15)		
1−3years	13	12	.88 (.30-2.61)		
</=1year	12	12			
Education			1.9 (1.31-2.76)	1.8(1.25-2.66)	0.002
Degree and above	5	1			

Secondary school	10	4	.5 (.04-5.74)		
< Grade 8	21	15	.28 (.03-2.65)		
Basic writing and reading	9	15	.12 (.01-1.20)		
Illiterate	8	20	.08 (.01-.80)		
Current occupation			1.9 (0.97-3.72)	1.5(.078-4.20)	0.233
Farm owner	11	17	.22 (.05-.98)		
Animal attendant (workers)	33	35	.31 (.08-1.26)		
Others	9	3			
Marital status					
Married	22	30	1.6 (0.79-3.62)	.85(0.65-1.11)	0.238
Single	31	25			

5. DISCUSSION

The overall prevalence of bTB was found to be 18.37% and 46.87%, respectively, at the animal and herd levels in the study areas. The overall animal-level prevalence of bTB in this study was consistent with previously reported prevalence of 18% and 20.3% in other studies in different sites in Ethiopia (Mamo *et al.*, 2013; Kemal *et al.*, 2019). Previous studies reported lower results compared to the present study (Ameni *et al*., 2003; Ameni *et al*., 2013; Kemal *et al*., 2019; Mekonnen *et al*., 2019). , In a similar area, a previous study reported an overall prevalence of 7.0% in southern Ethiopia (Gumi *et al.*, 2011) and 6.8% in Meskan, Gurage Zone, central Ethiopia (Tschopp *et al.*, 2011). However, it is considerably lower than the previous studies of 30% (Firdessa *et al.*, 2012) and 39.3% (Tulu *et al*., 2021).

The herd-level prevalence of bTB (46.87%) observed in the study area agreed with the findings of Lemu *et al*., 2020, and Dejene *et al.*, 2016, who reported prevalence of 46.8% and 46% in Oromia and Afar regional states, respectively. However, the herd-level prevalence of bTB in the current study was lower than the values found by Mekonnen *et al.*, 2019; and Almaw *et al.*, 2021, who found prevalences of 65.5% in the emerging dairy belts of regional cities and 54.4% in central Ethiopia, respectively. The difference in prevalence between this study and previous reports could be associated with differences in sampling design, environment, breed compositions, husbandry practices, the subject measuring the skin test, and the nature of the tuberculin itself.

In this study, older cattle (>9 years) were known to have bovine TB more often than the younger ones (1–3 years), which could be justified by the fact that the older the animal, the greater the potential exposure to pathogen infections and the longer the antibody remains circulating. Besides, older cattle could have had more frequent exposure to *M. bovis* over their lifetime when compared to younger ones. This finding was consistent with previous research in Ethiopia (Mekonnen *et al*., 2019; Almaw *et al.*, 2021), which confirmed increased age as associated with increasing the likelihood of bTB infection in cattle.

The source of animals was also identified as another risk factor for bTB positivity at the animal level. In the present study, purchased animals had 3.1 times the odds of being bTB reactors (95% CI: 1.24–8.12) compared to cattle bred on their own farm. In addition, the purchase of older cattle, particularly from high-risk areas, could increase the risk of introducing bTB into a herd. Instead, sourcing animals from bTB-free herds, reducing cattle trade in general, and prioritizing the trade of young animals before adults have previously been suggested as effective steps to reduce the spread of bTB (Reilly and Courtenay, 2007). Our data supports the prompt implementation of regulation on the trade of animals in Ethiopia, should arresting the spread of bTB truly be a priority for the nation (Adkin *et al*., 2016).

Results presented in this study suggest that the management condition of dairy farms was significantly associated with bTB positivity at the herd level. The study revealed that herds managed in poor farm conditions had a higher risk of being bTB positive (OR 4.7; 95% CI: 1.11–19.92) compared to herds managed on farms with good management. This observation was consistent with the results of earlier studies (Elias *et* *al.*, 2008; Romha *et al.*, 2014; Zeru *et al.*, 2014; Kemal *et al.*, 2019). Similarly, Mekonen *et al.* (2019) reported that farm management systems are one of the risk factors significantly associated with the bTB at herd level. Farms with poor management conditions may facilitate the persistence of *M. bovis* infection, creating a conducive environment for easy proliferation and transmission.

The present study has identified that the introduction of new cattle into apparently bTB-free herds is one of the risk factors for the transmission of bTB. As of the time of writing, the country has no legislation or regulation in place for bTB control, including traceability, accountability, and animal movement. Therefore, animals moved from one region to another without any regulatory checks, such as animal health certification, might insidiously promote the bTB's dissemination into wider areas. The present data demonstrate that the movement of animals into herds is associated with an increased risk of bTB positivity at the herd level (OR 2.8, 95% CI: 1.01–8.18). The finding concurs with previous studies (Tschopp *et al.*, 2009; Proano-Perez *et al.*, 2009). Continuing to allow the free movement of animals without any precaution could increase the risk of bTB spreading into wider areas. Similar findings have been reported in the UK, Italy, Tanzania, and Michigan, USA (Dejene *et al*., 2016; Gopal *et al*., 2006; Johnston *et al*., 2011; Kaneene *et al*., 2002; Marangon *et al*., 1998; Shirima *et al*., 2003).

In this study, stocking density and overcrowding due to disproportionately housed herds contributed to the bTB positivity of a herd (Humblet *et al*., 2009). The study revealed that herds managed under high stocking density showed higher odds of bTB positivity (OR 6.3, 95% CI: 1.96–20.24) than herds with less stocking density, implicating the deleterious effect of stress on the animals' resistance to the disease. High stocking density created as the result of poor housing conditions and overcrowding has been reported as a potential cause for the increased bTB positivity (Costello *et al*., 1998; Ameni *et al*., 2006; Elias *et al*., 2008). This is because, as stated by Rastits *et al*. (2000), bovine TB is a disease of overcrowding.

Based on the questionnaire survey about the awareness of the farm owners and/or workers regarding bTB and its zoonotic transmission, a significant number of the respondents did not know the zoonotic importance of bTB. The proportion of the respondents who had knowledge of bTB and its zoonotic implications was 32.14%. This result is in line with the reports of Ameni and Aklilu, 2007; Tigre *et al.*, 2012; Tamiru *et al.*, and Zeru *et al. (*2014), who indicated 35%, 37.1%, 29.7%, and 30.8%, respectively. In Eastern Ethiopia, Kemal *et al.* (2019) recorded a similar finding to the present study, where 33.55% of the respondents had knowledge of bovine tuberculosis and 23.25% were aware of the zoonotic importance of the disease. Furthermore, the respondents reported that the practice of consuming raw animal products and sharing the same house with cattle was very common. Previous studies by Ameni *et al*. (2003) and Mekonnen *et al*. (2019) also demonstrated a gap in the awareness of farm owners in this regard. This low awareness may be a limiting factor in the implementation of control and prevention of the disease in the country.

The findings of this study revealed that education status had a statistically significant association with knowledge level. This result is consistent with previous reports in Ethiopia (Mesfin *et al*., 2005; Bati *et al*., 2013; Kerorsa, 2019) and Nigeria (Ismaila *et al*., 2015). Respondents who did not receive formal education were 8 times less likely to have good knowledge about bovine tuberculosis than those with a degree and above education (95% CI: .01–.80) as compared to those with a degree and above educational level. The possible reason could be that as education increases, people will have better access to information about the disease.

6. CONCLUSION AND RECOMMENDATION

Using the SICCT test, the present study determined high bTB prevalence in the emerging dairy regions. In addition, this study identified farm management conditions, stocking density, introduction of new cattle from other herds, age and animal origin as important risk factors. The outcome of this study revealed that communities in the study area lack awareness of bTB and its transmission routes, which could allow for the spread of the pathogen between communities and animals. The questionnaire survey of this study revealed that the majority of pastoralists in the region are unaware of bTB and its public health importance. As a result, a large part of the community had a habit of drinking raw milk and being in close contact at the same shelter, suggesting the possible potential for transmission from bTB-positive animals. This is important data for designing future control strategies in south-central Ethiopia.

Based on the results of this study, the following recommendations are forwarded:

- Implementation of a control program in this city at this stage could be effective in further reducing or potentially stopping bTB, preventing transmission between cattle and reducing the likely zoonotic impact. This may include registering each dairy herd, controlling dairy herd movement and regularly testing herds.
- Community health education about the impact of the disease, transmission, control and prevention should be integrated into one health oriented education.
- Further investigation involving post-mortem pathological examination and molecular characterization of mycobacterial isolates from tuberculin reactor cattle should be done to complement the findings of the present study.

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