Prevalence of Bovine Tuberculosis in Dairy Cattle at Dairy Farm of Holeta Agricultural Research Center, Central Ethiopia

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Abstract: Bovine tuberculosis, caused by *Mycobacterium bovis*, which belongs to *Mycobacterium tuberculosis* complex (MTC), is a globally distributed zoonotic disease in cattle and remained an economic problem in Ethiopia. This study was conducted from November 2015 to May 2017 on 502 dairy cattle managed under the Dairy Farm at Holeta Agricultural Research Center. The objectives of the study were to estimate the prevalence, evaluate gamma interferon and comparative intradermal tuberculin test (CIDTT) for the diagnosis of bovine tuberculosis, and isolate, identify, and characterize causative agents of bovine tuberculosis using RD4 deletion typing molecular technique. Data collected on study animals were analyzed using STATA version13 software. Out of 502 cattle examined using different methods, 148 (29.5%, 95% CI: 25.5 - 33.7%) were positive for bovine tuberculosis using CIDTT, 173 (34.5%, 95% CI: 30.3 -38.8%) for Gamma-Interferon and 97 (19.3%, 95% CI: 15.9 – 23.1%) were positive for both tests. The results indicated moderate Kappa agreement between the two tests. Among 60 slaughtered animals that were positive for both test, 48 (80.0%) had gross tuberculosis lesion while the rest 12 (20.0%) had no such lesions. Out of 48 gross lesions cultured on Löwenstein–Jensen medium, 24 samples were culture-positive (19 on pyruvate enriched media that were suspected as *Mycobacterium bovis* while the 5 on glycerol which suspected as *Mycobacterium tuberculosis*). All of the isolates were subjected to PCR amplification for RD4 deletion typing technique that confirmed 8 (33.3%) of the isolates to be *M. bovis*. The rest being other members of the genus *Mycobacterium*. From the study it was concluded that bovine tuberculosis is highly prevalent in the studied farm. The comparison of evaluation techniques indicated the presence of moderate Kappa agreement between gamma interferon (ELISA) and tuberculin test (PPD). Therefore, it is recommended that an urgent need for regular surveillance, implementation of strict biosecurity measures and further large scale molecular epidemiological studies need to be implemented for effective prevention and control of bovine tuberculosis.

Keywords: *Bovine tuberculosis, Cattle, Culture, Diagnosis, Gamma-interferon, Molecular typing, PPD*

Introduction

Bovine tuberculosis (bTB) is a globally distributed zoonotic disease caused by *Mycobacterium bovis*, which belongs to the *Mycobacterium tuberculosis* complex (MTC) (Michel *et al*., 2010). The MTC includes *Mycobacterium bovis*, *M. tuberculosis, M. africanum, M. microti, M. canetti*, and *M. caprae* (Cousins *et al*., 2003; Good and Duignan, 2011; Atkins and Robinson, 2013). The disease is characterized by nodular granuloma or tubercle formation with resultant caseation and calcification in many of the vital organs (Hardstaff *et al*., 2013; Le Roex *et al*., 2013). The prevalence of bTB in Ethiopia ranges from 1.1% (Regassa *et al*., 2009) to 48% (Ameni *et al*., 2008) in peri-urban /intensive dairy production systems. The risk factors for the transmission of the disease vary in a different area of Ethiopia (Ameni *et al*., 2013; Mamo *et al*., 2013). The current increasing incidence of tuberculosis in humans, particularly in immune-compromised individuals, has given a renewed interest in the zoonotic importance of *M. bovis*, especially in developing countries (Firdessa *et al.,* 2013). Even though, routine meat inspection procedures with qualified professionals are practiced in a handful of municipal and export abattoirs throughout Ethiopia; estimates show that more than half of slaughtered animals each year are illegally processed in backyard system without undergoing proper inspection, thus posing a great health risk to the consumers (Biffa *et al*., 2009). Moreover, further diagnostic techniques such as gamma interferon and molecular methods like spoligotyping (Wood *et al*., 1990; Kamerbeek *et al*., 1997) and deletion typing (Brosch *et al.,* 2002) allow for the identification of *M. bovis* isolates from many livestock and wildlife species. The transmission of bTB to humans constitutes a public health problem (Ayele *et al*., 2004; OIE, 2009). In many developing countries, bTB remains endemic causing significant economic losses (Zinsstag *et al*., 2006). Hence, some countries of the world suggest the need for the control and eradication of the disease (Zumarraga *et al.,* 1999,

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Vordermeier *et al*., 2004). Many developed countries achieved eradication of bovine tuberculosis through national control programs, based on test-and slaughter policy. Among several diagnostic approaches of bovine tuberculosis, gamma interferon assay is the fastest and safest technique. Direct isolation of causative agents or indirect methods such as the assessment of developed antibodies against causative organisms and measurement of the cell-mediated immune response is also used for diagnosis (Santos *et al.,* 2010). Bovine TB infection in cattle is usually diagnosed in live animals based on delayed hypersensitivity reactions, elicited by intradermal inoculation of tuberculin and assessed after 72 hours (Wood and Jones, 2001). These tests are the most widely used tests for field surveillance of bTB (Monaghan *et al.,* 1994; Ameni *et al.,* 1999).

The reported sensitivity of the single intradermal tuberculin test (SITT) in cattle ranged between 80.2% and 100% (de la Rua-Domenech *et al.,* 2006; Schiller *et al.,* 2010). Reactivity against the tuberculin usually develops between 3 to 6 weeks post-infection. Some studies have suggested the pre-allergic phase as a cause of false-negative reactions and therefore, the lack of sensitivity in recently infected animals (Monaghan *et al.,* 1994; Alvarez *et al.,* 2012a). More recently, using a Bayesian approach, the estimate for the sensitivity of the SITT ranged between 53% and 69.4% depending on the interpretation criteria used by Alvarez *et al.,* 2012b).

In 2001, the QuantiFeron test became the first interferon-gamma release assay (IGRA) approved by the Food and Drug Administration (FDA) as an aid for the diagnosis of *M. tuberculosis* infection (Mazurek and Villarino, 2003). Cytokine release assays, particularly the gamma interferon (γ-IFN) tests are being prominently used internationally for the diagnosis of tuberculosis in animals (Schiller *et al.,* 2010). To improve specificity, new IGRAs were developed. The IGRAs assess response to synthetic overlapping peptides that represent specific *M. tuberculosis* proteins, such as early secretory antigenic target-6 (ESAT-6) and culture filtrate protein 10 (CFP-10) are absent from BCG vaccine strains and most nontuberculous mycobacteria (Andersen *et al.,* 2000). It has been reported that the specificity of the IFN- test is higher than that of tuberculin skin tests (96.8%) (Cagiola *et al*., 2004).

Molecular typing is conducted using PCR amplification for detecting *Mycobacterium* species DNA to improve the control of bTB. Complementary tests may be required, particularly in the final stages of eradication programs (Kolk *et al.,* 1992; Carlos and Opin, 2006). To this end diagnostic tests based on methods other than the commonly used allergic skin tests such as enzyme linked immunosorbent assay (ELISA) based gamma interferon assays that detects infected animals at early stage of the disease (Lilenbaum *et al*., 1999) are essential and need to be introduced. Other diagnostic tests such as spoligotyping are highly recommended to characterize *M. tuberculosis* complex species at strain level. This method is based on the polymerase chain reaction method for simultaneous detection and molecular typing (Molhuizen *et al.,* 1998). In Ethiopia, tuberculosis has been reported from camel, sheep, goats and swine among domestic and buffalo, and deer among the wild animals (Ameni *et al*., 2003; Ayana *et al*., 2013; Deresa *et al*., 2013; Zerom *et al.,* 2013; Dejene *et al.,* 2016; Jibril *et al*., 2016). However, the bTB status of Holeta Agricultural Center Dairy Farm was not known. The objectives of this study were: i) estimate the prevalence of bTB in the farm; ii) to evaluate the gamma interferon and comparative tuberculin test for the diagnosis of bovine tuberculosis; and iii) to isolate, identify and molecularly characterize causative agents of bovine tuberculosis from test positive animals.

Materials and Methods

Study Area Description

This cross-sectional study was conducted at dairy farm at Holeta Agricultural Research Center starting from November 2015 up to May 2017. Holeta Agricultural Research Center (HARC) was established in 1966 GC under the Institute of Agricultural Research (IAR), now the Ethiopian Institute of Agricultural Research (EIAR). The farm is located in Holeta town and has two sub-centers located at 9.11 latitude, 37.05 longitude and DMS 9˚6'53.08 N and DMS 37˚33' E (Fig.1), at an altitude of 2400 meters above sea level. The average annual temperature of the center is 14ºC (minimum 6ºC and maximum 22ºC). The center receives an annual rainfall of 1144 mm. Holeta town is found in Oromia National Regional State, in Oromia Special Zone Surrounding Finfine at a Distance of 29 Km from Addis Ababa, the capital city of Ethiopia (Figure 1).

Study Animals

The study animals were Holstein-Friesian crossbreed dairy cattle kept under a semi-intensive management system. Heifer calves, heifers, and cows aged one year and above were included in the study.

Study Methods

Ante-mortem inspection of the live animals: Ante-mortem inspection of the live animals was done at the dairy farm. The body condition of the animals was scored following the recommendations of Nicholson and Butterworth (1986). The age of the animals was determined according to Amstuz *et al.* (1998).

Comparative tuberculin skin test: Two sites on the right side of the mid-neck skin of the animals, 12-15 cm apart, were shaved and skin thickness was measured using a digital caliper before injection of purified protein derivative (PPD). The sites were recorded as A_1 for avian PPD site and B_1 for bovine. Each site was injected with an aliquot of 0.1ml of 2,500 Iu/ml PPD bovine into dermis toward the shoulder of animal and another site was similarly injected with 0.1ml of 2,500 Iu/ml of avian PPD toward the head. After 72 hours,

the skin thicknesses at the injection sites were again measured and recorded as A2 for avian PPD and B2 for bovine. The results were interpreted according to the recommendations of the Office International des Epizooties (OIE, 2009). Briefly, when the change in skin thickness was greater at the avian PPD injection site, the animals were considered as positive for *Mycobacterium* species other than the mammalian type (*M. tuberculosis* and *M. bovis*). When the increase in the skin thickness was observed at both sites of injection the difference was considered between the two sites of reactions, thus if an increase in skin thickness at the injection site for bovine PPD (B) was greater than the increase in skin thickness at the injection site of avian PPD (A) the manipulation of the change was done by subtracting the measured value at A from that at B. When the change was minus and less than 1 mm it was recorded as negatives, between 1 mm and 4 mm suspected, and when it is ≥ 4 mm as positives for bovine tuberculosis (Ameni and Tibbo, 2002; OIE, 2009).

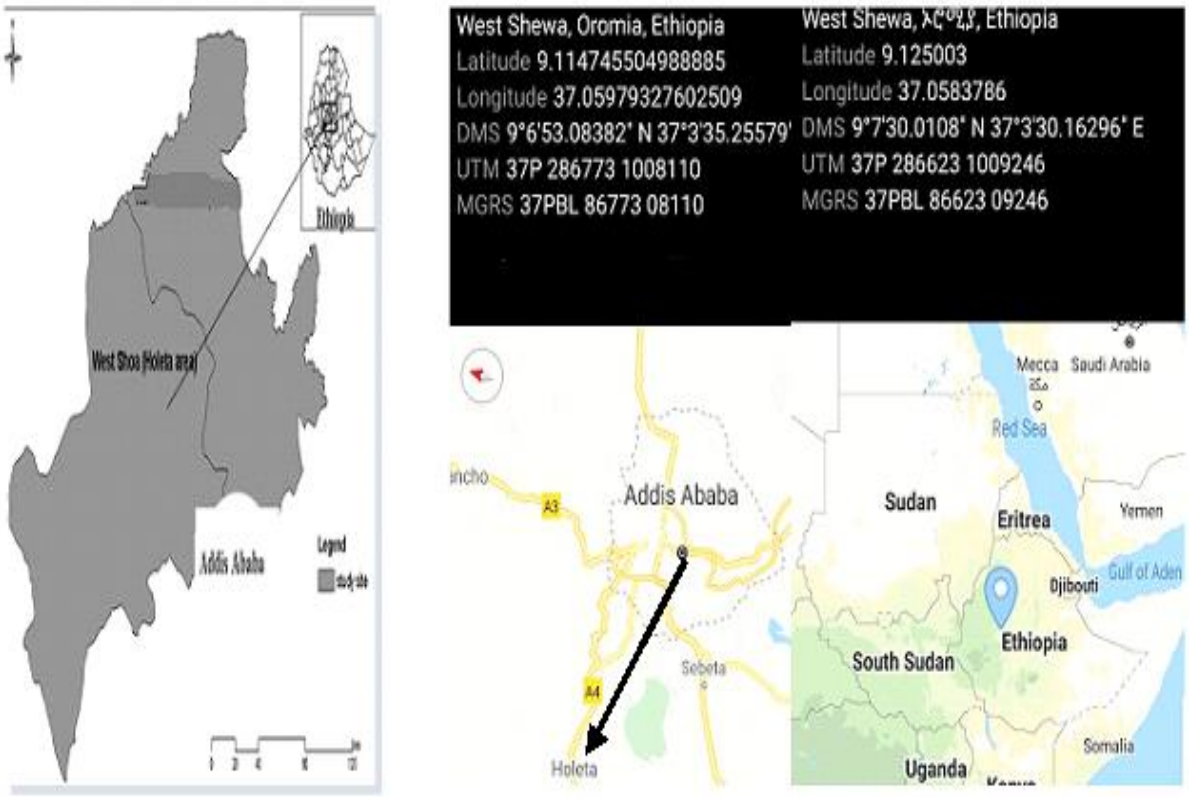

Figure 1. Map of the study area (Holeta within especial Zone of Finfine).

Gamma interferon assay (γ-IFN assay): A lithium-heparinized tube was used to collect a 5 ml blood sample from each animal immediately following the application of the intradermal tuberculin skin test. The blood samples were brought to the laboratory within 8 hours of collection and processed according to the methods described by Rothel *et al.* (1990). The whole blood samples collected from each animal were dispensed in 3x1.5 ml into a 24 well tissue culture plate. Then, the addition of 100 µl nil antigen (Phosphate Buffered Solution) as non-stimulating control to the first well, 100 µl bovine PPD to the second well and 100 µl avian PPD to the third well of each sample was added, and the plates were incubated in a humidified atmosphere at 37°C for 16-24 hours. Then, plasma samples were harvested from the cultures and tested with the Bovigam ELISA test kit (Prionics, Switzerland) according to the instructions supplied with the kit. Whole blood cultures were performed in 96 well plates in 0.20 ml/well aliquots by mixing 0.1ml of heparinized blood with an equal volume of PPD-A and PPD-B antigen-containing solution. Supernatants were harvested after 24 hours of culture, and IFN-γ was determined using the bovigam enzyme immunoassay (EIA) kit (CSL, Melbourne, Australia (Vordermeier *et al.,* 2001). The samples in the ELISA were run in duplicate. Positive and negative controls were used in each plate. The absorbance within 5 minutes of terminating the reaction was recorded at 450 nm. The mean absorbance values of positive and negative controls were determined for the test validation and compared with positive and negative values provided by the kit for validation of the test (negative bovine γ-IFN control <0.130; positive bovine γ-IFN control

>0.700). The mean nil antigen, avian and bovine PPD optical density (OD) for each sample were calculated and compared with the mean absorbance values of the nil antigens, avian, and bovine PPD controls. A sample was considered as positive when the difference between OD value of a sample stimulated with bovine PPD and OD value of the same sample stimulated with avian PPD and nil antigen is equal or higher than 0.100 and considered as negative when this difference is less than 0.100 (Rothel *et al.,* 1990; Wood *et al.,* 1991).

Post mortem inspection and sample collection: Postmortem examination was performed on 60 slaughtered animals randomly selected among those positive for both CIDT and γ-IFN tests. The examinations were conducted according to the procedures described by Corner (1994). Postmortem samples were collected from all the 60 slaughtered animals. The lung and lymph nodes were sliced into thin sections of similar thickness and inspected for the presence of visible lesions (Patterson and Grooms, 2000). Specimen collection was conducted using 0.9% normal saline in a sterile universal bottle according to the standard procedure of tissue sample collection recommended by Asseged *et al.* (2004). Generally, these organs were visualized in detail, palpated and incised, to assess bTB lesions. Simultaneously, observed lesions were cut at 2 mm thickness using a sterile scalpel blade for bacterial culture (Patterson and Grooms, 2000).

Mycobacterial culture and isolation of mycobacteria: Samples from suspected TB lesions were further processed for isolation of mycobacteria following the World Organization for Animal Health (OIE) protocols (OIE, 2009). Tissue samples for culture were collected into sterile universal bottles in 5 ml of 0.9 percent saline solution. Those collected samples were handled with cold chain and transported to Aklilu Lemma Institute of Pathobiology TB laboratory for culture. The samples were processed and cultured on Löwenstein–Jensen (LJ) media.

Molecular characterization of isolated mycobacteria: Acid-fast positive samples were harvested from the colony and heat-killing procedure was employed for the extraction of DNA. The DNA samples were amplified with polymerase chain reaction (PCR) for the process of denaturation, annealing, and extension. In this study, the RD4 deletion type was conducted to confirm suspected *M. bovis* from both pyruvate and glycerol enriched grown samples (Cadmus *et al*., 2006).

***RD4 deletion*:** Region of difference 4 (RD4) is a 12.7 kb deletion previously characterized as a region absent from *M. bovis* and the *M. bovis* BCG sub-strains Pasteur (Brosch *et al*., 1998). The proteins encoded by the region's 11 open reading frames (ORFs) show little similarity to known proteins, but bear some resemblance to enzymes involved in lipopolysaccharide synthesis. For this deletion typing, the procedure of Cadmus *et al.* (2006) was followed. Primers that were used include RD4 FlankF 5'-CTC GTC GAA GGC CAC TAA AG-3', RD4 FlankR 5'-AAG GCG AAC AGA TTC AGC AT-3', and RD4 IntF5'-ACA CGC TGG CGA AGT ATA GC-3' to check for the presence of the RD4 locus. The Hot StarTaq Master Mix system from Qiagen was used for PCR with the primers described above. *M. tuberculosis* H37Rv and *M. bovis* were used as positive controls while Qiagen water served as a negative control. A reaction mixture consisting of 10µl of Hot StarTaq Master Mix, 0.3µl each of the three primers (RD4 FlankR, RD4 FlankF, and RD4 InternF), 2µl DNA template and 7.1µl H2O Qiagen, adding up to a final volume of 20µl, were heated in a thermal cycler (Applied biosystem; PTC-100™) to 95°C for 15 minutes. Then, the reaction underwent 35 cycles consisting of denaturation at 95°C for 1minute, annealing at 55°C for 1minute, and extension at 72°C for 1minute. Finally, the reaction mixture was maintained at 72°C for 10 minutes. Electrophoresis of the PCR products was done in 1.5% agarose gel in 10× TAE running buffer with ethidium bromide at a ratio of 1:10. A 100bp DNA ladder (Promega Cooperation, USA) and Orange 6× loading dye were also used for visual tracking of DNA migration during electrophoresis. The gel was visualized in UV light illuminator. Isolates were confirmed as *M. bovis* by deletion typing of the RD4 region according to a previously described PCR protocol (Brosch *et al.,* 2002).

Data Collection, Management, and Analysis

For all 502 comparative intradermal tuberculin injected and a whole blood sample collected animal results were recorded on the Microsoft Excel spreadsheet for Windows 2007. STATA version 13 (STATA Corporation, College Station, TX, USA) software was used to analyze the data. Prevalence was calculated by dividing the number of test-positive animals by the total number of animals examined multiplied by 100. Pearson Chi-square (X^2) test was used to assess the associated risk factor with bovine tuberculosis. The prevalence of study animals was calculated based on the diagnostic tests and the age of animals. The results of gross pathology and culture were compared using the Chi-squared test and their concordances were determined by calculating the Kappa coefficient (k) using the formula: k= (P(A) - P(E) / 1 - P(E), where P(*A*) is the relative observed agreement between gross pathology and culture sample and P(*E*) is the hypothetical probability of chance agreement. Following the recommendations of Dohoo *et al.* (2003), which states Kappa values as: <0.2: slight agreement, 0.2–0.4: fair agreement, 0.4–0.6: moderate agreement, 0.6–0.8: substantial agreement and >0.8: almost perfect agreement. Similarly, the agreement between PPD and ELISA was computed using the Kappa test of agreement (Dohoo *et al*., 2003). A P-value of ≤0.05 at 95% confidence interval was considered as significant.

Results

Ante-Mortem Inspection

At the time of antemortem inspection, animals were inspected for physical and clinical examination as well as body condition scoring. All animals found on the farm had good body condition and a similar management system was practiced. However, some of the animals showed clinical signs of tuberculosis like coughing, respiratory distress, and weight loss although their physical condition was good.

Comparative Tuberculin Skin Test

Of the 502 animals, 148 were positive for the comparative tuberculin test (29.5%, 95% confidence [CI]: 25.5 – 33.7%) at a cut off value of ≥4 mm skin reaction thickness. As the age of cattle increases, there was a significant increase in the prevalence of bTB using PPD ($X^2_{(3)} = 14.6708$, $P = 0.002$, Table 1).

Gamma Interferon Assay

The prevalence of bTB based on PPD and ELISA tests was presented in Table 1. From 502 animals tested using the gamma interferon test, 173 cattle (34.5% 95% CI: 30.3 -38.8%) were positive (Table 1). Ninety-seven animals tested positive for both PPD and gamma interferon test giving a prevalence of 19.3% (95% CI: 16.0 –23.1%, Table 1).

Two-hundred seventy-eight animals (55.4%) tested negative for both tests. Fifty-one animals (10.2%) were ELISA negative but PPD positive while 76 animals (15.2%) were PPD negative but ELISA positive. The Kappa test of agreement between PPD and ELISA was 0.42 that indicated moderate agreement (Test agreement=74.7%, kappa = 0.42, P≤0.0001).

Table 1. Comparison of the age-based prevalence of bTB with PPD and ELISA results

Age	No. tested	*ELISA		**PPD		Both ELISA and PPD	
		No. positive	% prevalence	No. positive	% prevalence	No. positive	% prevalence
1-2 years	43	12	27.9	6	14.0	4	9.3
3- 4 years	173	57	33.0	42	24.3	31	17.9
5 -6 years	204	70	34.3	65	31.9	38	18.6
≥ 7 years	82	34	41.5	35	42.7	24	29.3
Total	**502**	**173**	**34.5**	**148**	**29.5**	**97**	**19.3**

** $X^2_{(3)} = 2.7753$, P=0.428; ** $X^2_{(3)} = 14.6708$, P=0.002 (significant).*

Table 2. Age-based percentage of gross lesions of bTB in slaughtered dairy cattle

Age in years	Number examined	Gross pathological lesions	
		Number positive	%
1 - 2	2	2	100
3 - 4	20	17	85
5 - 6	22	19	86.4
≥ 7	16	10	62.5
Total	**60**	**48**	**80.0**

Postmortem Inspection

From 60 randomly selected and slaughtered animals, detailed postmortem inspection revealed that 48 (80.0%) had gross pathological lesions suggestive of bTB. The remaining 12 animals (20.0%) were non-visible lesion reactors (Table 2).

The frequency and distribution of lesions were slightly higher in organs of the thoracic cavity including lung, mesenteric, and mediastinal lymph nodes (Figure 2). The Kappa test of agreement between culture and gross pathology was 0.286 that indicated fair agreement (Table 3).

Isolation of Mycobacteria

Of the 60 tissue samples from reactor-slaughtered animals with visible and non-visible lesions cultured on LJ media, 24 (40.0%) were positive for *Mycobacterium*. All of the isolates were obtained from samples with gross pathological lesions. Nineteen (79.2%) out the 24 isolates grew on pyruvate enriched media, while the rest five (20.8%) grew on glycerol enriched media.

Table 3. Kappa agreement between gross pathology and tissue culture (N=60)

		Value	Asymp. Std. Er.	Approx. T	Approx. Sig.	Exact Sig.
Interval by Interval	Pearson's R	0.408	0.066	3.406	0.001	0.002
Ordinal by Ordinal	Spearman Correlation	0.408	0.066	3.406	0.001	0.002
Measure of Agreement	Kappa	0.286	0.079	3.162	0.002	0.002

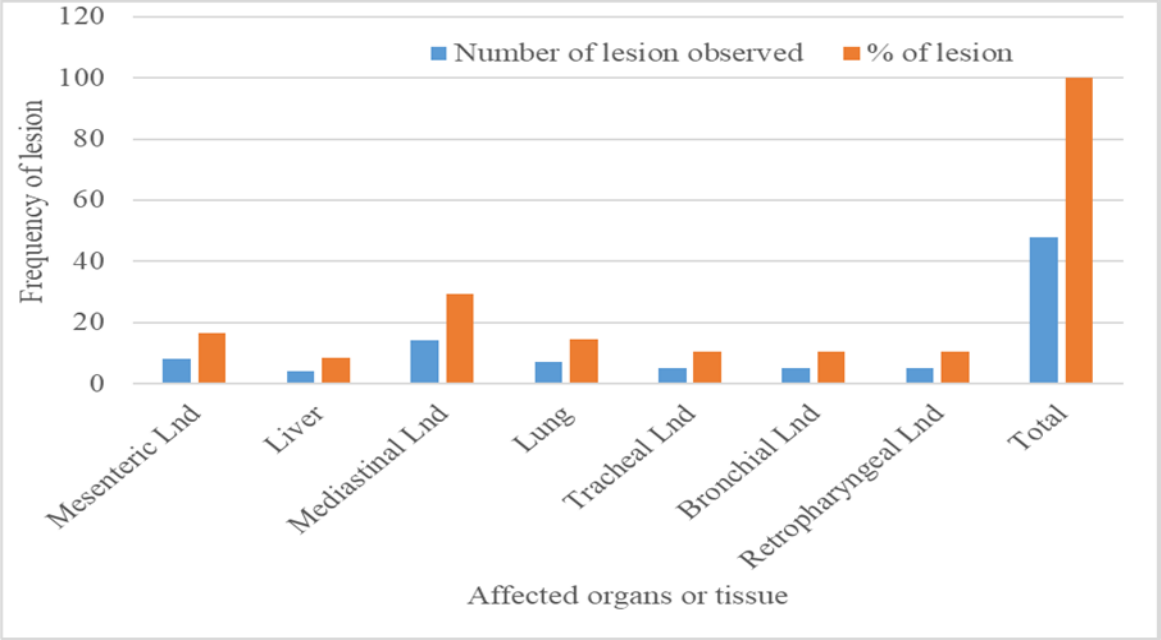

Figure 2. Distribution and frequency of suspected tubercular lesions in different tissue and/ or organs of 48 gross lesion positive samples collected.

Molecular Characterization of Mycobacteria Isolates

RD4 deletion typing: All the 24 isolates were subjected to RD4 deletion typing procedure and 8 isolates (33.3%) were RD4 positive at 446 base pairs confirming them to be *M. bovis* (Figure 3). The rest of the isolates were considered to be other members of the genus *Mycobacterium*.

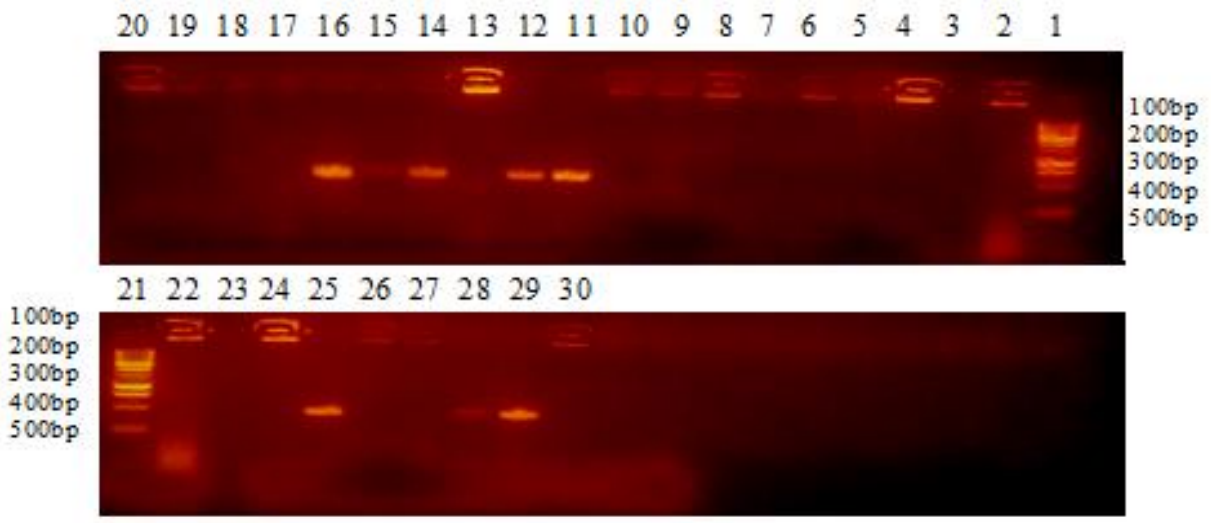

Figure 3. Electrophoretic separation of PCR products of RD4 deletion typing. Lanes: 1 and 21 = 100 bp DNA Ladder, 2 and 22 = *M. tuberculosis* (positive control), 3 and 23 = Qiagen H_2O (negative control), 4 and 24 = *M. bovis* (positive control), Lanes 5–20 and 25-30 were isolates which generated a PCR product of 446 bp in Genus typing, 8 RD4 Positive samples were=lane 11, 12, 14, 15, 16, 25, 28 and 29).

Discussion

Bovine tuberculosis is a global disease, and individuals living in countries where bTB is not eradicated may be infected with this disease because of the sharing of the same or common environment and the ease of spread between animals and the owner of animals (Ameni *et al.*, 2003). The prevalence of bTB recorded by comparative intradermal skin test (29.5%) is within the range of prevalence of 1.1% to 48.0% reported from Ethiopia (Ameni *et al.*, 2008; Regassa *et al.*, 2009). The findings of the present study are in close agreement with 25.9% (Biffa *et al.*, 2010) and 34.1% (Tsegaye *et al.*, 2010) prevalence previously reported in Ethiopia. The present prevalence of bTB is high when compared with the 9.4% (Ameni *et al.*, 2013), 11.0% (Mamo *et al.*, 2013), and 3.8% (Mamo *et al.*, 2012) prevalence

reported from various parts of Ethiopia. In contrast, the present prevalence was slightly lower when compared to the 46.8% (Ameni *et al.*,2003), 48.0% (Ameni *et al.*,2008), and 46.0% (Dejene *et al.*, 2016) prevalence reported from Ethiopia using comparative intradermal test and single intradermal tuberculin tests. The prevalence of bTB recorded with gamma interferon (34.5%) was high as compared to the 15.4% prevalence previously reported from Ethiopia (Ameni *et al.*, 2010). The high prevalence of bovine tuberculosis in the studied dairy farm might be related to the weak biosecurity measures practiced, the large herd size and close contact of animals during grazing and housing, the high percentage of exotic blood of the animals, and the long age of the dairy farm (Firdessa *et al.*, 2012; OIE, 2009). The prevalence of bovine tuberculosis was seen to increase while the age of the animals' increases (Table 1), which is related to an intrinsic property of bTB transmission.

The comparison of the results of gamma interferon (ELISA) and tuberculin test (PPD) indicated moderate Kappa agreement. This agrees with the previous reports from Ethiopia (Ameni *et al.,* 2010). Gamma interferon test was capable of identifying tuberculosis positive animals 60-120 days earlier than the skin test (Lilenbaum *et al.*, 1999). This might be the reason for the high positive cases detected using gamma interferon assay than PPD.

Post-mortem examinations conducted on 60 reactor animals showed that 17 animals (35.4%) were positive for gross lesions in the lung, bronchial, and tracheal lymph nodes. Lesions were observed with high frequency in both left and right diaphragmatic lobs of the lung and less in the apical lobe. Generally, 5 (10.4%) of these cows also had lesions in the bronchial lymph nodes, 5 (10.4%) in tracheal lymph nodes of the thoracic cavity, which is similar to Ameni *et al.* (2008), Regassa *et al.* (2009) reported bronchial and retropharyngeal lymph nodes as the most frequently and severely affected lymph nodes. There was no lesion found in the mandibular lymph node (Figure 2). Lesions were also found in the mesenteric lymph nodes of 14 cows 29.17% and liver tissue suggesting the possible excretion of the bacilli through feces for subsequent transmission through the ingestion of contaminated feed or water. The visible tuberculous lesions detected during the postmortem inspection of slaughtered animals were predominantly hard, bulging, and white to yellowish nodules giving caulis flower-like appearance mostly in the lymph nodes deep in the lungs and mediastinal lymph node, and sometimes-calcified (Jubb *et al.*, 1991). Bulging in the lymph nodes may be due to the difference of the tissues in their consistency. The high frequency of occurrence of suspected tubercular lesions in the lungs and associated lymph nodes in the present study might suggest the likelihood of transmission through aerosol favored by the cool weather condition, large herd size, and close contact of the animals. Despite the good body condition of the studied animals, it was revealed that the characteristic lesions and the causative mycobacteria could be harboured in the respiratory and digestive systems being asymptomatic. This corroborates with the findings of Firdessa *et al.* (2012).

The molecular characterization of the 24 culture-positive samples showed that 8 (33.3%) were RD4 deletion typing positive for *M. bovis* at 446bp. This is similar to reports of Mekibeb *et al.* (2013). Other authors have also isolated and reported RD4 positive *M. bovis* (Ameni *et al.*, 2008) and RD9 *M. tuberculosis* (Deresa *et al.,* 2013; Zerom *et al.*, 2013) in Ethiopia. In the present study, mycobacterial strains from dairy cattle were confirmed through characterizing the lesions, culture, acid-fast staining, and polymerase chain reaction.

Conclusion

From the study it was concluded that bovine tuberculosis is highly prevalent in the studied farm. The prevalence of bTB obtained using the comparative tuberculin test and gamma interferon assay was high. Gamma interferon assay identified more bTB positive animals than the comparative tuberculin test. As the age of the dairy cattle increases, the prevalence of bovine tuberculosis also increase. The high prevalence of bovine tuberculosis might have great economic and public health risks to the farm and farm workers with the potential transmission to surrounding areas. Therefore, there is an urgent need for regular surveillance, implementation of strict biosecurity measures and further large scale molecular epidemiological studies need to be conducted for effective prevention and control of bovine tuberculosis.

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Conflict of Interests

The authors declare that they have no competing interests.

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