academicJournals

Vol. 8(24), pp. 2352-2358, 11 June, 2014
DOI: 10.5897/AJMR2013.6325
Article Number: CEC093A45332
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

*Full Length Research Paper*

# Epidemiology and public health significance of bovine tuberculosis in and around Sululta District, Central Ethiopia

**Akililu Biru[1], Gobena Ameni[2], Teshale Sori[1], Fanta Desissa[1]*, Akafate Teklu[1] and Ketema Tafess[2]**

[1]School of Veterinary Medicine, Addis Ababa University, P. O. Box 34, Debre Zeit, Ethiopia.
[2]Aklilu Lemma Institute of Pathobiology, Addis Ababa University, P. O. Box 1176, Addis Ababa, Ethiopia.

Received 3 September, 2013; Accepted 19 May, 2014

**A cross-sectional study was conducted on 858 dairy cows, 1107 slaughter animals and 58 dairy workers in and around Sululta District to investigate the epidemiology and public health importance of bovine tuberculosis (BTB). To that end, comparative intradermal tuberculin test (CIDT), post-mortem examinations, bacteriological analysis, molecular typing and questionnaire survey were employed. The herd and individual animal level prevalence were 11.4% (98/858) and 20% (9/45), respectively. The individual animal prevalence was affected by farming system, herd size, management system, sex, age, breed and body condition (P<0.05). Abattoir survey showed a prevalence of BTB to be 3.5% (39/1107) based on suspicion of tuberculous lesion. Culture positivity in primary culture media was confirmed in 7.7% (3/39) of tissue samples, 11.1% (5/55) of milk samples and 2.5% (1/40) of nasal swab samples. Genus typing of the nine positive isolates indicated that only 11.1% (1/9) one isolate was positive for the genus Mycobacterium. Among the farm attendants, only 6.9% (4/58) of the farm attendants had awareness on the existence of BTB, 10.3% (6/58) had awareness that milk and meat could be a source of BTB and 79.3% (46/58) had habit of raw milk and raw meat consumption. The study reveals the importance of BTB and poor awareness on the existence, source and transmission of the diseases in the study area call for urgent intervention. Conventional preventive measures and large scale collaborative action to design cost effective preventive and control measures at national level is recommended.**

**Key words:** Bovine tuberculosis, cattle, comparative intradermal tuberculin test (CIDT), epidemiology, public health, Sululta, Ethiopia.

## INTRODUCTION

Bovine tuberculosis (BTB) is chronic infectious disease of cattle caused by intracellular bacterium, *Mycobacterium bovis* characterized by progressive granulomatous lesions or tubercle in the lung tissues, lymph nodes

Corresponding author. E-mail: fantadesissa@yahoo.com. Tel: +251 911 03034 12.

and other organs. *M. bovis* is a member of the *Mycobacterium tuberculosis complex* (MTC). The complex encompasses *M. tuberculosis, M. bovis, Mycobacterium microti, Mycobacterium canetti, and Mycobacterium africanum* (Smith et al., 2006). Bacteria of the *M. tuberculosis* complex are aerobic, non-motile, non-spore-forming, slow-growing and acid-fast bacilli (Thoen et al., 2004).

*M. bovis* is the most ubiquitous pathogen among mycobacterium species. It has been known to infect many vertebrate animals of all age groups and humans although cattle, goats and pigs are found to be most susceptible (Radostits et al., 2000; Thoen et al., 2006). Among MTC, *M. bovis* has a wide range of target organs (lungs, gastrointestinal tract, mammary gland, kidney and reproductive organs) as well as mammalian hosts (wild animals, domestic animals and humans) (Phillips et al., 2002).

Infection of cattle with *M. bovis* constitutes a human health hazard and economic implications in terms of trade restrictions and productivity losses (Phillips et al., 2002; Villarreal-Ramos et al., 2003). Infected animal loses 10 to 25% of their productive efficiency (Radostits et al., 2000). Direct losses due to the infection become evident by decrease in 10 to 18% milk and 15% reduction in meat production (Radostits et al., 2000).

Human tuberculosis caused by *M. bovis* is becoming increasingly important in developing countries where humans and animals share the same microenvironment and waterholes (Cosivi et al., 1998). It is estimated that in countries where pasteurization of milk is rare and bovine tuberculosis is common, 10 to 15% human cases of tuberculosis are caused by *M. bovis* (Cosivi et al., 1998; Ameni et al., 2007).

The existence of BTB in Ethiopian cattle has long been documented in different parts of the Ethiopia and it is one of the major constraints to the country's socio economic development (Ayele et al., 2004). Various previous fragmented studies have shown that its prevalence range from 0.02-7.96% by abattoir survey while it varies from 4.7-68.6% by tuberculin test in cattle from different parts of Ethiopia (Shitaye et al., 2006).

Sululta is one of the towns located in North Shewa zone of Oromia Regional State having large number of dairy sector that supply milk to a large communities in the Addis Ababa and its suburb. However, there is no information on the epidemiology and public health significance of BTB in the district. Therefore, this study was aimed to investigate the epidemiology of BTB and its public health significance in Sululta District.

## MATERIALS AND METHODS

### Study site

The study was conducted in and around Sululta District, part of the Oromia National Regional State, Central Ethiopia. Sululta District, representing a highland agro-ecology, is located about 25 km North west of Addis Ababa, the capital of Ethiopia. It is located at 9°3' to 9°31'N latitude and 38°29' to 38°58'E longitude. It has a total area of 1587 km$^2$. The District is inhabited by 129,000 people of which 64,516 are men and 64,484 are women; 15,145 or 11.74% of its population are urban dwellers. The district is one of the main dairy sheds of Addis Ababa. The district livestock population is estimated at 210,210 cattle, 80,900 sheep, 16,491 goats, 32,862 equines and 75.936 poultry (CSA, 2007).

### Study population

The study populations were dairy cows, slaughtered animals and farm attendants in the study area.

### Study design and sampling

A cross sectional study design with census (from all volunteer farm owners) sampling technique was employed to sample 858 dairy cows for CIDT from 45 dairy farms, 1107 slaughter animals for abattoir survey and 58 farm attendants for questionnaire survey. Sample size for dairy cows was determined based on the expected prevalence of 13.5% obtained by Ameni et al. (2007) in central Ethiopia according to Thrusfield (2007) by increasing the size fourfold for better precision. Purposive sampling was used for the abattoir survey based on the accessibility and availability of logistics as many animals as possible during the study period. The sample size of farm attendants was determined on the basis of the number of farm attendants and farm size included in the study. Furthermore, 55 milk samples from tuberculin reactor milking cows, 40 nasal swabs from dry cows, heifers and calves and 39 tissue samples from lymph nodes, liver, kidneys and lungs containing suspected TB lesion were collected.

### Comparative intradermal tuberculin test

Purified protein derivatives (PPDs), which are crude proteins extracted from both bovine and avian mycobacteria were used. For the CIDT test, two sites on the right side of the mid-neck, 12 cm apart in a horizontal line were shaved, cleansed with a swab immersed in 70% alcohol and dried and the initial skin thicknesses were measured with calipers. One site was injected into the upper site with an aliquot of 0.1 ml containing 2,500 IU/ml PPD-A (avian tuberculin, Lelystand, Biologicals BV, Lelystand, the Netherlands). Similarly, 0.1 ml of 2,500 IU/ml PPD-B (bovine tuberculin, Lelystand, Biologicals B, Lelystand, Netherlands) was injected into the lower site. After 72 h, the skin thicknesses at the injection sites were measured and registered. Test results were interpreted according to the recommendations of the Office International des Epizootics (OIE, 2009).

### Post-mortem examination

Detailed post-mortem examination was performed on lymph nodes, liver, kidneys and lungs for the investigation of tubercle lesion following the procedure described by Corner (1994). Incision was made into the parenchyma of the lung and other organs that was suspected to contain tuberculous lesions. The tuberculous lesions were cut into slices of about 2 cm using separate surgical blades. The slices were then examined for the presence of suspected tuberculous lesions and samples collected for mycobacteriological culture.

### Isolation of Mycobacteria

Milk and nasal swabs from tuberculin positive and tissue samples from suspected tuberculous organs were collected aseptically and processed for isolation of mycobacteria according to the standard methods described by Roberts et al. (1994). The nasal swab samples were first stirred well to release the samples from the swab and centrifuged at 3,000 rpm for 15 min and mixed with an equal volume of 4% NaOH, concentrated by centrifugation and the sediments neutralized with 1% (0.1 N) HCl as described by WHO (2002). Neutralization was checked by using phenol red as indicator and the solution was said to be neutralized when the color changed from purple to yellow. Thereafter, the sediment was cultured using conventional Löwenstein-Jensen (LJ) egg slant medium containing 0.6% sodium pyruvate and glycerol. Then, the culture was incubated at 37°C for 12 weeks and examined on weekly basis for the presence of mycobacterial colonies. Similar procedure was used for culturing of milk samples and tissue samples being tissue samples were homogenized prior to culturing. All the laboratory activities were carried out under Safety Cabinet world class II. Cultures were considered negative if no visible growth was detected after 12 weeks of incubation. In the presence of visible growth of colonies, microscopic examination of cultures using the Ziehl-Neelsen (ZN) staining method was performed to select acid-fast bacilli (AFB) positive isolates. AFB confirmed positive cultures were sub cultured on other LJ media and the isolates killed in water bath at 85°C for 45 min. Then, the heat killed isolates were kept at -20°C for molecular analysis.

### Molecular typing

Multiplex polymerase chain reaction (m-PCR) was used to confirm the presence of genus Mycobacterium in the isolate and to differentiate *M. tuberculosis* complex from *M. avium* complex, and other mycobacterial species following the procedure developed by Wilton and Cousins (1992). Heat killed AFB positive samples were used as source of DNA template. DNA amplification was done in a thermocycler with 20 µl reaction volumes consisting of 5 µl of genomic DNA as a template, 8 µl HotstartTaqMaster and ($MgCl_2$, dNTP, Taq polymerase), 0.3 µl of forward and reverse primers for each sample. The primers used were MYCGEN-F, 5' AGA GTT TGA TCC TGG CTG AG 3' (35 ng/µl); MYCGEN-R, 5' TGC ACA CAG GCC ACA AGG GA 3', (35 ng/µl); MYCAV-R, 5' ACC AGA CAT GCG TCT TG 3', (35 ng/µl); MYCINT-F, 5'-CCT TTA GGC GCA TGT CTT TA 3', (75 ng/µl); TB1-F, 5'-GAA CAA TCC GGA GTT GAC AA 3', (20 ng/µl); TB1-R, 5'-AGC ACG CTG TCA ATC ATG TA 3; (20 ng/µl) and 5.2 µl per sample of Qiagen water. The PCR reaction was carried out using Thermal Cycler (VMR Thermcycler 732-1200 Leicestershire, UK) based on the following amplification programme: 95°C for 10 min for enzyme actvation; 95°C for 1 min for denaturation; 61°C for 0.5 min for annealing; 72°C for 2 min for extension, involving 35 cycles all in all; and final extension at 72°C for 10 min. *M. avium*, *M. tuberculosis* and *M. bovis* strain NCTC 8559, H37 Rv and 2122/97 were used respectively as positive controls while $H_2O$ (Qiagen, USA) was used as a negative control. The PCR product was then electrophoresed in 1.5% agarose gel in TAE running buffer 10X containing ethidium bromide at concentration of 0.5 µg/ml in 1.5% agarose gel, 100 bp DNA ladder (USA), and orange 6x loading dye (USA) were used in gel electrophoresis. All members of the Genus Mycobacterium produced a band of 1030 bp where as *M. avium* or subspecies such as *M. avium* sbsp. paratuberculosis, *M. intracellularae* and members of *M. tuberculosis* complex produce a band, 180, 850and 372 bp, respectively.

### Questionnaire survey

Farm attendants were interviewed using a pre-structured questionnaire to assess possible associated risk factors for the occurrence and spread of BTB between cattle and people.

### Data management and analysis

The generated data were recorded and coded using Microsoft Excel spread sheet (Microsoft Corporation) and analyzed using STATA version 11.0. Multivariate logistic regression was used to analyze the data and to identify the risk factors. The odds ratio (OR) was calculated to assess the strength of association of different risk factors with the prevalence of BTB. The prevalence was calculated by dividing the number of positive reactor or harboring suspected tuberculous lesions cattle by the total number of cattle. P-value less than 0.05 and 95% confidence interval (CI) for odds ratio not including 1 were taken statistically as significant association.

## RESULTS

Out of the 45 dairy farms tested by CIDT, 9 (20%) (95% CI: 19.74-20.26%) of them were positive. Among 858 dairy cows tested by CIDT, 98 (11.4%) (95% CI: 9.24-13.56) were positive. Table 1 shows various risk factors affecting the prevalence BTB and the degree of association of each factor with the disease.

### Abattoir survey

Out of 1107 cattle slaughtered at Sululta Co-operative Abattoir Enterprise, 3.5% (95% CI: 2.422-4.578) were suspected to have tuberculous lesion. Table 2 shows the distribution and frequency of lesion detection from different lymph nodes.

### Isolation of Mycobacteria

Out of 39 tissue samples suspected of having tuberculous lesion, only 7.7% (3/39) were positive for the growth of mycobacteria. Among 55 milk samples cultured from tuberculin-positive lactating cows, only 9% (5/55) were positive for mycobacterial growth. Similarly, out of 40 nasal swab samples collected from tuberculin-positive non-lactating dairy cattle, only 2.5% (1/40) were positive for mycobacterial growth.

### Molecular typing

Mycobactreium genus typing was conducted on the 9 culture positive isolates (3 from tissue, 5 from milk and 1 from nasal swab isolates). Multiplex PCR result showed that out of the nine culture positive isolates, only 11% (n=1) isolate were positive for the genus Mycobacterium.

**Table 1.** Multivariate analysis showing risk factors for the occurrence of BTB at animal level in Sululta District.

| Risk factor | No. (%) of animals | | OR (95%CI) |
|---|---|---|---|
| | Examined | Positive | |
| **Sex** | | | |
| Female | 649 | 94(14.5%) | 1 |
| Male | 209 | 4(1.9%) | 0.11(0.10-0.12) |
| **Age (years)** | | | |
| <3 | 302 | 6% | 1 |
| 3-5 | 295 | 14.2% | 0.38(0.36-0.39) |
| 6-8 | 178 | 17.98 | 0.29(0.28-0.30) |
| >8 | 61 | 4.9% | 1.23(1.23-1.23) |
| **Herd** | | | |
| ≤15 | 249 | 1.2% | 1 |
| 16-35 | 359 | 1.4% | 0.19(0.18-0.19) |
| >35 | 227 | 38.5% | 1.08 (1.06-1.09) |
| **Breed** | | | |
| Zebu | 111 | 1.8% | 0.12(0.12-0.13) |
| Cross | 724 | 12.8% | 1 |
| **Body condition** | | | |
| Good | 622 | 4% | 0.17(0.15-0.18) |
| Medium | 134 | 19.4% | 0.18(0.17-0.19) |
| Poor | 79 | 55% | 1 |
| **Animal origin** | | | |
| Out | 140 | 7.9% | 1 |
| Farm | 695 | 12% | 0.62 (0.60-0.63) |
| **Production system** | | | |
| Extensive | 486 | 2.1% | 1 |
| Semi-intensive | 206 | 4.9% | 0.02(0.01'-0.03) |
| Intensive | 143 | 52.44% | 0.45(0.42-0. 49) |
| **Management system** | | | |
| Good | 199 | 0.5% | 1 |
| Medium | 493 | 3.9% | 0.04(0.04-0.05) |
| Poor | 143 | 52% | 0.13(0.12-0 .13) |

### Questionnaire survey

From fifty-eight farm attendants interviewed, only 6.9% (4/58) had awareness on BTB and knew the transmission of TB from cattle to humans and vice versa and 10.3% (6/58) knew that milk and meat are sources of BTB) (Table 3). Among the farm attendants, 79.3%( 46/58) had habit of raw milk and raw meat consumption (Table 4). Only 10.3% (6/58) of the respondents consume and feed their families and babies on boiled milk. Large number, 72.4% (42/58) of the respondents shared the same shelter with farm cattle.

## DISCUSSION

In the present study, animal and herd level prevalence of 11.4 and 20% were recorded in dairy herds using CIDT-test. These findings are in agreement with the study conducted in other parts of the country (Fikre, 2011). Although, the finding is contrary to the study conducted by other researchers (Elias et al., 2008, Tsegaye et al., 2010). In addition, the animal prevalence reported in the present study is much lower than those reported

**Table 2.** Distribution and frequency of suspected tuberculous lesion in cattle slaughtered at Suluta.

| Anatomic site | Frequency | Percent (%) |
|---|---|---|
| Retropharyngeal LN | 5 | 12.82 |
| Apical LN | 2 | 5.13 |
| Bronchial LN | 2 | 5.13 |
| MandibularLN | 2 | 5.13 |
| Mediastenal LN | 11 | 28.21 |
| Mesentric LN | 7 | 17.95 |
| Intestinal LN | 10 | 25.64 |
| Total | 39 | 100 |

LN = Lymph nodes.

**Table 3.** Farm attendants' awareness of bovine tuberculosis and its mode of transmission.

| Farm attendants' knowledge | Number of respondents |
|---|---|
| Awareness of BTB | 4 (6.9%) |
| Know that cattle transmit BTB to humans | 4 (6.9%) |
| Know that humans transmit TB to cattle | 4 (6.9%) |
| Know that milk and meat are source of infection | 6 (10.3%) |

**Table 4.** Milk and meat consumption habit of farm attendants

| Consumption habit | Number | Percent |
|---|---|---|
| **Milk drinking** | | |
| Raw milk | 46 | 79.3% |
| Only boiled milk | 6 | 10.3% |
| Not drinking | 6 | 10.3% |
| **Meat eating** | | |
| Only cooked meat | 4 | 6.9% |
| Raw meat | 46 | 79.3% |
| Not eating | 8 | 13.8% |

earlier by other workers (Ameni et al., 2003b; Shitaye et al., 2006). On the other hand, the animal prevalence recorded in the present study is much higher than the animal prevalence reported (Tschopp et al., 2009, 2010) in various parts of Ethiopia.

The differences among various studies might be attributed to the differences in herd sizes, age, body condition, sex, production system, breed of animals and the sample size considered. In the present study, majorityof the farms included have smaller herd sizes while majority of the previous studies were conducted on farms with relatively larger herd sizes. O'Reilly and Daborn (1995) have shown that the transmission of BTB from cattle to cattle is largely influenced by herd size, that is, the larger the herd size, the greater the chance of transmission. In Tschopp et al. (2009, 2010) studies, only Zebu breed that managed under extensive traditional cattle management system were included in the study where as in the present study, both cross and zebu breeds cattle managed under semi-intensive and intensive cattle production systems were included in the study. Substantiating the observations of the present study, it was previously documented that the prevalence of BTB is affected by cattle breed and husbandry (Radostitis et al., 2000).

Cattle groups with age greater than eight years were exposed 1.23 times than those with age of three years, to BTB. As reported by Radostits et al. (2000), as age increases, the probability of acquiring TB infection increases. The higher prevalence of BTB observed in female cattle than in male cattle could be due to the small number of male cattle in dairy farms. It may also be due

to the greater productivity stress and longer life span among female animals. In agreement with reports of Elias et al. (2008), body condition was associated with tuberculin reactivity. Similarly, animals kept under poor management system were two times more likely to be tuberculin test positive than those managed under good management system. Similar to the observation of the present study, previous studies also reported higher prevalence in animals with poor body condition as compared to those with good body condition scores (Cook et al., 1996; Kazwala et al., 2001; Asseged et al., 2004; Fikre, 2011). However, it is difficult to decide whether BTB has caused poor body condition or animals in poor conditions were susceptible than those in good body condition.

Abattoir inspection of carcasses revealed a prevalence of 3.52%. This is consistent with the report of Jemale (2005) and Ameni and Wudie (2003). In this study, the lesions were predominantly found in mediastenal lymph node which is consistent with the previous findings of Ameni and Wudie (2003), Asseged et al. (2004), Teklu et al. (2004) and Fikre (2011). A number of studies revealed that the majority of TB lesions are located in the thoracic cavity suggesting the inhalation route being the principal route of BTB transmission (Corner, 1994; Ameni and Wudie, 2003; Phillips et al., 2003; Teklu et al., 2004). In contrast, Cleveland et al. (2007) reported that 61.3% of carcasses in Tanzania had lesions in the gastrointestinal tract.

Out of 39 tuberculous lesions cultured, culture positivity in primary culture media was confirmed only in 5.12% (2/39), which was inconsistent with a report by Fikre (2011), who reported 44.2% culture positivity. Of these tissue samples, only 2.6% (1/39) were positive for genus Mycobacterium by multiplex PCR. This lower isolation rate of mycobacteria may have resulted from reduced sensitivity of culture arising from prolonged storage at field sites, and freeze-thaw cycles that occurred during transportation and contamination of tissue samples and overgrowth of *M. bovis* with environmental mycobacteria (Cleveland et al., 2007). On the other hand, out of 55 milk samples cultured, 9% (5/55) growth was observed. This was different from the findings (Yalelet, 2010; Hussein, 2009) in which no growth of *M. bovis* were observed in milk of tuberculin skin test positive cows. However, of the 55 milk and 40 nasal swab samples cultured, no isolate was confirmed to be a member of the genus Mycobacterium by multiplex PCR. This result is different from those reported by Fikre (2011) and Marlia et al. (2013) who reported 25 and 8% of the isolates were members of the genus Mycobacterium, respectively.

Among farm attendants interviewed, only 6.9% knew about BTB and its transmission from cattle to humans and vice versa. Close physical contact between the owner and cattle and the consumption of raw milk or milk products facilitate the transmission of BTB (Cosivi et al., 1998; WHO, 1993). Among the farm attendants, 79.3% (46/58) had habit of raw milk and raw meat consumption and only 10.3% (6/58) of the respondents consume and feed their families and babies on boiled milk. This finding was relatively consistent with the findings of Fikre (2011) who reported 94.2% of the interviewed households had habit of raw milk and milk products (yoghurt) consumption. This consumption habit might expose the public to risk of acquiring tubercle bacilli from animal (Cosivi et al., 1998). In conclusion, this study demonstrated the importance of BTB and various possible risk factors for the disease and its implication for public health in the Sululta District. The majority of the farm attendants in the area was not aware of the existence of BTB, its mode of transmission and had habit of raw milk and raw meat consumption behavior which is a potential risk factor for the transmission of the disease call for urgent need of intervention.

The authors recom-mended application of general conventional preventive measures such as improving farm management system, public awareness about the disease, its mode of trans-mission, importance of boiling or pasteurization of milk, adequate cooking of meat and strict meat inspection. Furthermore, on large scale, collaborative work is needed to establish clearly the impact of the disease on production and its zoonotic importance so as to design cost effective preventive and control measures at national level.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

## ACKNOWLEDGEMENT

The authors are grateful to the School of Veterinary Medicine, Aklilu Lemma Institute of Pathobiology for their financial and logistic supports.

## REFERENCES

Ameni G, Aseffa A, Sirak A, Engers H, Young DB, Hewinson RG, Vordermeier MH, Gordon SV (2007). Effect of skin testing and segregation on the prevalence of bovine tuberculosis, and molecular typing of Mycobacterium bovis in Ethiopia. Vet. Rec. 161:782-786.

Ameni G, Bonner P, Tibbo M (2003b). Across-sectional study of bovine tuberculosis in selected dairy farms in Ethiopia. App. Res. Vet. Med. 1:85-97.

Ameni G, Wudie A (2003). Preliminary study on bovine tuberculosis in Nazareth municipality abattoir of Central Ethiopia. Bull. Anim. Health Prod. Afr. 51:125-132.

Asseged B, Woldesenbet Z, Yimer E, Lemma E (2004). Evaluation of abattoir inspection for the diagnosis of Mycobacterium bovis infection in cattle at Addis Ababa abattoir. Trop. Anim. Health Prod. 36:537-546.

Ayele WY, Neill SD, Zinsstag J, Weiss MG, Pavlik I (2004). Bovine TB: an old disease but a new threat to Africa. Int. J. Tuberc. Lung Dis. 8:924-937.

Cleveland S, Shaw DJ, Mfinanga SG, Shirima G, Kazwala RR, Eblate E, Sharp M (2007). *Mycobacterium bovis* in rural Tanzania: risk

factors for infection in human and cattle populations. Tuberculosis 87:30-43.
Cook AJ, Tuchili LM, Buve A, Foster SD, Godfrey-Faussett P, Pandey GS, McAdam KP (1996). Human and bovine tuberculosis in the Monze district of Zambia. A cross-sectional study. Br. Vet. Med.152:37-46.
Corner LA (1994). Postmortem diagnosis of M. bovis infection in cattle. Vet. Microbiol. 40:55-63.
Cosivi O, Grange JM, Daborn CJ, Raviglione MC, Fujikura T, Cousins D, Robinson RA, Huchzermeyer HF, De Kantor I, Meslin FX (1998). Zoonotic tuberculosis due to Mycobacterium bovis in developing countries. Emerg. Infect. Dis. 4:1-17.
CSA (2007). Federal Democratic Republic of Ethiopia, Summary and Statistical Report: Population and Housing Census, Population Size by Sex and Age.
Elias K, Hussein D, Asseged B, Wondwossen T, Gebeyehu M (2008). Status of bovine tuberculosis in Addis Ababa dairy farms. Rev. Sci. Tech. 27:915-923.
Fikre Z (2011). Epidemiology and public health implication of bovine tuberculosis in and around Mekelle, northern Ethiopia. M.Sc. Thesis, Addis Ababa University College of Health Science and School of veterinary medicine, Debrezeit, Ethiopia.
Hussein N (2009). Study on Mycobacterium tuberculosis complex infection in livestock and humans in Amibara District of Afar Region. MSc Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre-zeit, Ethiopia.
Jemale M (2005). Evaluation o f Meat Inaspection Procedures for the Diaagnosis of Bovine in Awassa Municipality Abattoir. DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debrezeit, Ethiopia.
Kazwala RR, Kambarage DM, Daborn CJ, Nyange J, Jiwa SF, Sharp JM (2001). Risk factors associated with the occurrence of bovine tuberculosis in cattle in the Southern Highlands of Tanzania. Vet. Res. Com. 25:609-614.
Marlia M, Junqueira F, Antonio C (2013). Occurrence of mycobacteria in bovine milk samples from both individual and collective bulk tanks at farms and informal markets in the southeast region of Sao Paulo, Brazil. Vet. Res.9: 4.
O'Reilly LM, Daborn CJ (1995). The epidemiology of Mycobacterium bovis infections in animals and man: A review. Tuberc. Lung Dis.76:1-46.
OIE (2009). Bovine Tuberculosis. Manual of diagnostic tests and vaccines for Terrestrial animals, Part 2 Section 2.3. Chapter 2.3.3. World Organisation for Animal Health, Paris.
Phillips CJC, Foster CRW, Morris PA, Teverson R (2002). Genetic and management factors that influence the susceptibility of cattle to Mycobacterium bovis infection. Anim. Health Res. Rev. 3:3-13.
Phillips C, Foster C, Teverson R (2003). The transmission of Mycobaterium bovis infection to cattle. Res. Vet. Sci. 74:1-15
Radostits OM, Gay CC, Blood DC, Hinchelift KW (2000). Disease caused by bacteria -Mycobacterium. In: Veterinary Medicine: A Text Book of Disease of Cattle, Sheep, Pig, Goat and Horses. 9th ed. Harcourt Publisher Ltd., London. pp. 909-918.
Roberts GD, Koneman E W, Kim YK (1994). Mycobacterium. In: Balow A. ed. Manual of Clinical Microbiology. Washington DC. pp 304-339.
Shitaye JE, Getahun B, Alemayehu T, Skoric M, Treml F, Fictum P, Verbs V, Pavlik I (2006). A prevalence study of bovine tuberculosis by using abattoir meat inspection and tuberculin skin testing data, histopathological and IS6110 PCR examination of tissues with tuberculous lesions in cattle in Ethiopia. Vet. Med. 51:512-522.
Smith NH, Kremer K, Inwald J, Dale J, Driscoll JR, Gordon SV, van Soolingen D, Hewinson RG, Smith JM (2006). Ecotypes of the *Mycobacterium tuberculosis* complex. J. Theo. Biol. 239:220-225.
Tschopp R, Schelling E, Hattendorf J, Assefa A, Zinsstag J (2009). Risk factors of bovine tuberculosis in cattle in rural livestock production systems of Ethiopia. Prev. Vet. Med. 89:205-211.
Tschopp R, Assefa A, Schelling E, Berg S, Hailu E, Gadissa E, Habtamun M, Argaw K, Zinsstag J (2010). Bovine tuberculosis at wildlife-livestock-human interface in Hamer Woreda, South Omo, Southern Ethiopia. PLoS ONE. 5:12205.
Teklu A, Aseged B, Yimer E, Gebeyehu M , Woldesenbet Z (2004). Tuberculous lesions not detected by routine abattoir inspection: the experience of the Hosanna municipal abattoir, Southern Ethiopia. Review of Science and Technology, Office International des Epizooties. pp. 957-964.
Thoen CO, Philip L, Isabel DK (2006). Importance of Mycobacterium bovis as a zoonosis. Vet. Microbiol. 112:339- 345.
Thoen CO, Barletta RG, Prescott JF, Songer G. (2004). Pathogenesis o f bacterial infections in animals. 3rd ed. Ames, Iowa: Blackwell Publish ing, in press. pp. 46-87.
Thrusfield M (2007). Veterinary Epidemiology 3rd ed. London: Blackwell Science Ltd. PP 227-247.
Tsegaye W, Aseffa M, Mengistu Y, Bewrg S, Ameni G (2010). Conventional and Molecular Epidemiology of Bovine Tuberculoosis in dairy farms in Addis Ababa City, the Capital of Ethiopia. Inte. App. Res. Vet. Med. 8:143-151.
Villarreal-Ramos B, McAulay M, Chance V, Martin M, Morgan J, Howard CJ (2003). Investigation of the role of CD8+ T Cells in Bovine Tuberculosis *in vivo*. Infect. Immuniol. 71:4297-4303.
WHO (2002). Summary on Tuberculosis. http://www.who.int/tb/ publication/ global_report/ en/. Accessed on 23rd June, 2009.
WHO (1993). Report of the WHO meeting on zoonotic tuberculosis (Mycobacterium bovis, Geneva, 15 November 1993) with the participation of the Food and Agriculture Organization (FAO). WHO/CDS/VPH/93.130. WHO/FAO, Geneva.
Wilton S, Cousins D (1992). Detection and identification of multiple mycobacterial pathogens by DNA amplification in a single tube. PCR Meth. Appl. 1:269-273.
Yalelet W (2010). Epidemiology and species characterization of Mycobacterium tuberculosis complex in pastoralist and their livestock in two districts of the Afar region, North Eastern Ethiopia. MSc Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre-Zeit, Ethiopia.