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**STUDY ON MYCOBACTERIUM BOVIS INFECTION IN UMANS
AND ANIMALS IN NORTH WEST SHOA ZONE, OROMIA
NATIONAL REGIONAL STATE**



BY

ALEMAYEHU REGASSA

**A thesis submitted to the school of Graduate Studies of Addis Ababa
University in partial fulfillment of the requirement for the Degree of
Master of Science in Veterinary Epidemiology**

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June, 2005**

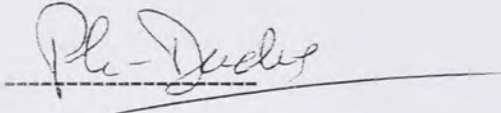
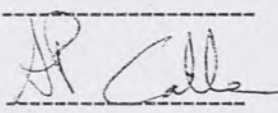
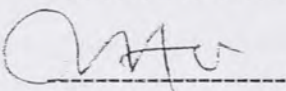
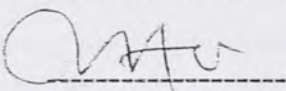
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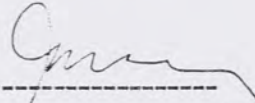
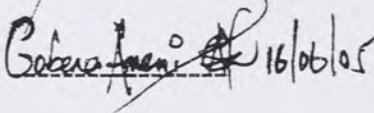
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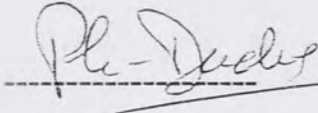
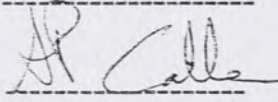
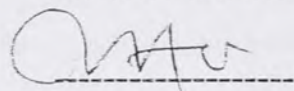
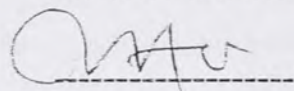
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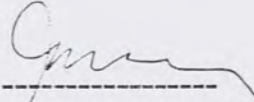
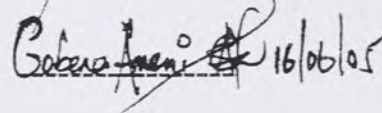
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TABLE OF CONTENTS

LIST OF TABLES	III
FIGURE	IV
LIST OF ABBREVIATIONS	V
ACKNOWLEDGEMENTS	VI
1. INTRODUCTION	1
2. LITERATURE REVIEW	4
2.1. ETIOLOGY	4
2.1.1. Taxonomy	4
2.1.2. Morphology	4
2.1.3. Chemistry and staining	5
2.1.4. Growth requirement and cultural characteristics	5
2.2. EPIDEMIOLOGY	7
2.2.1. Source of infection and mode of transmission	7
2.2.2. Risk factors: Animal Population	8
2.2.2.1. Environment	8
2.2.2.2. Agent	9
2.2.2.3. Host	9
2.2.3. Risk Factors: Human Population	10
2.2.3.1. Close physical contact	10
2.2.3.2. The increase in the demand for milk	10
2.2.3.3. Feeding habit	10
2.2.3.4. HIV Infection	11
2.2.3.4. Absence of control mechanism	11
2.2.4. Distribution	12
2.3. DIAGNOSIS OF TUBERCULOSIS	13
2.3.1. Clinical examination	13
2.3.2. Tuberculin skin test	14
2.3.3. Postmortem examination	15
2.3.4. Bacteriology	15
2.3.4.1. Direct Microscopy	15
2.3.4.2. Culture	16
2.3.4.3. Identification of mycobacterial species	16
2.3.5. Blood-based laboratory tests	17
2.3.5.1. Lymphocyte proliferation assay	17
2.3.5.2. Gamma-interferon assay	17
2.3.5.3. Enzyme-linked Immuno-Sorbent assay	18
2.4. CONTROL OF BOVINE TUBERCULOSIS	18
2.4.1. Treatment and vaccination	18
2.4.2. Test and slaughter	19
2.4.3. Sanitation	19

3. MATERIALS AND METHOD	21
3.1. STUDY AREA	21
3.2. STUDY DESIGN.....	21
3.2.3. STUDY TYPE.....	22
3.3. STUDY METHODOLOGY	23
3.3.1. <i>Human Studies</i>	23
3.3.2. <i>Study Animals</i>	24
3.3.2.1. Comparative interadermal tuberculin test.....	25
3.3.2.2. Milk sample collection.....	27
3.4. DIFFERENTIAL TESTS	28
3.5. DATA ANALYSIS.....	28
4. RESULT	30
4.1. INDIVIDUAL ANIMAL PREVALENCE	30
4.1.1. <i>Risk factors of bovine tuberculosis in cattle</i>	31
4.1.1.1. Breed.....	31
4.1.1.2. Age.....	31
4.1.1.3. Sex	32
4.1.1.4. Body condition.....	32
4.1.2. <i>Multivariate analysis of effect of risk factors on BTB prevalence in cattle</i>	33
4.2. HERD PREVALENCE	35
4.3. TUBERCULOSIS IN CATTLE OWNERS AND ASSOCIATED RISK FACTORS	36
4.4. BACTERIOLOGICAL FINDINGS.....	37
5. DISCUSSION	39
6. CONCLUSION AND RECOMMENDATIONS.....	42
7. REFERENCES.....	44
8. ANNEXES	51



LIST OF TABLES

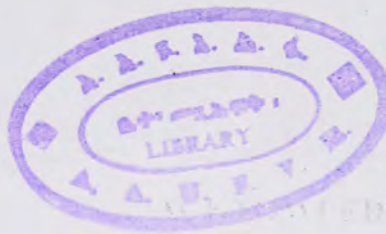
Table 1. Tuberculin reactivity of cattle owned by TB patients and controls -----	30
Table 2. Univariate analysis of different risk factors for bovine tuberculosis -----	33
Table 3. Multivariate analysis of factors associated with tuberculin positive status in Cattle owned by tuberculous and non-tuberculous patients in Fiche (2004/05) -----	34
Table 4. Multivariate analysis of factors associated with tuberculin positive status in cattle owned by tuberculous and non-tuberculous patients in Fiche (2004/05) -----	35
Table 5. Tuberculin reactivity of herds owned by tuberculous non-tuberculous patients -----	36
Table 6 Analysis of risk factors with tuberculosis status in humans treated at Fiche Hospital-----	37
Table 7. Culture results of the specimens on Lowenstein Jenson media -----	38
Table 8. Species of mycobacteria isolated from sputum of human tuberculous patients and intradermal tuberculin test positive animals in Fiche, (2004/05) -----	38

FIGURE

Fig1: Comparative interadermal tuberculin test positive cow-----26

LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
AFB	Acid-fast bacilli
BTB	Bovine Tuberculosis
BCG	Bacillus Calmette Guerin
CIDT	Comparative Intradermal Tuberculin Test
ELISA	Enzyme Linked Immuno-Sorbent Assay
FNA	Fine Needle Aspiration
HIV	Human Immunodeficiency Virus
HPC	Hexadecylpyridinium Chloride
HTB	Human Tuberculosis
IFN- γ	Interferon Gamma
LJ	Lowenstein-Jensen
OIE	Organization for International Epizootics
OR	Odds Ratio
PPD	Purified Protein Derivative
SIDT	Single Intradermal Test
TB	Tuberculosis
TCH	Thiophene-2- Carboxylic acid Hydrozide
WHO	World Health Organization



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ABSTRACT

A case control study was conducted between October 2005 and April 2005 to assess the *Mycobacterium bovis* and other *Mycobacterium tuberculosis* complex infection in cattle and their respective owners in selected districts of North Shoa Zone of Oromia National Regional State, central Ethiopia. A total of 174 cattle owners (87 tuberculosis cases and 87 controls), and 1041 heads of cattle (506 owned by cases while 535 owned by controls) were included in the study. The methods used were comparative interadermal cervical tuberculin test (CIDT), mycobacteriology, acid fast staining, chest x-ray, biochemical tests and drug susceptibility test. The prevalence of tuberculosis was significantly ($\chi^2=48.4$, $P<0.001$) higher in cattle owned by tuberculosis patients (24.3%) than in cattle owned by controls (8.6%), and thus, cattle owned by a tuberculosis patient was about four times more likely to be infected (Odds Ratio, OR=4.2, 95% CI=2.79-6.2) with tuberculosis than cattle owned by a tuberculosis free owner. Further, cattle owners who consumed raw milk were at higher risk ($\chi^2=14.12$, $P<0.001$, OR=3.34) of acquiring tuberculosis than those who consumed boiled milk. The prevalence of tuberculosis in cattle was significantly higher in exotic breeds ($\chi^2=101.3$, $P<0.05$, OR = 7.4), animals older than 4 years ($\chi^2=39.2$, $P<0.001$, OR = 5), and animals in poor physical condition ($\chi^2=15.3$, $P<0.005$, OR = 2.5). Of the 42 human isolates, 31(73.8%) were *Mycobacterium tuberculosis*, 7 (16.28%) were *Mycobacterium bovis* while 4 (9.5%) were atypical mycobacteria as demonstrated by biochemical and drug sensitivity tests. On the other hand, of the 11 cattle isolates, 2 (18.1%) were *M. tuberculosis*, 5 (45.5%) *M. bovis* and 4 (36.4%) were atypical mycobacteria. In conclusion, the results of the present study showed the transmission of mycobacterial species between cattle and their owners.



1. INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by mycobacterium that has been a major health risk to man and animals for more than a century. It is widely distributed throughout the world affecting all age groups of humans and animals. In humans, it is being responsible for more deaths than any other bacterial diseases ever today (Bhatia and Ichhpujani, 1994). Worldwide, 10 million cases of Human Tuberculosis (HTB) are recorded annually and 3 million people die of the disease every year (Bedard *et al.*, 1993). The World Health Organization estimated human TB for years 1990-1999 to be 88 million cases and 30 million deaths, most of which had occurred in developing countries (Cosivi *et al.*, 1998). The situation is worsened by the fact that HIV-AIDS pandemic is associated with mycobacterial infections. HTB of animal origin, particularly *M. bovis* is becoming increasingly important in developing countries. *Mycobacterium bovis* is one of the etiological agents of mammalian TB that antigenically inter-related and are more often grouped as *Mycobacterium tuberculosis* complex. In sub-Saharan Africa, humans and animals share the same microenvironment and waterholes, especially during draught and dry season. According to Cosivi *et al.* (1998), 60% of the African, 47% of the Asian and 38% of the Latin American and Caribbean countries reported the occurrence of BTB from sporadic to enzootic. In such countries, where BTB is still common and pasteurization of milk is not practiced, an estimated 10-15% of HTB is caused by *M. bovis* (Ashford *et al.*, 2001). Approximately 85% of the cattle and 82% of the human population of Africa live in areas where BTB is either partly controlled or not controlled at all (Cosivi *et al.*, 1998). In developed countries, before the control and elimination of BTB and the wide introduction of milk pasteurization, *M. bovis* was responsible for more than 50% of the cases of the cervical lymphadenitis in children (Cosivi *et al.*, 1995) and the proportion of human TB cases due to *M. bovis* was between 5-20% (Grange, 1995). Infection of human due to *M. bovis* is mainly through drinking of raw milk and, hence HTB due to *M. bovis* does occur in extra-pulmonary form particularly in the cervical lymphadenitis form. However, cases of pulmonary TB due to *M. bovis* were also reported particularly in patients from rural areas that live in close contact to cattle (O'Reilly and Daborn, 1995). Transmission of TB due to *M. bovis* or *M. tuberculosis* from HTB patients to cattle is also possible mainly due to contamination of pasture from urogenital TB cases in man (O'Reilly

and Daborn, 1995). Even though man-to-man transmission of *M. bovis* is very rare; there are reports from Paris hospital in which HIV patients with open pulmonary TB due to multidrug resistant *M. bovis* acted as source of infection for other five HIV patients in the hospital (Bouvet *et al.*, 1993).

Ethiopia is a country where the impact of Bovine TB is particularly important and it is one of the 13 countries singled out by WHO (1997) as home for 75% of the world's TB cases. Recent reports indicated that it is one of the top three in Africa, by the record of the number of TB patients (Dye *et al.*, 1999). The current information on the epidemiology of HTB in Ethiopia is based on case notification and as a result it is very difficult to know the incidence of the disease. The prevalence of BTB in Ethiopia is also high ranging from 3.4% in smallholder production system to 50% in peri-urban (intensive) dairy production system (Ameni and Roger, 1998; Kiros, 1998; Ameni *et al.*, 2001; Bogale *et al.*, 2001; Regassa, 2001).

The high prevalence of TB in cattle, close contact of cattle and human in rural areas, habit of the community to drink raw milk and increasing HIV epidemics suggest the significant role of *M. bovis* in HTB. However the role of *M. bovis* in human TB has been undermined because of the following reasons:

- Laboratory confirmation of human TB cases is based mainly on smear microscopy of specimens from patients rather than on culture. Consequently, presence of acid-fast bacilli is considered as confirmation for TB and often assumed to be due to *M. tuberculosis*,
- Failure to set up appropriate cultures for bacterial growth and perform biochemical tests to differentiate *M. bovis* and *M. tuberculosis* often due to lack of facilities and logistics,
- Type of sample collected is mainly sputum where in the majority of cases is *M. tuberculosis*, whereas biopsies are rarely taken from extra pulmonary cases for culture of mycobacterium, which are expected to be mostly due to *M. bovis*. In rare cases when it is done, it is mainly for research purpose.

Therefore, the present study is formulated with the general objective of investigating *M. tuberculosis* complex infection in humans and animals in and around Fiche.

Specific objectives:

1. To isolate mycobacterial species from raw milk of cattle and human TB cases
2. To estimate the association between human TB cases and tuberculin reactor cattle.
3. To generate some base line data that could be useful for the control of bovine tuberculosis in cattle and the prevention of its zoonotic transmission.

2. LITERATURE REVIEW

2.1. Etiology

Both human and animals tuberculosis are caused by the bacterial genus *Mycobacterium*.

2.1.1. Taxonomy

The genus *Mycobacterium* is classified under the order actinomycetales and family Mycobacteriaceae. The genera *Rhodococcus* and *Nocardia* are also grouped under this order. The genus, *Mycobacterium* includes a number of species, some being pathogenic to man and animals, some are opportunistically pathogenic while others are essentially saprophytic living in water and soil (Thoen, 1984). The classic species of *Mycobacteria* that cause disease in man and animals include *M. bovis*, *M. tuberculosis*, *M. avium*, *M. leprae* and *M. lepraemarium* (Bhatia and Ichhpujani, 1994). The etiological agents of mammalian tuberculosis (*Mycobacterium tuberculosis* complex) are *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, *M. canetti* and *M. bovis* sbspp *caprae* (Haddad *et al.*, 2004). *Mycobacterium* species other than the *Mycobacteria tuberculosis* complex that cause TB like diseases in man and animals are commonly known as atypical mycobacteria. Based on the growth rate and formation pigments, these species are classified into four groups as photochromogenic, scotochromogenic, nonchromogenic and rapid growers (Carter and Chengappa, 1991).

2.1.2. Morphology

Mycobacteria are slender, non-capsulated, non-motile and non-spore forming aerobic rods, (Quinn *et al.*, 1999). *M. Tuberculosis* is a thin, straight or slightly curved rod measuring from 2 to 5 μm by 0.3 μm . By contrast, *M. bovis* tends to be somewhat shorter and thicker. However, it impossible to distinguish them from one another based on morphological features alone (Grange, 1995). In the animal's body, Mycobacteria are typically slightly curved rods,

about 2 μm - 4 μm long and 0.2 -0.5 μm wide. The organisms may stain uniformly or present a beaded irregularly stained appearance. In the tissues, the bacilli occur singly, in pairs arranged at an angle or in clusters of organisms usually lying parallel to one another (Pritchard, 1988). The morphology in culture may vary between species; cells of *M. tuberculosis* are often arranged in "serpentine" cords, while those of *M. avium* are coccoid (Grange, 1995).



2.1.3. Chemistry and staining

The chemical structure of the cell wall is complex that contains peptidoglycans, arabinogalactans, mycolic acids and superficially, a variety of lipids including mycosides, cord factor, and the sulpholipids (Thoen and Bloom, 1995). The components of the cell wall, particularly the mycolic acid gives a wax like structure making it strongly hydrophobic. This structure is responsible for the ability of the bacteria to retain basic dyes such as "carbol fuschin" and resist decolourization with strong decolourizing agents such as alcohol and strong acids (Pritchard, 1988). The high lipid content of the mycobacteria is also responsible for a low permeability to water-soluble compounds, which might explain the resistance of tubercle bacilli to chemical disinfection. Furthermore, it plays a major part in determining the type of tissue reaction it evokes in the animal body, in addition, the wax-D component plays a decisive role in inducing delayed type hypersensitivity (Thoen and Himes, 1986).

2.1.4. Growth requirement and cultural characteristics

The *Mycobacteria* have fastidious nutritional growth requirements and will not grow on simple laboratory media (Krebs, 1997). Culture media for isolation can be classified as egg-base (coagulated egg), agar-based (solidified agar), selective media (media containing antimicrobial agents) and liquid media. An egg based culture media such as Lowenstein-Jensen (LJ) media with a composition of coagulated egg, potato meal, bone marrow infusion, citrate,

glycerol, and malachite green is commonly used for mycobacteria cultivation (Herbert and Sommers, 1987). The addition of glycerol suppresses growth of *M. bovis* but stimulates other mycobacteria. Malachite green inhibits growth of bacterial contaminants and provides a green background against which colonies of Mycobacterium are more clearly visible. Stone brick's media is another commonly used culture medium for the isolation of *M. bovis* where the glycerol in LJ medium is replaced by 0.2% pyruvate to enhance growth of *M. bovis* (Grange, 1995).

Mycobacteria grow slowly and its generation time range from 12hr upward (Hirsh and Zee, 2000) and colonies appear after 2-8 weeks at 37°C. On primary culture, *M. bovis* grows more slowly than *M. tuberculosis*, requiring more than 8 weeks. Tubercle bacilli are obligate aerobes (Thoen, 1984), but growth of *M. tuberculosis* and *M. bovis* can be enhanced at 5-10% CO₂ (Vestal, 1981). In contrast to *M. tuberculosis*, *M. bovis* fails to produce niacin or reduce nitrate and is inhibited by thiophene-2-carboxylic acid hydrazide (TCH). Most strains of *M. tuberculosis* are inhibited by para-aminosalicylic acid, isonicotinic acid, hydrazide, streptomycin, pyrazinamide and/or ethambutol (Thoen and Bloom, 1995). *M. bovis* is resistant to pyrazinamide.

Optimal temperature for growth of *Mycobacteria* varies among species, depending on their natural habitat or host that they parasitize. For *M. tuberculosis* and *M. bovis* the optimum temperature is 37°C and for avian strains the optimum temperature is 40°C. For strains affecting cold-blooded creatures such as fish and reptiles, 25°C is the optimal growth temperature (Herbert and Sommers, 1987). On primary culture, *M. tuberculosis* and *M. bovis* show characteristic differences in growth characteristics. On egg media the human type produces a dry wrinkled, warty growth; luxuriant colonies are yellowish roughened surface. The bovine type grows much less luxuriantly and cohesive than that of human strain. This type of growth is referred to as dysgonic in contrast to the more profuse growth of *M. tuberculosis*, which is described as eugonic (Krebs, 1997). *M. avium* is moist slimy, glistening, luxuriant, frequently, yellow to gray (Grange, 1995).

2.2. Epidemiology

2.2.1. Source of infection and mode of transmission

The main reservoir of *M. bovis* is cattle, which can transmit the infection to many mammalian species including man (Acha and Szytres, 2001). Organisms leave the host in respiratory discharges, faeces, milk, urine, semen, and genital discharges. These body excretions may contaminate grazing pasture, drinking water, feed, water and feed troughs or fomites, which may act as sources of infection to other animals. The common routes of infection by which, tubercle bacilli gain entrance into the host are respiratory and alimentary (Russell, 2003). Rarely animals can also be infected through the cutaneous, congenital and genital routes. Tuberculosis is transmitted among cattle mainly via aerosols (Radostits *et al.*, 1994). The transmission of TB between cattle via the respiratory route is ideally facilitated by natural cattle behavior especially in communities with high stocking densities and substantial cattle movement through markets and between farms (Neill *et al.*, 1994). The alimentary route of infection is common in young calves ingesting infected milk from tuberculous udder. Animals can also be infected through uterine pipette, teat siphons or by means of contaminated milking machine (Pritchard, 1988).

The widespread distribution of *M. bovis* in farm and wild animal population represents a large number of reservoir hosts to the microorganism (Cosivi *et al.*, 1998). The spread of the infection from affected wild animals to susceptible domestic animals occurs when wild animals such as badgers and possums share pasture or territory with domesticated animals. Neill *et al.*, (1994) recognized that wild animals represent a permanent reservoir of infection and pose a serious threat to control and elimination programs

Transmission of *M. bovis* infection from humans to cattle is usually direct and by the respiratory route, but indirect spread via bedding contaminated with urine from renal excretions of human TB patients has been reported (Cosivi *et al.*, 1998). Infection can also occur when animals inhale eructated rumenal aerosols following ingestion of contaminated grass or hay

(O' Reilly and Daborn, 1995). Occasionally iatrogenic transmission occurs by the use of surgical instruments such as teat siphons, urinary catheters and hypodermic needles (Neill *et al.*, 1994).

Man contracts the infection primarily by ingesting the agent in raw milk and milk products, and secondly by inhaling it. Humans most commonly acquire TB infection by inhaling aerosolized bacteria as droplet nuclei each containing 1-3 bacteria. The infectious dose is very low; 1-3 viable bacteria are considered sufficient as infectious inoculums (Anderson, 1997).

2.2.2. Risk factors: Animal Population

The probability of infection with *M. bovis* is influenced by factors, which are linked to environment, host and the pathogen itself.

2.2.2.1. Environment

The risk of *M. bovis* infection is higher in housed animals than animals on pasture. This may show that dairy farms and intensive fattening units suffer more from the effects of BTB than farms with extensive production system (Barwinek and Taylor, 1996). Although the prevalence of the disease within a country varies from area to area, the highest incidence of bovine TB is generally observed where intensive dairy production is most common, notably in the milk sheds of larger cities (Acha and Szytres, 2001). This problem is exacerbated where there is inadequate veterinary supervision, as is the case in most developing countries. However, even under extensive pastoral conditions, husbandry factors such as the congregation of livestock from different sources at watering points or the gathering together of animals in enclosure overnight may lead to increased transmission of Bovine TB leading to high prevalence (O'Reilly and Daborn, 1995). Calves, which are housed with cows, are exposed to a constant risk of infection by the aerogenous route, but when calves are not housed with cows about 90% of them reach maturity without being infected (Morris *et al.*, 1994).

Malnutrition and other stress factors are predisposing factors to the infection. Some of the variations in prevalence between herds and regions are due to the management and environmental factors, which offer opportunity for the transmission of infection and development of the disease (Pritchard, 1988).

2.2.2.2. Agent

Mycobacteria are moderately resistant to heat, desiccation, and to many disinfectants (Hirsh and Zee, 2000). *M. bovis* is an obligate pathogen but it can survive for substantial periods in the environment under favorable conditions. Drying is only effective if they are exposed to direct sunlight but they may survive for several weeks, even months in a dark and moist environment (Radostits *et al.*, 1994). Persistence of the organisms in the carcass depends on the speed of decomposition and degree of environmental protection given to the carcass and this may act as source of infection for scavengers and cattle grazing around the site of decomposition (Morris *et al.*, 1994).

2.2.2.3. Host

It has been suggested that zebu type cattle are more resistant to the effect of TB than exotic breed under the same husbandry conditions (Hirsh and Zee, 2000). Genetically improved cattle are more susceptible to BTB than local races because they may suffer more severely from deficient housing and malnutrition that can expose the animals to the disease. The effect of the disease on local animals is much less severe but in intensive feedlot conditions, a morbidity rate of 60% and a depression of weight gain can be experienced in tuberculosis zebu cattle (Cosivi *et al.*, 1998).

Although cattle are considered as the main hosts of *M. bovis*, the disease has been reported in several other species of both domestic and wild animals (O'Reilly and Dabron, 1995)

2.2.3. Risk Factors: Human Population

2.2.3.1. Close physical contact

Close physical contact between humans and potentially infected animals is present in some communities, especially in developing regions. For example, in many African countries cattle are an integral part of human social life; they represent wealth and are at the center of many events and, therefore, gatherings. In addition, with 65% of African, 70% of Asian, and 26% of Latin American and Caribbean populations working in agriculture, a significant proportion of the population of these regions may be at risk for bovine TB (Cosivi *et al.*, 1998).

2.2.3.2. The increase in the demand for milk

The demand for milk was increasing at an estimated rate of 2.5% per year over the period of 1970-1988 in sub-Saharan Africa (Walshe *et al.*, 1991). This rise demand for milk consumption will be met by increasing number of productive animals and intensifying animal production. Accordingly in all countries of sub-Saharan Africa, there is active competition between large-scale, often state-run, processing and marketing enterprises and the informal sector. The informal sector can ignore standards of hygiene and quality, and producers often sell directly to the final consumers. Cosivi *et al.* (1998) observed that the occurrence of BTB have close links with intensive management. Thus, intensification of animal production can play a key role in the maintenance and spread of *M. bovis*.

2.2.3.3. Feeding habit

Consumption of raw or soured milk is mainly practiced in some part of the world. Approximately 90% of the total volume of milk produced in sub-Saharan Africa is consumed fresh or soured, and only a very small proportion follows official marketing channels (Walshe *et al.*, 1991). It is known that consumption of milk contaminated by *M. bovis* is regarded as the

principal mode of TB transmission from animals to humans (Acha and Szytres, 2001). In regions where BTB is common and uncontrolled, milk borne infection is the principal cause of cervical lymphadenopathy (Scrofula) and abdominal and other forms of extra-pulmonary TB (Cosivi *et al.*, 1998). *M. bovis* was isolated from seven (2.9%) of 241 samples of raw milk in Ethiopia WHO (1997). Both *M. bovis* and *M. tuberculosis* have also been found in milk samples in Nigeria (Idrush and Schnurrenberger, 1977) and Egypt (Nafeh *et al.*, 1992). Thus, serious public health implications of potentially contaminated milk and milk products should not be underestimated.

2.2.3.4. HIV Infection

In many developing countries, TB is the most frequent opportunistic disease associated with HIV infection (Cosivi *et al.*, 1998). HIV sero-prevalence rates greater than 60% have been found in TB patients in various African countries (Raviglione *et al.*, 1995). Persons infected with both pathogens have annual risk of progression to active TB of 5% to 15%, depending on their level of immuno-suppression; approximately 10% of non-HIV infected persons newly infected with TB become ill at some time during their lives. In the remaining 90%, effective host defenses prevent progression from infection to disease (Cosivi *et al.*, 1998).

The HIV induced immune suppression could lower the host's defense mechanism and the occurrence of HIV-TB co-infection in one person makes TB infection very likely to progress to active disease. Grange *et al.* (1994) indicated that HIV-*M. bovis* co-infection appears to be more serious in areas where a cattle rearing is the livelihood of the community. TB cases due to *M. bovis* in HIV-positive persons also resemble disease caused by *M. tuberculosis*. Thus, they manifest as pulmonary disease, lymphadenopathy, or, in the more profoundly immunosuppressed, disseminated disease (Bouvet *et al.*, 1993).

2.2.3.4. Absence of control mechanism

BTB can be controlled/eliminated from a country or region by implementing the test and slaughter policy. However, because of financial constraints, scarcity of trained man power, lack of political will, as well as the under estimation of the importance of BTB by national governments and donor agencies, control measures are not applied or are applied inadequately in most developing countries (Cosivi *et al.*, 1998).

2.2.4. Distribution

The global distribution of *M. bovis* infection in animals and humans varies widely. The animal population of the world exceeds 3 billion, excluding poultry; more than 1 billion are cattle, of which possibly one-third live in countries where BTB is under control. Another third are in areas where the disease is widespread and the remaining third are in regions where the prevalence of BTB is high (Steele, 1996; Cosivi *et al.*, 1998)

In Africa, of the 55 nations, 25 reported sporadic/low occurrence of Bovine TB, 6 reported enzootic disease, 2 (Malawi and Mali) were described as having a high occurrence, 4 did not report the disease and the remaining 18 countries did not have data. Of all nations in Africa, only 7 apply disease control measures as part of a test and slaughter policy and consider BTB of notifiable disease; the remaining 48 control the disease inadequately or not at all. Almost 15% of the cattle population is found in countries where Bovine TB is not notifiable and test and slaughter policy is used. Thus, approximately 85% of the cattle and 82% of the human population of Africa are in areas where BTB is either partly controlled or not controlled at all (OIE, 1996; WHO, 1997).

In Ethiopia, most of the surveys carried out on Bovine TB have been based on abattoir reports and tuberculin testing of animals in a particular locality. Abattoir data has indicated that the condemnation rate of cattle meat from BTB ranged between 1 and 1.5% out of the 6940 animals slaughtered within a period of 17 months in Dire Dawa in 1971 (Yehualshet, 1993). A summary of the report by the same author on the number of BTB in different parts of the

country indicate a prevalence of 0.05% to 1.83% out of the 210,000 animals slaughtered in 1975 based on lung condemnation.

2.3. Diagnosis of tuberculosis



Mycobacterial diseases have always been difficult to study and data on bovine tuberculosis is always underestimated due to lack of diagnostic facilities and epidemiological investigations (Cosivi *et al.*, 1998). A presumptive diagnosis of TB in cattle and other susceptible species is often made based on history, clinical findings, and tuberculin skin test and necropsy findings (Radostits *et al.*, 1994). In addition to these, modern diagnostic techniques such as the *in vitro* lymphocyte proliferation assay, interferon gamma assay (OIE, 2000) and enzyme inked immuno-sorbent assays (ELISA) have been developed for the detection of Mycobacterial infection (Barwinek and Taylor, 1996).

Similarly in case of routine work the diagnosis of tuberculosis in humans is usually based on history, clinical findings (Symptoms, Chest x-ray, Ultrasound, Erythrocyte sedimentation rate, etc.), direct microscopy of sputum, and histopathological examination of affected lymph nodes. Microscopy of direct smears for acid-fast bacilli (AFB) is the most commonly used method for diagnosis of TB but its major disadvantage is discouragingly low sensitivity. Based on the study undergone in several African laboratories, Gebre *et al.* (1995) indicated that the sensitivity of direct microscopy ranged from 8.8% to 46.4%.

2.3.1. Clinical examination

Because of chronic nature of the disease and the multiplicity of signs caused by the variable localization of the infection, tuberculosis is difficult to diagnose based on clinical examination. The main clinical findings observed in animals in advanced cases are a capricious appetite and fluctuating temperatures, pulmonary blow characterized by a chronic cough, which is stimulated by squeezing the pharynx or by exercise and is common in the morning or during cold weather. Affected animals tend to become more docile and sluggish (Radostits *et al.*, 1994).

Uterine TB is characterized by drops of yellow fluids, which may have high influence on reproductive performance of the cow. Tuberculous mastitis can be recognized as a granulomatous inflammation of the mammary gland with hard swelling on the bottom of the udder. This is of major importance from the public health point of view and as means of transmission to the calf (Prichard, 1988).

2.3.2. Tuberculin skin test

Since Robert Koch used it for the first time in 1891 in his effort to develop a treatment to TB, tuberculin tests have successfully been used worldwide for the diagnosis of tuberculosis in cattle (O'Reilly and Daborn, 1995). Over the years, different reagents and techniques have been used, ranging from subcutaneous injection of Koch's Old Tuberculin prepared from *M. tuberculosis*, with assessment by determining the animal's temperature on repeated occasions, to measuring the degree of tissue reaction in millimeter at the injection site. The most important types of tuberculin used for this purpose is Purified Protein Derivative (PPD) whose major antigenic component is probably a heat shock protein (Tizard, 1996). When PPD is injected intradermally into normal animal, there is no significant local response but when they are inoculated into exposed animals, there is an allergic skin reaction, which is typically the delayed type hypersensitivity reaction (Monaghan *et al.*, 1994).

There are two types of tuberculin skin tests, the single intradermal skin test (SIDT) and comparative intradermal test (CIDT). The choice between the two tests generally depends on the actual prevalence of tuberculosis and on the environmental level of infection with other sensitising organisms (Thoen and Himes, 1986).

2.3.3. Postmortem examination

On gross necropsy examination tuberculosis may be provisionally diagnosed when caseous or calcified foci are observed in various tissues of the body, but this is difficult in the initial stages of the disease (Jones *et al.*, 1997)

Early lesions consist of small caseous and calcified granulomatous nodules mainly in the retropharyngeal, bronchio-mediastinal and occasionally the mesenteric lymph nodes. In advanced and generalized type of tuberculosis which is known as miliary TB, lesions are the size of millet seeds distributed all over the visceral organs of the body mainly in the lungs, liver, spleen, mediastinal and retropharyngeal lymph nodes, mammary gland, and intestine (WHO, 1997).

Post-mortem examinations should be supported by a histological examination of samples stained with haematoxylin and eosin (OIE, 2000). Typically, lesions caused by *M. bovis* in cattle are described as having a center of caseous necrosis with some calcification and a boundary of lymphocytes, neutrophils and epitheloid cells. Some of the epitheloid cells may fuse together and form multinucleated giant cells. An outer border of fibrous connective tissue is usually present, giving the lesion a focal appearance and providing encapsulation to some extent, which may limit the spread of infection. Since the lesions are not conclusive, it is necessary to demonstrate the etiological agent using Ziehl-Neelsen stain (Thoen and Bloom, 1995).

2.3.4. Bacteriology

2.3.4.1. Direct Microscopy

Examination of smears prepared from sputum, tissue, milk, urine and other samples taken from suspected individuals are stained with the Zeihl Neelsen staining method and organisms appear

as red due to their acid-fast property (Quinn *et al.*, 1999). However, direct microscopic examinations of nasal discharge and tissue sections from animals have limited diagnostic value.

2.3.4.2. Culture

The definitive diagnosis of TB depends on the isolation and identification of the mycobacteria in specimens taken from suspected individuals. The main factors that influence the success of primary isolation of *M. bovis* from clinical specimens are the culture media, the decontamination procedure and incubation condition (Corner, 1994). Before inoculation, the samples are digested, decontaminated and concentrated. The commonly used decontaminants for *M. bovis* preparation are hexadecylpyridinium chloride (HPC) at both 0.075% W/V and 0.75% W/V, benzalkonium chloride (Zephiran) 0.25% W/V, oxalic acid 5% W/V, NaOH 2% and 100% sodium hypochlorate (OIE, 2000; Corner, 1994).

Corner (1994) indicated that growth on the agar base media is much faster than on the egg based media with mean time to the first appearance of colonies being 27 days and 28 days on B83 and 7H11, respectively compared to 36 days on Stonebrink's medium. It has been demonstrated that using modified 7H11 agar medium, *M. bovis* from infected tissues can be isolated within 3 weeks. However, the agar medium is highly liable to contamination even after decontamination of the specimen, so egg base media is preferable for mycobacterial growth (Vestal, 1981).

2.3.4.3. Identification of mycobacterial species

Identification of mycobacterial isolates depends upon colony morphology, staining characteristics as well as biochemical tests such as niacin production, nitrate reduction, urease test and pyrazinamidase activity (deamination of pyrazinamide to pyrazinoic acid in 4-7 days) and drug sensitivity tests, etc (Vestal, 1981). *M. bovis* grows slowly and subculture requires a minimum of 14 days for colonies to become visible on media (Corner, 1994). The most useful biochemical tests for a *M. bovis* are susceptibility to Thiophene-2- carboxylic acid hydrozide

(TCH) and to isonized. These biochemical tests take up to 3 to 4 weeks to complete (OIE, 2000).

2.3.5. Blood-based laboratory tests

2.3.5.1. Lymphocyte proliferation assay

This *in vitro* assay detects cellular reactivity to tuberculin antigen in whole blood samples. The assay has scientific value, but is not used for routine diagnosis because the test is time consuming and the logistics and laboratory execution is complicated. It requires long incubation times and the use of radioactive nucleotides (OIE, 2000; WHO, 1997). The method involves incubating lymphocytes in the whole blood diluted with tissue culture media for 3-5 days, and then using radioactive nucleosides to detect the level of cell proliferation (Wood and Rothel, 1994).

2.3.5.2. Gamma-interferon assay

In this test, the release of a lymphokine in a whole blood culture system is measured. The assay is based on the release of gamma-interferon from sensitized lymphocytes during a 16-24 hrs incubation period with specific Ag (PPD tuberculin). A sandwich ELISA which utilizes two monoclonal antibodies to bovine gamma-interferon is used for the detection of the IFN- γ released by sensitized T-lymphocytes (Russell, 2003). The sensitivity and specificity varies from 76.8% to 93.6% depending on interpretation method (Wood and Rothel, 1994).

The advantage of this assay over tuberculin test is that animals need only be captured once, but serious disadvantages include the relatively high costs and the fact that the examination of blood samples has to be started within 8 hrs after collection. Moreover, this test only works

with the family bovidae and hence should only be used on cattle, buffalo, etc (Barwnek and Taylor, 1996).

2.3.5.3. Enzyme-linked Immuno-Sorbent assay

There have been numerous attempts to develop clinically useful sero-diagnostic tests for TB. The ELISA appears to be the best choice and can be a complement, rather than an alternative, to tests based on cellular immunity (Andersen, 1997). Improvement may be possible by using different antigens, including proteins (e.g. MPB 70, which is very specific) and (peptido) glycolipids. Moreover, in *M. bovis*-infected animals, an anamnestic rise has been described, resulting in better ELISA results 2-8 weeks after a routine tuberculin skin test. Zerihun (1991) used ELISA to differentiate *M. bovis* from other mycobacteria and related microorganisms. The indirect ELISA detects antibodies to *M. bovis* in the serum. Its specificity is relatively high but its sensitivity can be very low. It is useful for detecting infectious cases in the short term because it yields good results in advanced cases of pulmonary and disseminated TB (OIE, 2000).

2.4. Control of bovine tuberculosis

2.4.1. Treatment and vaccination

The treatment of TB with drugs for animals has been of limited success and is forbidden in most countries. This is particularly because of the high cost of treatment, the frequent recurrence of the disease when the treatment is stopped and the possibility of the development of multi drug strains of *M. bovis* (WHO, 1997).

Vaccination of animals against TB would be important in reducing the prevalence of the disease to an acceptable level before the test and slaughter policy in domesticated animals is applied (OIE, 1996). However, since the results obtained globally with BCG have been

suboptimal and efficacy varied considerably from region to region a highly effective vaccine needs to be developed. There are recent trials on the use of the vaccine in calves (Anderson, 2001). Vaccination with BCG may interfere with the diagnostic test and would invalidate the key diagnostic tool used in control programs. Vaccination of human with strain BCG has been practiced in humans but its efficacy in protecting the subject from infection is lower (OIE, 1996).

2.4.2. Test and slaughter

Bovine TB can be eliminated from a country or a region by implementing the test and slaughter policy, if other reservoir hosts of infection do not exist. This policy is effective only in areas with relatively low Bovine TB prevalence and effective control of animal movement. In developing countries, however, this method is not socially and economically accepted; so alternative strategies have to be used. These are, slaughterhouse surveillance and trace back of tuberculous animals to herds of origin (Barwinek and Taylor, 1996) and varying forms of test and segregation with the test and slaughter program applied only in the final stages of eradication (WHO, 1997).

2.4.3. Sanitation

Once reactor animals to BTB test are identified, hygienic measures to prevent the spread of infection should be instituted. Feed troughs should be cleared and thoroughly disinfected with hot 5% phenol or equivalent cresol disinfectants. Suspicious reactors being held for retesting should be isolated from the remainder of the herd (Barwinek and Taylor, 1996). The usual source of *M. bovis* in milk is udder infection; it can also occur by contamination with faeces, uterine discharge or by air or dust borne bacilli. Such contamination can be much reduced by better dairy design and practices. Effective meat inspection and proper disposal of tuberculous lesions are also paramount important (Pritchard, 1988).

Careful management may raise disease-free stock from an infected herd. Each calf should be taken away from its dam at birth; on no account should the dam be allowed to lick or suckle the calf. The calf should then be placed in a thoroughly disinfected calf pen and be given its dam's colostrum preferably pasteurized, in a clean bucket. Farm attendants should be checked as they may produce a source of *M. tuberculosis* and of *M. bovis*, and cause transient positive reactions in cattle (WHO, 1997).

3. MATERIALS AND METHOD

3.1. Study area

The study was conducted in Fiche Hospital and the surrounding two districts of North west Shoa (Selalle) Zone of Oromia region named as Girar Jarso and Yaya gulale districts which found at about 115km north of Addis Ababa in the central highlands and they are located at $9^{\circ}48'N$ and $38^{\circ}44'E$ with an average altitude of 2800masl. The North west Shoa (Selalle) Zone has 12 districts but these two districts were selected as the study areas based on previous records showing higher number of TB patients treated in the Hospital. Accessibility was also considered so that animals could be traced back for tuberculin testing and sampling. The laboratory work was conducted at laboratory of Fiche Hospital (direct AFB) and the Armauer Hansen Research Institute (AHRI) in Addis Ababa.

3.2. Study Design

3.2.1. Study population

The study was conducted on human TB patients (cases) and patients that visited Fiche Hospital for other health problems not related to tuberculosis (control group). Cattle owned by both groups were also investigated for reaction to the interadermal tuberculin test.

Human TB patients (cases): -A case was defined as patients visited Fiche Hospital TB clinic showing either pulmonary or extrapulmonary signs that a physician suspected as being due to tuberculosis. Further tests including direct staining of sputum, lymphnode aspiration, chest x-ray, ultrasound and other indicative diagnostic techniques were undergone.

Control group: - In this study, control groups were selected based on the suggestion given by Martin, *et al.* (1994) who indicated the possibility of selection of controls from all noncase patients that visited the same clinics. So, the controls in this study were selected from those

individuals that visited Fiche Hospital for all cases other than tuberculosis. That is, control groups were patients visited Fiche Hospital for other diseases that clinically were excluded as not being TB patients; further tests such as sputum acid fast staining, chest x-ray, erythrocyte sedimentation rate, ultrasound and other tests were done and were not indicative of tuberculosis



3.2.2. Sample size

The average number of human TB patients that visited the Fiche Hospital was taken as the basis for sample size determination. The total number TB patients visited the Hospital during the last three years originating from the specific study area were 1044. Accordingly, the average annual number of human TB patients that visited the Hospital from the area was 348. However, during the study period of six months, 200 tuberculous patients visited the hospital. Of these patients, only 87 of them who had cattle holdings participated voluntarily and were considered as cases. An equal number of human patients visiting the hospital due to health problems that were not associated with tuberculosis that had cattle and accepted to participate in the study were included as controls. On average, an individual owned 6 cattle and thus a total of 1041 animals from human TB patients and controls were tested.

3.2.3. Study type

The study type was a case-control study. That is, human study subjects that screened as TB patient (cases) at the Hospital were registered and followed back to their home to trace back their animals for tuberculin testing and sampling. Similarly, the controls were also screened and their animals were traced back for tuberculin testing and sampling.

3.3. Study methodology

3.3.1. Human Studies

Tuberculosis clinic at Fiche hospital, which was visited by a number of human TB cases, was selected and used for this study. The patients were approached through their health personnel for their willingness to participate in this study. A total of 87 human TB patients (cases) were volunteer to participate in the study and accordingly, samples were collected as part of the routine diagnostic procedure from these cases. They were interviewed using a semi-structured questionnaire (annex 6) about the degree of their association with cattle, habit of consumption of meat and milk and other relevant information related to tuberculosis in their household which indicated in the questionnaire.

Specimen (Sputum and lymph node aspiration) was collected before the antimicrobial therapy commence using sterile, leak proof, disposable plastic materials labeled with the patients code number, type of specimen and date of collection. In case of TB lymphadenitis, fine needle aspiration (FNA) procedure was used for collecting samples for isolation and identification of mycobacterial species. The samples were kept in the cold chain until being transported to the lab in a cool box. A total of 111 specimens (87 sputum, 21 FNA and 3 ascitic fluid) from 87 human TB patients (63 pulmonary and 24 extra pulmonary tuberculosis) were collected and processed in the laboratory.

Eighty-seven sputum samples were collected from both pulmonary and extrapulmonary TB patients. The samples were decontaminated by adding 2% NaOH (1:3 ratio), agitated in a vortex mixer for 15 minutes at room temperature and centrifuged at 3,500 rpm for 15 minutes at 4°C. The supernatant was taken off into another container for proper disposal and the sediment was suspended in 2ml of sterile physiological saline solution (PBS). One to two drops of 0.05% phenol red indicator was added to indicate the PH change and then neutralized using concentrated hydrochloric acid until the colour changed to yellow. The sediment was inoculated into two slants of Lowenstein-Jensen media one with pyruvate and the other with

glycerol. Twenty-one FNA and 3 ascitic fluid samples were collected from 21 TB lymphadenitis and 3 TB peritonitis cases, respectively and processed as in sputum samples and inoculated to the same media (OIE, 2000).

Ethical consideration

Ethical issues were considered both in human and animal studies. The purpose of the study was explained to the subjects to be involved in the study and/or their guardians. Consent was obtained from the owners of animals, and patients and/or their guardians. Regarding human subjects, medical personnel collected specimens as part of the routine diagnostic procedure after consents were obtained. Participation was on voluntary basis and there was no inducement to encourage participation. Patients benefited from the free diagnostic support for their disease. Laboratory results were reported to their physician or health care provider. Owners were informed when their animals were found TB positive either using interadermal tuberculin test or culture and advised to take some measures of disease prevention (such as isolation of positive animals, boiling of milk, etc.).

3.3.2. Study Animals

All cattle holdings by human TB patients and controls were included in the study. They were thoroughly observed for their body condition. Local animals were scored according to the guidelines established by Nicholson and Butterwoeth (1986); nine scores were used in which the three main scores (Fat, Medium and Lean) were divided into three categories each having F^- , F , F^+ , M^- , M , M^+ , L^- , L , L^+ . Each score was given a number from 1 (L^-) up to 9 (F^+), the former representing the most emaciated animal and the latter well-fattened one. Scoring was done by looking at the structure of the tail, head, transverse process of the lumbar vertebrae, the ribs, the hump, the hips, the brisket, etc. Scoring of exotic breeds was done according to the guidelines forwarded by Richard (1993); all animals were graded as P, M, G. (poor, medium and good) based on the above mentioned anatomical structures. Each score was given a number from 0 (P_1) to 5 (G_2), i.e. the three main scores divided into two sub-categories as P_1 ,

P₂ (0, 1), M₁, M₂ (2, 3) and G₁, G₂ (4, 5) (annex 5). In addition to the body condition score other relevant data such as age, sex, breed, were also collected for each animal before tuberculin injection and sampling.

3.3.2.1. Comparative interadermal tuberculin test

This was conducted on cattle having contact with human TB cases and control group. A total of 1041 cattle (506 cattle of TB case and 535 cattle of control group) above six months of age were tested by comparative interadermal tuberculin test. Sixty milk samples were collected from comparative interadermal tuberculin positive cows for bacterial isolation and identification as mentioned below (OIE, 2000).

Test procedure: - Two sites on the skin of the mid-neck of the study animals, 12 cm apart, were shaved and skin thickness was measured in millimeters with digital calipers before the injection of tuberculins. Aliquots of 0.1 mL of 20,000 IU/ml bovine purified protein (PPD) (Veterinary Laboratories Agency, UK), and 0.1 ml of 25,000 IU/ml avian PPD (Veterinary Laboratories Agency, UK) were injected into the dermis at these sites. A correct injection was confirmed by palpating a small pea-like swelling at each site of injection. After 72 h, the thickness of the skin at the injection sites was again measured. The result was interpreted as follows:

Interpretation: - The difference in the skin thickness before and after injection at both sites was used for the interpretation of results. When differences in skin thickness were greater at the site of injection for avian PPD than bovine PPD, the animal was considered as positive for mycobacterial species other than *M. bovis*; but when the change in the skin thickness was increased at both injection sites, differences in thickness of the two sites was considered and results were interpreted according to the standards set by the manufacture of the products. In the interpretation of the interadermal comparative test, the following guideline was employed.

- **Positive** if the bovine reaction was positive and more than 4 mm greater than the avian reaction, i.e. $\Delta B - \Delta A > 4\text{mm}$.

- **Inconclusive (doubtful)** if the bovine reaction was positive and from 2 to 4 mm greater than the avian reaction, i.e. $\Delta B - \Delta A$ is between 2 and 4mm both values inclusive.
- **Negative** if the bovine reaction was negative or if the bovine reaction is positive, but equal to or less than a positive avian reaction, i.e. $\Delta B - \Delta A < 2\text{mm}$.

Key $A_2 - A_1 = \Delta A$ and $B_2 - B_1 = \Delta B$

A_1 = Skin thickness before injection of avian PPD

A_2 = Skin thickness after 72 hours at injection site of avian PPD

ΔA = Difference in skin thickness before and after injection of avian PPD

B_1 = Skin thickness before injection of bovine PPD

B_2 = Skin thickness after 72 hours at injection site of bovine PPD

ΔB = Difference in skin thickness before and after injection of bovine PPD

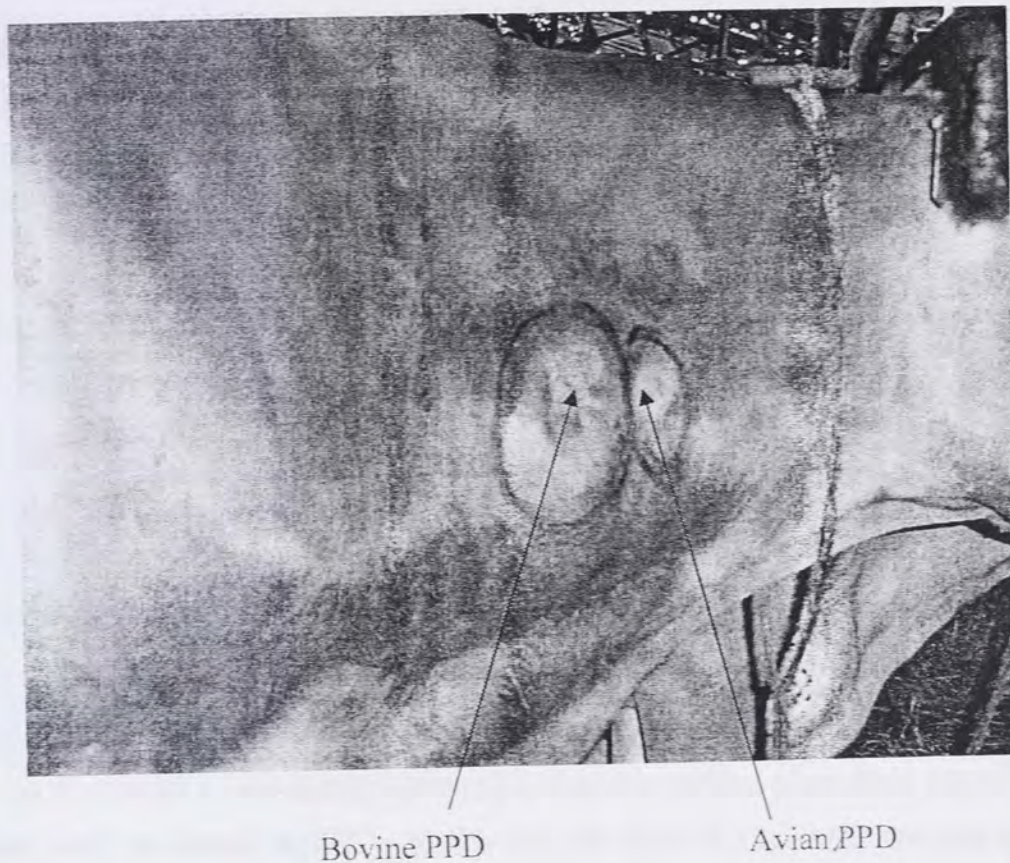


Fig 1. Comparative interadermal tuberculin test positive cow

3.3.2.2. Milk sample collection

A total of 60 milk samples were collected from 41 tuberculin positive milking cows of case owners and from 19 reactors cows of control owners and processed for culture. About 30 ml of the last few streams was collected into sterile universal bottles by the owners themselves towards the end of milking from each quarter of tuberculin positive dairy animals in the herds having contact with human TB cases and control group and then placed in cool boxes and stored at 4°C.

Sample processing and isolation of Mycobacteria: - The procedure indicated by Kazwala *et al.*, (1998) was followed for culturing milk. Milk samples were centrifuged at 3000 rpm for 15 minutes and the supernatant was discarded. The sediments were suspended in 2ml of sterile physiological saline solution and decontaminated with equal volume of sterilized 4 % sodium hydroxide solutions. One to two drop of 0.05% Phenol red indicator was added and then neutralized using concentrated hydrochloric acid. The suspension was centrifuged at 3000 rpm for 15 minutes at 4°C and the sediment was used for microscopic and cultural examination.

Microscopic examination: Eighty-seven sputum samples were subjected to direct microscopic examination before processing for culture and stained with Ziehl-Neelsen stain (annex 1). The stained smears were examined under oil-immersion lens.

Cultural examination: All specimens collected from human TB patients (111 samples of sputum, FNA and ascitic fluid) and from tuberculin positive cattle (60 milk samples) were processed and prepared for mycobacterial culture as shown above. Primary isolation was done onto two LJ media favoring growth of *M. bovis*, with the addition of 1% sodium Pyruvate. Thick inoculums of sediments were smeared on the surface of medium slopes and the cultured tubes were incubated at 37°C and 5% CO₂ for about 8-12 weeks. Growth for Mycobacteria was checked every week. Positive cultures were sub cultured onto another set of media and incubated for another 3 to 4 weeks for further identification.

3.4. Differential Tests

Generally identification of mycobacterial isolates was based on colony morphology, biochemical (Nitrate reduction test) and drug sensitivity tests (pyrazinamidase and thiophene-2- carboxylic acid hydrazide tests). These tests were applied according to procedures to differentiate *M. bovis* and other species of *Mycobacterium*. The test procedures are given in appendix (annexes 2, 3 and 4).



3.5. Data analysis

In the analysis, descriptive and analytical statistics were used. That is, percentages (prevalence) were applied to summarize the distribution of tuberculin reactivity. Individual animal level prevalence was defined as the number of positive reactors per 1041 (506 cases and 535 controls) animals tested. Herd level prevalence was computed as the number of herds with at least one-reactor cattle divided by the total number of herd tested (174 herds, i.e., 87 cases and 87 controls); that is, the herds were considered as positive if at least one animal among them was found tuberculin positive. In this study, herd is the number of cattle owned by the human study subjects and the number of cattle in the herds varies from two up to 20 animals.

Logistic regression was used for univariate and multivariate analysis using STATA statistical software, Stata Corporation, 702 University Drive East College Station, Texas 77840 USA. The variations between different factors were analyzed using Chi-square (χ^2) test. Odds ratio (OR) was calculated to assess strength of association of different factors to the occurrence of BTB in cattle and its potential risk to humans and to see the effects of different risk factors on human tuberculosis

The effects of risk factors on tuberculin reactivity summarized using univariate (Table 2) and multivariate analysis with different models (Table 3 and 4). The standard tuberculin result in bovine was reported in three categories as positive, doubtful and negative. Therefore two

models were developed for multivariate risk factors analyses. In the first model the doubtful reactors were considered as negative and in the second model as positive. For all the models backward stepwise process was used to fit the final model. The final models contain variables that have $p < 0.05$.

4. RESULT

4.1. Individual animal prevalence

The result of the interadermal tuberculin test in cattle owned by tuberculous and non-tuberculous human patients is given in Table 1. The overall prevalence, considering doubtful reactors as negative was 16.2% (169/1041). When doubtful reactors were considered as positive, prevalence increased to 24.9% (259/1041).

Table 1: Tuberculin reactivity of cattle owned by TB patients and controls

Household	Tuberculin reactivity			Total
	Positive	Doubtful	Negative	
TB Positive	123 (24.3%)	35 (6.9%)	348 (68.8%)	506
TB Negative	46 (8.6%)	55 (10.3%)	434 (81.1%)	535
Total	169 (16.2%)	90 (8.7%)	782(75.1%)	1041

In the case group, which comprised of 506 animals owned by tuberculous human patients, 123 (24.3%) and 35 (6.9%) were positive and doubtful for the interadermal tuberculin test, respectively; the remaining animals were negative to the test. When doubtful reactors were added up to the positive group, the prevalence of tuberculin reactivity in this group was 31.2%. The prevalence in the control group, which comprised of 535 cattle owned by non-tuberculous patients visiting FICHE hospital, only 46 (8.6%) and 55 (10.3%) of the animals were positive and doubtful with the interadermal tuberculin test, respectively. In this group, more than 75% were negative.

The difference in the prevalence of tuberculin reactivity to the comparative interadermal tuberculin test in cases and control were significant ($\chi^2 = 48.4$, $P < 0.001$). Univariate analysis of risk factors were performed by considering doubtful reactors as positive and negative for both cases and controls, respectively. Tuberculin reactivity was more likely to occur in animals owned by tuberculous patients compared to cattle owned by non-tuberculous patients.

When doubtful reactors were considered as tuberculin positive, the tuberculin positivity was more likely to occur in cattle owned by TB patients (OR = 2.0; $p < 0.001$) compared to control. Even it was more likely (OR = 3.41, $p < 0.001$) when doubtful reactors were considered as negative.

4.1.1. Risk factors of bovine tuberculosis in cattle

The univariate analysis of the different likely risk factors considered for tuberculin positivity in both cases and control group of cattle are given in Table 2. For risk factors analysis, both models i.e. doubtful reactors as being negative and positive were considered, respectively.

4.1.1.1. Breed

Out of 1041 animals included in the study 485 (46.6%) were local, 391 (37.6%) crosses and the remaining 165 (15.9%) were exotic animals. Without regard to doubtful reactors, the prevalence was high in exotic breeds (40.6%); relatively lower in cross breed and local cattle at 13.8% and 9.9%, respectively. This difference in prevalence was significant ($\chi^2 = 101.3$, $p < 0.05$) between the breeds. The univariate analysis given in Table 2 considers doubtful reactors as negative (model I) and as positive (model II). In model I, bovine tuberculosis was more likely in exotic breeds (OR = 6.2, $p < 0.05$) and crossbreeds (OR = 1.5, $p < 0.05$) of cattle when compared to local Zebu cattle. This likelihood was relatively lower in exotic (OR = 3.6, $p < 0.05$) and non-significant in crossbreed cattle (OR = 0.8, $p > 0.05$), in model II.

4.1.1.2. Age

Animals were grouped into 4 age groups as calf (<2), heifer/bull (2-4), Adult (4-7) and old animal (≥ 7). The distribution of tuberculous cattle among different age groups is shown in Table 2. The tuberculin reactivity increased with age up to seven years (6.1%-22.4%) and then decreased slightly (19.4%). This difference in tuberculin reactivity among the different age

groups was significant ($\chi^2 = 39.2, p < 0.001$). In model I, group 2, 3, and 4 were 1.43, 4.4 and 3.7 times more likely when compared to animals younger than 2 years. Similarly, in model II, this likelihood was 0.9, 2.6 and 2.6, respectively.

4.1.1.3. Sex

As indicated in Table 2 the recorded rates of positivity with sex was 15.6% and 16.7% for male and female animals, respectively. However, this difference on prevalence of BTB between male and female animals was not significant ($\chi^2 = 0.16, P > 0.05$).

4.1.1.4. Body condition

The rate of tuberculin positivity was 11.4%, 17.9%, and 28.4% in animal with poor, medium and good body condition, respectively. Using the univariate analysis and taking doubtful reactors as positive, the difference in prevalence among these three groups was statistically significant ($\chi^2 = 15.3, p < 0.005$). In the respective model I and II, animals in good condition were 3.2 and 2.3 times more likely to react to the tuberculin test than those in poor condition. By a similar procedure, animals in medium body condition were 1.6 and 1.4 times more likely when compared to animals in poor body condition.

Table 2: Univariate analysis of different risk factors for bovine tuberculosis

Risk factors		No of animal s	Model I			Model II		
			% Positive	Crude OR	95%CI	% Positive	Crude OR	95%CI
Sex	Male	429	15.85	1		24.2	1	
	Female	612	16.50	1.05	0.8-1.5	25.3	1.06	0.8-1.4
Age	<2	79	6.10	1		14.6	1	
	2-<4	271	8.49	1.43	0.5-3.9	13.7	0.9	0.5-1.9
	4-<7	255	22.35	4.43	1.7-11.5	30.6	2.6	1.3-5.0
	>7	436	19.40	3.71	1.5-9.6	24.9	2.6	1.3-4.9
Breed	Local	485	9.9	1		21.4	1	
	Cross	391	13.81	1.5	1.0-2.2	18.7	0.8	0.6-1.7
	Exotic	165	40.61	6.2	4.0-9.6	49.7	3.6	2.5-5.3
Body condition	Poor	370	11.76	1		20.2	1	
	Medium	604	17.39	1.6	1.1-2.3	26.4	1.4	1.0-1.9
	Good	67	30.16	3.2	1.7-6.1	36.5	2.3	1.3-4.0
Owner Status	Control	535	18.8	1		18.8	1	
	Case	506	31.2	3.41	2.4-4.9	31.2	2.0	1.5-2.6

4.1.2. Multivariate analysis of effect of risk factors on BTB prevalence in cattle

Table 3 shows the association between the prevalence of tuberculosis in cattle, and different risk factors by considering doubtful reactors as negative reactors (Model I). Accordingly, individual animal prevalence was significantly differed between the status of the owner ($\chi^2 = 48.4$, $P < 0.001$), among the age group ($\chi^2 = 39.2$, $P < 0.001$), breed ($\chi^2 = 101.3$, $P < 0.05$), and body condition ($\chi^2 = 15.3$, $P < 0.05$). Thus, cattle owned by TB patients (OR = 4.2), age group over four (OR = 4.7), exotic breed (OR = 7.4), and cattle with better physical condition (OR = 2.5) had a higher odds of tuberculin reactivity than those owned by TB free individuals, age group under four, local breed, and poor physical condition, respectively.

Table 3: Multivariate analysis of factors associated with tuberculin positive* status in cattle owned by tuberculous and non-tuberculous patients in Fiche (2004/05)

Risk Factors		No of animals	% Positive	χ^2	Adjusted OR	95%CI	p-value
Sex	Male	429	15.85		1		
	Female	612	16.50	0.16	1.1	0.74-1.61	0.654
Age	<2	79	6.10		1		
	2-<4	271	8.49	39.2	1.6	0.58-4.62	0.355
	4-<7	255	22.35		5.0	1.83-13.5	0.002
	>7	436	19.40		4.7	1.77-12.64	0.002
Breed	Local	485	9.9		1		
	Cross	391	13.81	101.3	1.9	1.2-3.0	0.007
	Exotic	165	40.61		7.4	4.6-11.8	0.000
Body condition	Poor	370	11.76		1		
	Medium	604	17.39	15.3	1.5	0.99-2.32	0.058
Owner Status	Good	67	30.16		2.5	1.2-5.0	0.012
	Control	535	18.8		1		
Owner Status	Case	506	31.2	48.4	4.2	2.79-6.17	0.000

* Only positive reactors were considered as positive and doubtfuls considered as negative

Table 4 shows the association between the prevalence of tuberculosis in cattle, and different risk factors by considering doubtful reactors as positive reactors (Model II). Accordingly, individual animal prevalence was significantly differed between the status of the owner ($\chi^2 = 48.4$, $P < 0.001$), among the age group ($\chi^2 = 39.2$, $P < 0.001$) and, breed ($\chi^2 = 101.3$, $P < 0.05$). Thus, cattle owned by TB patients (OR = 2.1), age group over four (OR = 2.7), exotic breed (OR = 3.8) had a higher odds for tuberculin reactivity compared to those owned by TB free individuals, a ge group under four, local breed, r espectively. With this model sex and body condition were not found to affect the disease prevalence.

Table 4: Multivariate analysis of factors associated with tuberculin positive* status in cattle owned by tuberculous and non-tuberculous patients in Fiche (2004/05)

Risk Factors		No of animals	% Positive	χ^2	Adjusted OR	95%CI	P-value
Sex	Male	429	24.2	0.16	1	0.9-1.7	0.204
	Female	612	25.3		1.23		
Age	<2	79	14.6	39.2,	1	1.4-5.4	0.004
	2-<4	271	13.7		1.0		
	4-<7	255	30.6		2.7		
	>7	436	24.9		2.6		
Breed	Local	485	21.4	101.3	1	2.5-5.6	0.001
	Cross	391	18.7		1.9		
	Exotic	165	49.7		3.8		
Body condition	poor	370	20.2	15.3,	1	0.8-2.9	0.158
	Medium	604	26.4		1.3		
	Good	67	36.5		1.6		
Owner Status	Control	535	18.8	48.4	1	1.5-2.8	0.001
	Case	506	31.2		2.1		

* Tuberculin positive + doubtful reactors

4.2. Herd prevalence

In each of the case and control groups, 87 herds were tested. Herd prevalence in cattle owned by TB patients ranged from 62.1% (54/87) to 72.4% (63/87) while it varied from 28.7% (25/87) to 45.9% (40/87) in those owned by TB free individuals (Table 5). This variation in prevalence was due to the doubtful reactor herds; that is when we consider the doubtful herds as negative we are calculating the minimum prevalence and when doubtful herds added to positive herds the herd prevalence increases. In both cases the herd prevalence was significantly ($\chi^2=19.5$, $P < 0.001$) higher in herds owned by TB patients than those owned by TB free individuals. The tuberculin positivity was more likely to occur in herds owned by TB

patients compared to cattle herds owned by non-TB patients. When doubtful herds were considered as tuberculin positive, the tuberculin positivity was more likely to occur in cattle owned by TB patients (OR = 3.1; $p < 0.001$) compared to control. Even it was more likely (OR = 4.06, $p < 0.001$) when doubtful herds were considered as negative.

Table 5: Tuberculin reactivity of herds owned by tuberculous non-tuberculous patients

Household	Tuberculin reactivity			Total
	Positive	Doubtful	Negative	
TB positive	54 (62.1%)	9 (10.3%)	24 (27.6%)	87
TB negative	25 (28.7%)	15 (17.2%)	47 (54.1%)	87
Total	79 (45.4%)	24 (13.8%)	71 (40.8%)	174

4.3. Tuberculosis in cattle owners and associated risk factors

Summary of the questionnaire survey are shown in Table 6. Of 87 TB cases, 63 (72.4%) and 24 (27.6%) were diagnosed as pulmonary and extrapulmonary tuberculosis, respectively. About 83.9% (73/87) of the patients were consuming raw milk and milk products, while only 6.9% (6/87) were consuming boiled milk; the rest 9.2% (8/87) did not like to consume milk at all. Classification of the cases on the basis of age indicated that 56.4 % of the patients were between the ages 15 and 39 while 19.5% and 24.1% were below 15 and above 39 years, respectively. Classification of the patients on the basis of residence showed that 76% of them were rural dwellers and 79.2% of extrapulmonary tuberculosis were also from rural areas of the districts.

Most of the assessed risk factors (sex, age, physical contact and the origin of cattle owners) did not differ between cases and controls. However, a significant ($\chi^2 = 14.12$; $P < 0.001$) difference was observed between the milk consumption habit of the case and control groups. Cattle owners who consumed raw milk were at higher risk (OR = 3.34) of infection with tuberculosis than those who consumed boiled milk.

Table 6: Analysis of risk factors with tuberculosis status in humans treated at Fiche Hospital

Risk factors		Case		Control	χ^2	P-value	Odds Ratio
		Pulmonary TB	Extra-Pulmonary TB				
Sex	Male	33	17	56	0.87	0.351	1
	Female	30	7	31			1.25
Age	<15	9	8	18	0.04	0.98	1
	15-39	37	12	48			1.11
	>39	17	4	21			1.10
Contact	No	6	3	7	0.28	0.60	1
	Yes	57	21	80			1.14
Origin	Rural	47	19	64	0.12	0.73	1
	Town	16	5	23			0.48
Milk consumption behavior	Boil+NDM*	11	3	8	14.12	0.001	1
	Rarely	20	3	7			1.81
	Frequently	32	18	72			3.34

NDM* = Not drink (like) milk



4.4. Bacteriological findings

Primary growth was observed in 45.9% (51/111) of the specimens from human TB patients while 26.7(16/60) of the milk sample from tuberculin positive were positive on primary culture. But after sub-culturing 53 (42 human and 11 cattle) isolates were able to be recovered from the specimens (Table 7).

Table 7: Culture results of the specimens on Lowenstein Jenson media

Type of sample	Growth on L-J media		
	Positive	Negative	Total
Sputum	36 (41.4%)	51 (58.6%)	87
FNA	6 (28.6%)	15(71.4%)	21
Peritoneal fluid	0	3	3
Milk	11 (18.3%)	49 (81.8%)	60
Total	53(31%)	118(69.0%)	171

Table 8 shows summary of mycobacterial species isolated from the sputum of human TB patients and milk samples of tuberculin positive cows. Subjecting both human and cattle isolates to biochemical and drug sensitivity tests indicated that 73.8% (31/42) and 16.7% (7/42) of the human isolates indicates *M. tuberculosis* and *M. bovis*, respectively. The rest 49.5% (4/42) were other mycobacterial species. On the other hand, 18.1% (2/11) and 45.5% (5/11) of the cattle isolates indicates were *M. tuberculosis* and *M. bovis*, respectively. The rest 36.4% (4/11) were other mycobacterial species.

Table 8: Species of mycobacteria isolated from sputum of human tuberculous patients and interadermal tuberculin test positive animals in Fiche, (2004/05)

Source of sample	Type of sample	<i>M. tuberculosis</i>	<i>M. bovis</i>	Other	Total
Human	Sputum	29(80.6%)	4(11.1)	3(8.3%)	36
	FNA	2(33.3%)	3(50.0%)	1(16.7%)	6
	Peritoneal fluid	0	0	0	0
Bovine	Milk	2(18.2%)	5(45.5%)	4(36.4%)	11
Total		33(62.3%)	12(22.6%)	8(15.1%)	53

5. DISCUSSION

Both individual and herd prevalence of BTB were higher in the cattle/herds owned by TB patients as compared to those owned by TB free individuals, suggesting the existence of transmission of mycobacterial species between the human TB patients and their cattle. This transmission is possible in both ways i.e. either human TB patient or the reactor cattle could be a source of infection for the other. The disease transmission may be cyclical: cow-to-man-to-cow (Cosivi *et al.*, 1998), underling the existence of higher risk dissemination of mycobacteria among the cattle and human populations. Similar findings were reported by Cook *et al.* (1996) and Ameni *et al.* (2001). In addition, Anderson (1997) indicated that human contracts the infection primarily by ingesting the agent in raw milk and milk products, and secondly by inhaling it when there is close physical contact between the owner and his/her cattle especially during the night. Cosivi *et al.* (1998) indicated that pulmonary as well as extra pulmonary cases of human TB of animal origin will continue to be of public health problem especially in areas where the prevalence of infection is high in cattle and where raw milk or its products are commonly consumed. Substantiating the findings of this study, Ashford *et al.* (2001) indicated that milk is still regarded as the principal vehicle for transmission to humans in countries where BTB is not controlled. The same author observed that ingestion of contaminated milk or other dairy products is more often associated with scrofula, abdominal tuberculosis and other extrapulmonary forms of the disease. The habit of consumption food of animal origin including raw milk, and the close physical contact between infected cattle and their owners as well as the isolation of *M. tuberculosis*, *M. bovis* from milk of reactor cows, indicated the potential danger of acquiring infection through consumption of raw milk and other animal products. Kazwala *et al.* (1998) suggested that the public health hazardous due to *M. bovis* infections is acquired mainly through consumption of contaminated milk. Furthermore, Sinha (1994) indicated that *M. bovis* accounts for 30% of tuberculosis in children and up to 10% of the extra pulmonary form of tuberculosis in humans as whole. Ashford *et al.* (2001) also stated that in countries where BTB is still common and pasteurization of milk is rare, an estimated 10% to 15% of human cases of TB are caused by *M. bovis* but in areas where milk is routinely pasteurized or boiled before consumption, *M. bovis* infection is responsible for 1% to 6% of human TB cases. Although contaminated milk

is the usual source of infection for the town dwellers, farm workers can also acquire lung disease directly by inhalation (O'Reilly and Dabron, 1995).

Analysis for the effect of risk factors revealed that the animal prevalence of BTB increased with age up to the age of seven years, and then observed to decrease slightly. This finding is in consistent with other works reported by Cook (1996), O'Reilly and Dabron (1995). O'Reilly and Dabron (1995) reported that the reaction to tuberculin test in cattle increases uniformly by 7.5% for every year of life reaching 40% at 6 to 7 years old. As explained by other workers (Barwinnek and Taylor, 1996), this could be due to the fact that as the age increases the probability of acquiring tuberculosis infection also increases. On the other hand the decrease in prevalence observed in the old age may not reflect the actual disease status of the animal. The probable reason may be the fact that reaction to tuberculin, on the basis of which prevalence is calculated, is associated with immune status of the animal. In other words, the level of reaction is directly related to the maturation and wasting of organs of immune system i.e. immature and old animals rarely react to tuberculin inoculation regardless of the status of infection (Hebert and Paterson, 1955). Further, Tizard (1996) stated that lowered response to interdermal tuberculin test in older animals is due to the immunodepression resulting from old age.

The finding of lower prevalence in Zebu breed is in line with the previous reports (Radostits *et al.*, 1994; O'Reilly and Dabron, 1995; Kiros, 1998; Ameni and Roger, 1998), which reported lower cases of BTB in Zebu animals followed by their crosses. The probable reason could be due to the fact that genetically improved cattle may suffer more severely from deficient housing and malnutrition; subsequently, become more prone to infection than local breeds.

Although multivariate analysis did not show significant difference in prevalence between cattle with good and poor physical condition, univariate analysis showed that the prevalence tend to be higher in cattle with good body condition. This could be justified by the fact that animals under good body condition are with good immune status that can respond to any foreign protein better than those with poor body condition, which can be immunocompromised due to other disease or malnutrition.

Though molecular characterization has not been performed on the isolates due to time limitation, biochemical and drug sensitivity tests were conducted for the speciation the isolates. Accordingly, the isolation of *M. bovis* from sputum of cattle owners suggests the possibility that man can acquire pulmonary TB from cattle. In a similar manner, the isolation of *M. tuberculosis* from cow's milk suggests that cattle owner is the source of infection for its cattle. Similar results have been reported by Boulahbal (1978) who identified seven *M. tuberculosis* out of 113 isolates in Algeria, and Idrisu and Schnurrenberger (1977) have also isolated *M. tuberculosis* from milk in Nigeria. Even in Ethiopia, Kiros (1998) isolated two *M. tuberculosis* out of 19 isolates from milk. These patchy findings need further and wider investigation to establish the contribution of *M. tuberculosis* in cattle TB. Further, the isolation of atypical mycobacterium from milk is also of public health significance because some of them are potentially pathogenic to man, especially in immunocompromised individuals (Grange, 1988).

In conclusion, although the biochemical and drug sensitivity tests are the definite identification tools of mycobacterial species, the isolation of both species of mycobacteria (*M. bovis* and *M. tuberculosis*) from both man and cattle suggests the existence of transmission of both species between cattle and their owners.



6. CONCLUSION AND RECOMMENDATIONS

The findings of the present study showed the widespread occurrence of tuberculosis both in cattle and humans and indicate its transmission between the two species.

The existence of significant difference in the prevalence of tuberculosis among the cattle owned by human TB patients and TB free individuals indicate transmission of the infection between humans and cattle. Furthermore, isolation *M. bovis* from tuberculin positive cows' milk and sputum of TB patients signifies the public health importance of *M. bovis* particularly in raw milk consumers; and the increase in human tuberculosis in the country may be partly attributed to *M. bovis*. The isolation of *M. tuberculosis* from milk indicates the possibility of cattle to act as a source of infection to humans. As people and cattle in the study area are living in close association and due to the fact that raw milk is consumed to a large extent in the area, the contribution of *M. bovis* in the human tuberculosis should not be undermined.

The information obtained from Fiche hospital and the study districts indicated that a large proportion of the human tuberculosis cases originated in rural parts of the district might be associated with poor awareness of the transmission of BTB by the consumption behavior of people and by the close interaction between humans and cattle.

Therefore any control programme against tuberculosis in humans should be designed parallel to control strategies in cattle. The most effective method of controlling tuberculosis in cattle is the test and slaughter policy. However, due to economic, cultural and infrastructural problems prevailing in the country, it cannot be practical in Ethiopia; therefore the alternate and possible recommendations are;

- Awareness should be created through public education on the potential risks of bovine tuberculosis to human health through consumption of raw milk and milk products, close contact and sharing the same house.

- Similar studies are lacking at national level; therefore, further studies should be initiated so that integrated control of tuberculosis in humans and animals is implemented.
- The importance of *M. bovis* as a public hazard is given little attention by the medical professionals and close collaboration between the medical and veterinary professionals is lacking. Therefore, close collaborations between the two professionals needs to be strengthened.
- Test and segregation supplemented with pasteurization of milk, isolation of calves immediately after birth and vaccination of calves could be a sound and acceptable control option particularly in intensive dairy herds and fattening farms.

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8. ANNEXES



Annex I: -test procedure of AFB (Vestal, 1981)

- ♣ Prepare smear, a 3mm loop to spread a loopful over an area of 2-3 square cm or smear a drop on the slide with a pipette.
- ♣ Fix smear using a Bunsen-flame and pass the slide 3 times slowly over its cone of heat. Do not scorch.
- ♣ Place a piece of filter paper over the smear. This holds the carbol fuchsin on the slide.
- ♣ Cover the filter paper with carbol fuchsin and heat the slide to steaming with a Bunsen flame
 - If a Bunsen-flame is used, heat gently to steaming (DO NOT BOIL) and allow standing 5 minutes without further heating. If the slide dries, keep it moist by adding more carbol fuchsin without additional heating.
- ♣ Remove paper strips and wash slides with tap water. Drain.
- ♣ Decolourize with acid alcohol until no more colour appears in the washing (about 2 minutes). Thick smears may require longer, but not over-decolourize, since some mycobacteria may lose their acid-fastness if this is done.
- ♣ Wash with distilled or tap water. Drain.
- ♣ Flood slide with methylene blue Counterstain for 1-2 minutes.
- ♣ Wash with distilled or tap water. Drain.
- ♣ Dry in air or over gentle heat. Do not blot.
- ♣ Examine smear with oil immersion lens, taking care to wipe the lens well after each examination, especially if the smear is positive. Three long lines the length of the smear or nine short lines the width of the smear are examined. The bacilli are stained red and the background material is stained blue.

Annex II: - Nitrate Reduction Test

Mycobacterium tuberculosis produces the enzyme nitroreductase, which catalyzes the reduction of nitrate to nitrite. Some of the other atypical mycobacterial species that reduce nitrate could be differentiated from *M. tuberculosis* by the Pyrazinamidase test. The development of a red colour upon addition of the reagents indicated the presence of nitrite and a positive test result for *mycobacterium tuberculosis*. Most mycobacterial cultures to be tested for nitrate reduction were examined 3 to 4 weeks after inoculation onto the subculture medium i.e. after visible colonies appear.

A) Procedure

1. Add 0.2 ml of sterile distilled water to a 16 x 125-mm screw cap tube.
2. Use a sterile spade or applicators -----the water 2 spadeful of growth from a 4 week old culture on Lowenstein-Jenson or some other egg-base medium.
3. Add 2 ml of the NaNO_3 substrate to the tube.
4. Shake by hand and incubate upright for 2 hours in a 37°C water bath.
5. Remove from the water bath.
6. Add one drop of reagent #1
7. Add two drop of reagent #2
8. Add two drop of reagent #3
9. Examine immediately for a pink-to-red colour

B) Results and interpretation

Positive = May range from pale pink (\pm) to deep red (5+) when compared with the colour standards. Only 3+ to 5+ are considered positive.

Negative = No colour. If no colour develops, the test is either negative or the reduction has proceeded beyond nitrite. Add a small amount of powdered Zink to all negative tests.

- a) If nitrate is still present, it will be catalytically reduced by the Zink, and a red colour will develop, indicating a true positive.
- b) If no colour develops when Zink dust is added, the original reaction was positive, but the nitrate was reduced beyond nitrite. Repeat the test in this case to confirm the observation.

Annex III: - Pyrazinamidase (PZA)

One of the most useful biochemical tests in the classification scheme for a *M. bovis* is susceptibility to Pyrazinamidase. The deamination of Pyrazinamidase (PZA) to pyrazinoic acid and ammonia is helpful in separating the weakly niacin-positive strains of *M. bovis* from *M. tuberculosis* and in distinguishing *M. bovis* from members of the *M. avium* complex. This enzyme acts to split pyrazinamide to pyrazinoic acid in 4 to 7 days. *Mycobacterium bovis* was Pyrazinamidase negative even at 7 days, whereas both *M. tuberculosis* and the *M. avium* complex were positive within 4 days.

A) Procedures

1. Inoculate the surface of two tubes of medium with a heavy loopful of growth from an actively growing culture (2 to 3 weeks old). The inoculum should be heavy enough to be visible.
2. Incubate cultures and controls at 37°C.
3. After 4 days, add 1.0 ml of freshly prepared 1% ferrous ammonium sulfate solution to each unknown culture, to the colour control standard, and to one each of the positive and negative controls.
4. Leave tubes at room temperature for 30 minutes and then examine for a pink band in the agar medium.
5. Refrigerate negative tubes for an additional 4 hours to minimize growth of contaminants from nonsterile ferrous ammonium sulfate solution and examine the medium again for a pink band in the agar. The colour is easiest to detect by examining the tube against a white background and using incident room light.
6. If the four day tubes is negative or doubtful, repeat the test at 7 day using the second tube.
7. If the four day tube is positive, the second tube may be discarded without further examination.

B) Result and interpretation

A pink band, which forms in the substrate agar and diffuses into the medium, indicates the enzymatic hydrolysis of PZA to free pyrazinoic acid.

Positive = Pink band in agar

Negative = No pink band in agar

Annex IV: - Thiophene-2- Carboxylic acid hydrozide (TCH)

Thiophene-2- carboxylic acid hydrozide sensitivity test was used to distinguish *M. bovis* from *M. tuberculosis* and other non- chromogenic slow growing mycobacteria. *M. bovis* is sensitive to low concentration of TCH. *M. tuberculosis* and other species of mycobacteria generally are resistant to this compound. The test procedure for TCH sensitivity is indicated in the annex---

A) Media and supplies

Middlebrook 7H-10 medium

Thiophen-2-carboxylic acid hydrazide (Aldrich Chemical Co.,Milwaukee,WI)

Strile screwcap tubes or four-section Petri plates (plastic quadrant plates).

B) Preparation

Prepare two batches of complete, enriched Middlebrook 7H-10 medium. One batch is poured as a drug-free control medium; to the other is added sufficient filter-sterilized TCH to make a final concentration of 2 µg/ml. Dispense each medium into sterile, screwcap tubes or, preferably, into four-sectioned Petri dishes.

C) Procedure

1. Dilute a 7-day-old liquid test culture to 10^{-3} and 10^{-5} (1:1000 and 1:100,000) in sterile saline or water.
2. Inoculate one control and one drug-containing medium with 0.1 ml of each dilution.
3. Incubate for 3 weeks at 35⁰C in an atmosphere of 10% carbon dioxide -90% air.

D) Result and Interpretation

Record the organisms as resistant to TCH if growth on the drug-containing medium is equal to or greater than 1% of that observed on the drug-free control medium.

Annex V: - Body Condition Scoring

A) For Exotic Cattle

0. Animals are emaciated with spinous processes, hipbones, tail head and ribs projected prominently. No fatty tissue can be detected, neural spines and transverse processes feel sharp.
1. Individual spinous processes are still fairly sharp to the touch and there is no fat around tail, head, hip bones, tail head and ribs and still prominent, but appear less obvious.
2. Spinous processes can be identified individually when touched, but feel rounded rather than sharp. There is some tissue cover rounded tail, over hip bones and flank individual ribs are no longer visually obvious.
3. Spinous processes can only be felt with firm pressure. Areas on either side on tail head now have a degree of fat cover which can be easily felt.
4. Fat cover around tail head is evident as slight rounds soft to touch spinous processes can not be felt even with firm pressure and folds of fat are beginning to develop over ribs and thigh of animal.
5. Bone structure is no longer noticeable and animal presents a blocky appearance. Tail head and hip bone are almost completely buried in fatty tissue and folds of fat are apparent over ribs and thighs. Spinous processes are completely covered by fat and animal's mobility is impaired by large amounts of fat carried.

B) For Zebu Cattle

1. Condition score 1 (L-) marked emaciation- the animal could be condemned ante mortem
2. Condition score 2 (L) transverse processes project prominently, spines appear sharply
3. Condition score 3 (L+) individual dorsal spines are pointed to the touch, hips, tail-head and ribs are prominent
4. Condition score 4 (M-) ribs, hips and pins are clearly visible, muscle mass between hooks and pins are slightly concave.
5. Condition score 5 (M) –ribs usually visible, little fat cover, dorsal spines are barely visible
6. Condition score 6 (M+) the animal is smooth, dorsal spines can not be seen, but are easily felt
7. Condition score 7 (F-) animal is smooth and well covered but fat deposits are not marked
8. Condition score 8 (F) fat cover in critical areas can easily be seen and felt; transverse processes cannot be seen or felt.
9. Condition score 9 (F+) heavy deposits of fat is clearly visible on tail-head, brisket, dorsal spines, ribs and hooks.

Annex VI: - Questionnaire format to assess risk factors of bovine tuberculosis in and around fische.

Respondents: human TB cases and control groups having contact with cattle.

Date-----

1. Name of the owner-----

Age (<15, 15-45, >45), Sex (M, F)

Address: a) Urban (city-----, wereda-----, kebele-----, house number-----)

b) Rural (wereda-----, PA-----, Village-----)

2. The last school he/she attended:

- a) No formal education c) Secondary school
b) Primary school d) College/ University graduate

3. Occupation: (farmer, Civil servants, others)

4. Which species of domestic animals do you own?

(Cattle, sheep, goats, Horses/mules/donkeys, Poultry, camel)

5. Which breeds of cattle do you own? a) Local b) cross c) exotic (Holstein)

6. Purpose of cattle keeping

- a) Draught b) Milk for home use c) Milk for sale d) Other

7. Number of cattle you own

- a) ≤ 10 b) 10-30 c) > 30

8. Have you noticed respiratory problems in your cattle herd? a) Yes b) No

9. If yes, do you want then to be tested for TB? a) Yes b) No

10. Do you share the housing with your animals? a) Yes b) No

11. Do you know bovine tuberculosis? a) Yes b) No

12. Do you know that tuberculosis can be transmitted from cattle to man and vice versa?

- a) yes b) No

13. If yes, would you please indicate some ways? -----

14. Habit of drinking milk a) Raw b) boiled c) mixed

15. Reason for boiling a) Fear of milk borne diseases b) due to culture

16. Do you boil milk for your baby? a) Yes b) No

17. Habit of eating meat a) Cooked b) raw c) mixed

18. If you sell milk/ milk products, who buys it?
 - a) Local people
 - b) Milk collection Unit
 - c) Others (specify) -----
19. Do you have (other) tuberculosis patient in your family or farm workers?
 - a) Yes b) No
20. If yes, how many? -----
21. If you have tuberculosis patient in your family or farm workers, indicate the type of tuberculosis? a) Pulmonary b) extra pulmonary c) do not know
22. How long have you been sick? a). More than a year b) Less than a year c) Other
23. Have you ever taken any treatment? a) Yes b) No
24. If yes, a). Traditional b) Modern

Clinical Record

1. Type of TB suspected. 1. Pulmonary 2. Extra pulmonary
2. IF extra pulmonary, specify? -----
3. Sample(s) taken -----
4. Result of direct smear a) positive b) negative
5. Result on culture a) positive b) negative
6. If positive type of mycobacterium on the biochemical and drug sensitivity tests
 - a) *M. bovis* b) *M. tuberculosis* c) Other atypical mycobacterium

SIGNED DEDICATION SHEET

I the under signed, declare that the thesis is my original work and has not been presented for a degree in any University

Name -----

Signature -----

Date of submission -----

This thesis has been submitted for examination with my approval as University Advisor

Name -----

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Bovins Infection in Umans & Animal.

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