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BOVINE TUBERCULOSIS: A CROSS-SCTIONAL STUDY IN AND AROUND ADDIS ABABA

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A thesis submitted in partial fulfilment for the degree of Master of Science in Tropical Veterinary Epidemiology at the Freie Universität Berlin and Addis Ababa University

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BOVINE TUBERCULOSIS: A CROSS-SECTIONAL STUDY IN AND AROUND ADDDIS ABABA

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LIST OF ABBREVIATIONS

AFB	Acid-Fast Bacilli
AHRI	Armaur Hansen Research Institute
AIDS	Acquired Immune Deficiency Syndrome
BCG	Bacille Calmette-Guerin
CI	Confidence Interval
CIDT	Comparative Intradermal Tuberculin test
CMI	Cell Mediated Immunity
CR	Complement Receptor
DAAD	Deutscher Akademischer Austauschdienst
DF	Degrees of Freedom
DNA	Deoxyribonucleic acid
DTH	Delayed Type Hypersensitivity
e.g.	Example
E. coli	Escherichia coli
EHNRI	Ethiopian Health and Nutrition Research Institute
FVM	Faculty of Veterinary Medicine
g	gram
GDP	Grand Domestic Product
GTZ	Gesellschaft für Technische Zusammenarbeit
HCSM	Heat Concentrated Synthetic Medium
HIV	Human Immunodeficiecy Virus
HSP	Heat Shock Proteins
IFN-Υ	Interferon Gamma
IU	International Unit
KDa	Kilo Dalton
Kg	Kilogram
LJ	Löwenstein-Jensen
Log	Logarithm
LR	Likelihood Ratio
Lt.	Liters
LTT	Lymphocyte Transformation Test

MHC	Major Histocompatibility Complex
ml	milliliters
mm	millimeters
NVL	No Visible Lesion
OIE	Office International des Epizooties
OR	Odds Ratio
OT	Old Tuberculin
PHAB	Department of Physiology, Pharmacology and Biochemistry
PPD	Purified Protein Derivative
SIDT	Single Intradermal Test
T_2H	Tiophene-2-Carboxylic acid Hydrazide
TB	Tuberculosis
w/v	weight by volume

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1. INTRODUCTION

Ethiopia is a country whose agricultural sector is the biggest contributor to its Grand Domestic Product, GDP, and the major contributor to its export earnings. Currently, the contribution of this sector to GDP and export earnings is 48% and 90%, respectively (Kebede, 1997). Livestock contributes 30% to the agricultural GDP and 19% to the export earnings. Besides, about six million oxen provide the draught power required for the cultivation of grain crops (Tegegne and Gebre wold, 1997). Although the contribution of the livestock sector to the national economy is quite high, animal productivity is extremely low.

The country possesses the highest number of livestock in Africa, with an estimated 33.8 million cattle. Despite this huge resource, Ethiopian livestock productivity is lower than the African average. Total herd off take is estimated at 7%, live weight gains are low at about 20Kg, and mortality is high at about 20%. Heifers don't reach maturity until 4 years of age, cows calve every second year and produce only 1.5-2.1 litres of milk per day over a 150-180 days of lactation period (Kebede, 1997). The major biological constraints contributing to low productivity include low genetic potential of the animals, poor nutrition and disease prevalence.

The low level of livestock productivity is also reflected in the very low per capita consumption of animal protein. In general, the per capita consumption of animal products in developing countries represents 15-30% of that of developed countries. For example, the average consumption of milk in developed countries is about 200Kg per person per year, as opposed to 27.5Kg for Africa, and 20Kg for Ethiopia (Tegegne and Gebre wold, 1997).

Programs in many developing countries are aimed at encouraging milk production through small holder and other dairy schemes to answer the increased demand for milk and milk products. This increased demand will be partly met by an intensification of animal production. Unfortunate enough, animal tuberculosis has shown close links with intensive management system, and can spread rapidly when there is inadequate veterinary supervision (Alhaji, 1976; Barwineck and Taylor, 1996).

Bovine tuberculosis, caused by *M. bovis*, has been eliminated as an animal health problem, in most of the developed countries, but still remains one of the most prevalent and devastating disease of cattle in developing countries throughout most of the world. From the estimated 1 billion cattle world-wide, one third lives in regions where the prevalence of bovine tuberculosis is high, the other third in areas where the disease is widespread but the incidence is unknown. Jointly with other diseases, bovine tuberculosis seriously affects the productivity of the livestock industry in these countries, by reducing milk production, the food value of carcass, and reproduction. Veterinary scientists have demonstrated that tuberculosis in cows reduces their production 30% or more; the losses, in calves are much higher because of high mortality (Thoen and Steel, 1995). While these bottlenecks relate to the development of the dairy industry throughout the world, the disease attains much of its importance from being a zoonosis, causing human tuberculosis.

In developed countries, the prevalence and the annual risk of human tubercular infection was shown to be closely associated with the prevalence of tuberculin positive cattle (Cook *et al.*, 1996), and therefore was successfully controlled by tuberculin test and slaughter schemes of cattle (Pritchard, 1988).

In developing countries, especially in Africa where *M. bovis* infection is present in various animal species, there is a substantial lack of knowledge of the distribution, epidemiological patterns, and zoonotic implication of this important disease. Animals gathering at watering points, markets, and in pens overnight, can play a key role in the maintenance and spread of *M. bovis* under more-traditional farming conditions. With increased size of cattle herds and intensified milk production in many developing countries, concurrent with the intensification of the livestock management systems, these classic conditions prevail and are aggravated in virtual absence of adequate monitoring and control measures for the widespread transmission of *M. bovis* to human populations. An additional factor that could affect the zoonotic aspects of *M. bovis* infection in developing countries is the close physical relationship between humans and potentially infected animals (Meslin and Cosivi, 1995). A survey of human sputum cultures from human tuberculosis patients in Malawi indicated that 42.8% of the culture positive specimens were *M. bovis* (Bedard *et al.*, 1993). Such situations are most evident in areas where European breeds of cattle have been used to establish a dairy industry.

A further cause of considerable concern is that a signifieant amount of the milk produced is being marketed through unsupervised, informal channels. In line with this, Idrisu and Schurrenberg (1977), cited by Meslin and Cosivi, (1995) found both *M. bovis* and *M. tuberculosis* in market milk in Nigeria. Yehualashet (1995) was able to isolate *M. bovis* from 4 out of 131 milk samples in Ethiopia, indicating the risk of transmission to human, especially to children; and an isolation rate of 0.4% of *M. bovis* from human sputum samples has been reported. Although the relationship needs further quantification, the risk of *M. bovis* as a zoonosis is strongly underlined. Conditions such as customs of consuming raw milk, keeping animals in the same dwelling with their owners, tethering cattle in close proximity to the owners´ house and using cow dung for plastering wall or floors and as a source of energy for cooking, do exacerbate the chance of spread of tuberculosis as zoonosis in Ethiopia.

It has been stated that tuberculosis infects a third of the world human population, and in some African countries the incidence is said to have doubled in the last decade (Reichel and Oloya, 1994). With the advent of the AIDS (Acquired Immune Deficiency Syndrome) pandemic there has also been a rise of HIV related tuberculosis, with HIV infected people being more likely to develop overt tuberculosis after infection than non-infected persons. So far the largest number of people who carry both these infections live in developing countries (Kleeberg, 1984).

On the one hand, *M. avium-intracellulare-complex* and *M. bovis* have been isolated from HIV/AIDS patients in Europe and United States (Meslin and Cosivi, 1995), on the other, *M. tuberculosis*, the human tubercle bacillus, has been isolated from exotic animals in areas where tuberculosis in humans was present. *M. avium* serovars 1 and 2 (of over 30 different serotypes of the *M. avium* complex) are most commonly isolated from cattle (Thoen and Steel, 1995). The isolation of these organisms from patients with AIDS has stimulated an increased interest in the sources of these bacteria.

The dramatic increase in reported cases of human tuberculosis caused by *M. tuberculosis* associated with HIV (Buddle *et al.*, 1995; Daborn *et al.*, 1996), has suggested that there might be a similar increase in the incidence of human tuberculosis caused by *M. bovis* and *M. avium* complexes, also in association with HIV infection. Such an increase would inevitably result in the transmission of the disease from human beings, not only to other human beings but also to animals, thereby further increasing the level of *M. bovis* (and other mycobacteria) in the environment. On the assumption that infection due to human, avian and other atypical mycobacteria might be common in cattle as well, this study is intended to investigate the types of mycobacteria causing infection in cattle.

The objectives of the present investigation were, therefore,

- to determine the prevalence of bovine tuberculosis in cattle, as indicated by the tuberculin test,
- to gather information on risk factors for tuberculosis in cattle,
- to isolate and identify mycobacteria from secretions of animals to establish whether mycobacteria species were being secreted, and;
- to relate the laboratory findings with some zoonotic aspects of the disease, and to ascertain the extent of the implications to the people engaged in the dairy enterprise and vice versa.

2. LITERATURE REVIEW

2.1 General Characteristics of Mycobacteria

2.1.1 Taxonomy

Mycobacteria are classified into the order of Actinomycetes which includes the genera *Mycobacterium, Rhodococcus and Nocardia.* The genus *Mycobacterium* includes a number of species, and according to E. J. Baron and co-workers, a tentative classification is proposed based on pathogenesis and natural history (Baron *et al.*, 1994). Species thus grouped include: obligate pathogens that cause exclusively human infections, facultative pathogens that are found primarily in animals or the environment but produce documented human infections, opportunistic pathogenic species that are found in the environment but produce documented human infections, and saprophytic species found in the environment that do not cause human infections (Table 1).

2.1.2 Morphology and Staining

Mycobacteria are thin rods of varying lengths, non-motile, non-sporing, aerobic and oxidative. Because, the cell walls are rich in lipids, mycolic acid constituting the bulk of these, they are characteristically acid fast; once a dye has been taken up by the cells, they are not easily decolourised, even by acid alcohol. In order to stain the bacillus, the smears must be placed in hot staining solution or remain in contact with the stain for several hours (Karlson, 1983). Several staining techniques are available but the Ziehl-Neelsen stain is the one commonly used in most laboratories.

Table 1: Currently recognized species of the genus Mycobacterium, isolated from humans

Group	obligatory	facultative	potential	saprophyte
Strict pathogens	M. africanum	M. bovis		
	M. leprae			
	M. tuberculosis			
	M. ulcerans			
Photochromogens		M. asiatucum		
		M. kansasi		
		M. marinum		
		M. simiae		
Scotochromogens		M. scrofulaceum	M. gordonae	
		M. szulgai	M. flavescens	
		M. xenopi		
Non-chromogens	M. genavense	M. avium	M. gastri	
		M. haemophilum	M. nonchromogenicum	
		M. intracellulare	M. terrae	
		M. malmoense	M. triviale	
		M. shimoidei		
Rapid growers		M. chelone	M. fallax	M. phlei
		M. fortuitum	M. smegmatis	M. vaccae
				M. thermosensible
Strict animal pathogens	M. farcinogens	M. microti		
	M. lepraemurium			
	M. paratuberculosis			
	M. porcinum	M. senegalense		

Source: Baron, *et al.*, 1994

2.1.3 Growth Requirements and Characteristics

Tubercle bacilli can be cultivated on artificial media directly from infected tissues, but the organism frequently is not present in a pure culture, due to contamination by organisms, which grow more rapidly than the tubercle bacilli. One of the methods commonly used to selectively inhibit the contaminants is to add an equal volume of 2-4% solution of NaOH to the tissue or exudate. Other methods also used for decontaminating tissue and body fluids include incorporation of gentian violet and malachite green in the media in order to prevent growth of contaminants not killed by treatment with alkali.

The mammalian tubercle bacilli grow slowly (10-14 days, up to 12 weeks on primary culture), while the development of the avian type is more rapid, viable growth occurring in 4-5 days, on media containing serum, potato or egg (Dorset egg medium). The addition of glycerine (Lowenstein-Jensen, LJ) enhances the growth of human and avian strains on primary culture, but has no effect, or even may inhibit the bovine strain; conversely, LJ with 0.4% pyruvate enhances the growth of *M. bovis*. On a solid medium, the human type produces a dry, rough, crumbly growth (eugonic), with warty, granular or cauliform morphology; and are easily picked from the media but are hard to emulsify. The bovine type produces a scant, dry growth (dysgonic), the colonies are buff, low and small; and emulsify easily in solution. The avian type produces a smooth, moist, grayish-white growth). The optimum temperature for growth is 37°C although the micro-organism does grow slowly at a temperature as low as 30°C. Avian tubercle bacilli grow well at temperatures between 25 and 45°C (Buxton and Fraser, 1977; Cernoch *et al.*, 1994; Karlson, 1983).

2.1.4 Tenacity

The presence of a lipid substance in the genus *Mycobacterium* increases its resistance to conditions that are detrimental to other non-sporing types of bacteria. When present in exudate, which is thoroughly dried, the microorganism may be viable for months. Although direct sunlight kills the bacillus after a few minutes of exposure, much of the animal exudates and faecal matter in which the microorganism is present usually protects it from the direct rays of the sun. The tubercle bacillus remains alive in putrefying materials and contaminated watering tanks for months, but is quite sensitive to high temperature, cresol compounds (2-3%), and absolute or denatured alcohol (Barwinek and Taylor, 1996). Table 2 shows the survival time of *M. bovis* under different environmental conditions.

Table 2: Survival time of *M. bovis* under different environmental conditions

Contaminated material	Conditions	Survival time
Purulent emulsion	Direct sunlight	>10 h but < 12 h
	Diffuse sunlight	at least 30 days (not tested further)
Cattle dung	Direct sunlight	> 6 h but <37 h
	Diffuse sunlight	15-150 days
	Covered	365-730 days
Pasture	Temperate climate, depending on season and climatic conditions	7-63 days
Water (experimentally contaminated)		180 days
	27.5 °C	230-237 days
	22.4 °C	245-295 days

Source: Mitscherlich *et al.*, 1984; cited by Barwinek and Taylor, 1996

2.1.5 Mycobacterial Antigens

A wide range of fractions of mycobacteria can elicit humoral and cell-mediated immune responses. Some of these antigens have been studied in relation to their effect on the immune response of the host, whereas others have been studied primarily in relation to the identification of mycobacteria. Detailed reviews of studies done on soluble antigens using immunodiffusion described four major groups of soluble antigens (Pritchard, 1988; Pritchard *et al.*, 1975):

Group I antigens are common to all mycobacterial species and related bacterial genera (*Nocardia, Corynebacteria*).

Group II antigens are restricted to the slow-growing mycobacteria.

Group III antigens occur in rapidly growing *Mycobacteria* and *Nocardia*.

Group IV antigens are specific to individual species.

The important consequence of this antigenic constitution is that the immune response to the pathogenic slow-growing mycobacteria is not specific since part of the response will be elicited by antigens shared with other species. Conversely, rapid-growing mycobacteria and related genera may elicit immune responses to antigens present in pathogenic mycobacteria (Grange and Collins, 1987).

Many protein antigens have been identified and characterised by the use of antibodies, particularly murine monoclonal antibodies. Although mycobacteria consist of several thousand proteins, the range of proteins recognised by antibodies was in fact quite restricted, leading to the concept of immunodominance, i.e., that there were certain proteins, which were selectively recognised by the immune system. Several of the antigens identified belong to a group of molecules, whose function is to protect the organism from environmental damage; thus the 70 kDa antigen belongs to the heat shock proteins (hsp) 70 family, the 65 kDa to the hsp 60 family, the 10 kDa antigen being homologous to the hsp Gro Es of E coli, and the 23 kDa being the enzyme superoxide dismutase, which protects organisms from damage induced by superoxide anions, are thought to be a major component of the antibacterial response of the macrophages (Colston, 1996).

2.2 Epidemiology of Bovine Tuberculosis

2.2.1 Aetiological Agents

Although the organism of principal importance to laboratory diagnosticians is *M. bovis*, numerous mycobacteria have been isolated from cattle. *M. tuberculosis*, the human tubercle bacillus causes pulmonary disease in primates and certain hoofed animals; it has also been isolated from exotic animals in areas where tuberculosis in humans is present. *M. avium* serovars 1 and 2 (of >30 different serotypes of the *M. avium-intracellulare complex*) are commonly isolated from cattle; *M. kansasi* has been isolated from pulmonary and extrapulmonary lesions and *M. scrofulaceum* from cervical lymph nodes. *M. fortuitum* also has been isolated from lymph nodes of cows, and has been associated with mastitis in cattle (Thoen and Steel, 1995).

2.2.2 Virulence

Development of mycobacterial disease in animals depends on the ability of mycobacteria to survive and multiply in macrophages of the host. The pathogenicity of mycobacteria is a multifactorial phenomenon, requiring the participation and cumulative effects of several components, and may vary from species to species. From the cell wall components, Trehalose-6, 6 dimycolate inhibits chemotaxis, induces disintegration of the rough endoplasmic reticulum and detachment of ribosomes in liver cells, and is leukotoxic. Sulphur-containing glycolipids (sulfatids) appear to promote the survival of virulent tubercle bacilli within macrophages by inhibiting phagolysosome formation and avoiding exposure to hydrolytic enzymes present in the lysosomes (Thoen and Chiodin, 1993).

Secreted proteins in the antigen 85 complex may play a role in development of cell-meditated responses and disease in the host. Fibronectin binds to antigen 85 components to effect monocytes phagocytose C3 sensitised cells, and the release of large amounts of this antigen could inhibit binding of fibronectin to tubercle bacilli (Thoen and Chiodin, 1993).

The elevated synthesis of stress proteins, in response to changes in physiological conditions within the phagolysosome may protect mycobacteria from hydrolytic enzymes, reactive oxygen radicals and myeloperoxidase-killing mechanisms. Superoxide dismutase produced and released by several mycobacterial pathogens, could protect the organisms from the toxic effects of reactive oxygen radicals generated during the oxidative burst by host macrophages (Thoen and Chiodin, 1993).

Different species follow different ways of evading the host defense mechanisms, particularly to avoid the effect of phagocytosis by macrophages. Thus, *M. tuberculosis* inhibits the fusion of lysosomes with the phagosomes; *M. avium* survives within the fused phagolysosomes, by virtue of their capsule-like coating material of mycosides; *M. bovis* eludes the bactericidal effects of macrophages, by escaping from fused into non-fused vacuoles in the cytoplasm (Grange, 1996; Grange and Collins, 1987).

2.2.3 Hosts Range

M. bovis combines one of the widest host ranges of all pathogens (Colston, 1996) with a complex epidemiological pattern which involves interaction of infections among human beings, domestic, and wild animals. An extensive range of mammals including various domesticated and feral hoofed animals, primates and a wide variety of exotic species both of captive and free-living have been implicated (Pritchard, 1988; Thoen *et al.*, 1995).

Following eradication of *M. bovis* in cattle, re-infection of cattle herds had occurred from man, imported cattle, and wildlife reservoirs. The importance of each reservoir depends on the degree of contact with cattle herds and the extent to which tubercle bacilli are excreted by these free-living species. In the Northern Territories of Australia, populations of water buffalo (*Bubalus bubalus*) are known to develop generalized tuberculosis, and are infective. In North America, the bison herds (*Bison bison*) have long been known to maintain *M. bovis*, and with elk (*Cervus canadensis*) they provide an important reservoir in certain extensive range conditions. In New Zealand, the brush tail possum (*Trichosuron vulpecula*), and in England and Republic of Ireland the bager (*Meles meles*), are implicated as significant reservoirs. In Africa a reservoir of M. bovis is known to exist in cape buffalo (*Syncern caffer*), lechwe (*Kabus lechwe*), the cape kudu (*Strepsicerus species*) and cape duiker (*Sylvicaproa species*) (Pritchard, 1988).

2.2.4 Environmental and Management Factors

The large body of field data arising from tuberculosis control in cattle show that there is substantial variation in both incidence and prevalence between geographical regions, and between farms within regions. One simple example of this is that incidence is typically higher in dairy than in beef herds. The evidence suggests that even when heifers are pastured with heavily infected cows the incidence remains low until they enter the cowshed (Morris *et al.*, 1994). Malnutrition, pregnancy and concurrent infections may depress the immune responsiveness in some cases and these may be important factors in cattle herds (Collins, 1994; Francis *et al.*, 1978; Tweddle and Livingstone, 1994).

2.2.5 Transmission

The routes by which cattle are infected are influenced by factors such as age, environment and pertaining farming practices. The alimentary route of infection would not be unexpected in very young calves ingesting infected milk from tuberculous udders. Tuberculosis transmission between cattle via the respiratory route is ideally facilitated by natural cattle behaviour, especially in communities with high stocking densities and substantial cattle movement through markets and between farms (McIlroy *et al.*, 1986; Roberts, 1998).

Less common routes of transmission have also been recorded. Cutaneous infection requires contamination of other primary lesion, in congenital infection transmission is via the umbilical vessels, genital transmission occurs in cattle if either the male or female sexual organs are tuberculous, iatrogenic transmission to the mammary gland has resulted from the use of contaminated intramammary infusion (Neill *et al.,* 1994).

2.2.6 Distribution

Tuberculosis is a widely spread disease throughout the animal kingdom. It is common among certain species of domesticated animals, and cattle are within the most frequently affected animals. It became widely disseminated in countries where cattle were housed for long periods and in large herds, and was particularly prevalent among dairy herds in the vicinity of large European cities. For instance the British Economic Advisory Council in 1934 reported that at least 40% of the cows in dairy herds in Great Britain were infected with tuberculosis (Alhaji, 1976), and 0.5% produced tuberculous milk (Collins and Grange, 1983). A prevalence as high as 50% was also reported in Germany in mid of the century (Pino, 1991; Pittler and Steel, 1995).

The progress, which has been made towards the total eradication of bovine tuberculosis, has changed the pattern of its present distribution. Countries which at the turn of the century had the highest prevalence of tuberculosis now have the lowest, while countries in South America, Asia and Africa, which probably acquired the disease by way of Europe, are today the problem areas (Alhaji, 1976).

It is estimated that there are 5 million cattle infected with *M. bovis* in a population of over 250-300 million cattle in South America. In Central America, the Caribbean and Mexico, in an estimated population of 50 million cattle, about 1 million are infected (Berrada and Rojas, 1995).

Apparently, tuberculosis had been rare in parts of Africa, where animals constantly live in the open. However, the introduction of European breeds of cattle and the development of intensive agriculture rapidly changed the distribution of the disease in these areas. For instance, prevalences greater than 6.6% among native cattle in some areas of Kenya, up to 50% in some areas in Malawi, as high as 25% in parts of northern Nigeria, and 4.2% in Libya have been reported by different workers (Alhaji, 1976; Sanousi and Orme, 1985).

In Ethiopia, although tuberculosis still remains a great problem in man, no data are available concerning the countrywide prevalence and pattern of the disease in cattle. Most of the surveys so far carried out are based on tuberculin testing of animals, and abattoir reports in a particular confined locality. Data from 1971, for the condemnation rate of carcasses with tuberculosis originating from one slaughter house (Diredawa) ranged between 1 and 1.5% (Yehualashet, 1995). A summarised report on the number of bovine tuberculosis from different parts of the country which included about 210,000 slaughtered cattle, in 1972 and 1973 was made, denoting a lung disease rate of 0.05-1.83% (Haile mariam, 1975). A total of 1,106,412 cattle were slaughtered at 6 abattoirs from 1985 to 1990. Generalised tuberculosis was the cause of whole-carcass condemnation in 0.008% (Yehualashet, 1995) but with wide differences (varying from 0.006% in Addis Ababa to 1.156% in Debre Zeit). In 1984, 4838 animals in selected farms were subjected to tuberculin test and 16.8% were found to be positive reactors. The Mojo farm had a high percentage of positive reactors (77.7%), followed by Kuriftu (50.6%). Reactors´ rates between 6.4% and 13.1% were found on six other farms. The rate for 1985, as determined with 3352 animals was 24.4% (this time 90% rate at Mojo and 35.5% at Debre Zeit). In 1991, the tuberculin test of 2000 cattle, 2 years of age and older, in small dairy herds (2-20 animals per holder) resulted in 5.1% and 1.0% of reactors in cross and local breeds, respectively (Yehualashet, 1995).

Table 3: Incidence of tuberculosis of the lung in 1972 and 1973, in cattle at slaughter in Ethiopia

Place	no. of animals	no. of lung affected	percent of affection
Asmara	15,936 (27,817)	223 (105)	1.39 (0.38)
Makale	25,661 (39,875)	819 (730)	3.10 (1.83)
Malguewonde	19,938 (38,303)	48 (207)	0.24 (0.54)
Addis Ababa	12,088 (81,944)	60 (123)	0.05 (0.15)
Kombolcha	47,077 (-------)	211 (---)	0.40 (-----)
Diredawa	21,443 (7,483)	190 (4)	0.88 (0.053)
Debre Zeit	-------- (3,934)	---- (7)	----- (0.017)
Gondar	------ (12,525)	---- (3)	----- (0.02)

Source: Haile mariam, 1975

Numbers in parentheses indicate respective figures for 1973.

2.3 Pathogenesis of Bovine Tuberculosis

Although tuberculosis in cattle can manifest itself in a great variety of ways depending upon the routes of infection and the subsequent immune response, the most frequently occurring form is lesions of the broncho-mediastinal lymph nodes and of the lung, associated with infection via the respiratory route. Microorganisms on small particles such as dust and water droplets that do not impinge against the mucocilliary layer can pass through terminal bronchioles, thus gaining access to alveolar spaces. Here the organisms are ingested by phagocytes, which pass through the lining of the bronchioles, enter the circulation, and then are carried to lymph nodes, and parenchyma of the lung. Following ingestion of the bacilli, lysosomes fuse to the phagosomes to form phagolysosomes, and it is there that phagocytes attempt to destroy the bacilli. The hydrolytic enzymes present and released into the phagosomes fail to kill the bacilli, the later multiply and destroy the phagocytes. The cell-mediated immune responses mediated by T-lymphocytes, in an attempt to control the disease, produce lymphokines which immobilize and activate additional blood borne mononuclear cells at the sites where virulent mycobacteria or their products exist, resulting in the accumulation of large numbers of phagocytes. The persistence of mycobacterial antigens and their indigestible waxes in macrophages and the high turnover rate of macrophages in lesions, result in chronic inflammation and granuloma formation characteristic of tubercle (Pritchard, 1988).

The persistence of tubercle bacilli in the macrophages also acts as a source of antigen for antibody production; these may result in antigen-antibody complexes, which trigger a variety of immuno-pathological manifestations. The cellular hypersensitivity that develops contributes to cell death and tissue destruction (caseous necrosis). In some instances, liquefaction and cavity formation occur due to enzymatic action on proteins and lipids. Rupture of these cavities into the bronchi allows aerosol spread of the bacilli (Thoen and Bloom, 1995).

2.4 Pathography

Signs exhibited by tuberculous animals depend up on the extent and location of lesions. The general signs are weakness, erratic appetite, dyspnoea, emaciation, and low-grade, fluctuating fever. Enlarged superficial lymph nodes provide a useful diagnostic sign. When lungs are extensively involved, there is commonly an intermittent, hacking cough. The principal sign of tuberculosis commonly is chronic wasting or emaciation that occurs despite good nutrition and care (Thoen and Bloom, 1995).

2.5 Immunity Against Tubercular Infections

Although mycobacteria initially encounter normal humoral components and granulocytes, the activities of activated mononuclear macrophages are considered to be more important in protecting the host. Macrophages are involved in processing mycobacterial antigens and in presenting antigens to T-lymphocytes. This involves a number of specific receptor-ligand interactions. The most widely studied of these involve complement receptors CR1, CR3 and CR4, which are considered to be key recognition units in the immune response to mycobacteria. The subsequent interaction of macrophages and lymphocytes with specific antigens stimulates the release of soluble substances, lymphokines, that attract, activate and increase the number of mononuclear cells at the site of infection (Thoen and Bloom, 1995; Thoen and Chiodin, 1993).

T-cells are regarded as pivotal in immune responses to mycobaceria, and all major T-cell subsets participate; CD8+ cells can cause lysis of cells infected with mycobacteria, cytotoxic activity has also been demonstrated with CD4+ cells, T-helper type-2 cells produce IL-4, necessary to regulate antibody production, and type-1 cells produce IFN-γ and IL-2, and are associated with resistance (Pollock and Anderson, 1997; Pollock *et al.*, 1996; Stanford and Stanford, 1996; Thoen and Chiodin, 1993). Type 1 and type-2 cells operate in a reciprocal fashion, whereby cellular and humoral immune responses are mutually antagonistic.

In general terms, cell-mediated immunity (CMI) occurs following T-cell recognition of processed mycobacterial antigens in association with major histocompatibility complex (MHC) products on antigen presenting cells. This interaction may cause activation of anti-mycobacterial capacities in macrophages, via the release of cytokines by the T-cells. Furthermore, blood monocytes, which have greater anti-mycobacterial capabilities than resident macrophages may be attracted into the lesions (Neill *et al.*, 1994; Thoen and Chiodin, 1993). Thus, CMI may benefit the host by initiating processes, which may clearly destroy or inhibit mycobacteria. Alternatively, similar cellular interactions may lead to a delayed-type hypersensitivity (DTH) with significant tissue necrosis. As in the case of beneficial CMI, DTH may also inhibit tubercle bacilli but in this macrophages containing replicating organisms are destroyed. This releases mycobacteria from their protective and favourable environment possibly allowing phagocytosis by the activated macrophages. However, the cellular destruction also contributes to pathogenesis through tissue damage and/or spread of organisms. Factors leading to necrosis include potentially toxic concentration of cytokines, release of toxic factors from degraded bacilli and disintegrating macrophages, ischaemia and local activation of complement by immune complexes (Neill *et al.*, 1994).

2.6 Pathology of Bovine Tuberculosis

Gross descriptions of lesions of bovine tuberculosis have been documented by Pritchard (1988). A primary lesion or focus of infection is established following interaction of host and pathogen. This primary lesion, together with the lesion in the regional lymph nodes is termed the 'primary complex'.

The routes by which cattle are infected, together with the host immune response and the virulence of the organism determine to a major extent the manifestation of this disease. Following aerogenous infection in cattle, the primary complex is usually found in a subpleural location in the dorsal portions of the diaphragmatic lobes of the lung and its associated lymph nodes. Initial foci may coalesce to form larger areas of caseous bronchopneumonia and further intrapulmonary dissemination may occur via airways, by lymphatic spread or by haematogenous spread. If haematogenous spread occurs, this may lead to further lesions in the lungs, spleen, bone marrow, liver, kidneys, adrenals, testes, uterus, udder, meninges or serous cavities. Infection through the skin will usually be limited to the initial site; in congenital tuberculosis, the primary complex is in the liver and portal lymph node. Intestinal lesions usually arise from ingestion of heavy bacterial load but also may arise from dissemination of primary complexes in the lung (Neill *et al.*, 1994). The gross appearance of the tubercle is usually that of a firm white or yellowish nodule. On section, a yellowish caseous necrosis is common (Thoen *et al.*, 1995).

2.7 Diagnosis of Bovine Tuberculosis

2.7.1 The Tuberculin Test

Intradermal tuberculin testing is the usual method recommended by the Office International des Epizooties (OIE) for detecting tuberculous infection in cattle. Tuberculin was used for the first time by Robert Koch in 1891 in an attempt to develop a treatment for tuberculosis. Over the years, different reagents and techniques have been used, ranging from subcutaneous injection of Koch's Old Tuberculin (OT) prepared from *M. tuberculosis*, with assessment of the test by determining the animal's temperature on repeated occasions, to measuring the degree of tissue reaction in millimeter at the injection site.

2.7.1.1 The Tuberculin

Koch`s Old Tuberculin (OT) was produced by growing tubercle bacilli on glycerol broth, removing the bacteria, reducing the volume of broth to one tenth by evaporating and passing it through a sterilising filter. This old tuberculin was diluted 1:4 to give the intradermal tuberculin used for testing cattle. Koch`s Old Tuberculin was known to be a complex mixture which varied considerably from batch to batch.

Heat Concentrated Synthetic Medium (HCSM) tuberculin was produced in the same way as OT (heat concentrating the filtrate to one third of its original volume) but the nitrogen for bacterial growth is produced by asparagine and the resultant tuberculin is unmixed with protein from the culture media.

Further improvement in quality followed the description of techniques for the precipitation of tuberculin with ammonium sulfate or trichloroacetic acid. This allowed for production of a more standardized product, and such tuberculin is described as `Purified Protein Derivative`(PPD). Although PPD tuberculins are described as `pure` they are a complex mixture of protein, lipids, sugars and nucleic acids including a great variety of antigens, many of which are common to several mycobacterial species (Francis *et al.*, 1973; Haagsma and Angus, 1995; Monaghan *et al.*, 1994).

2.7.1.2 Tuberculin Reactions

The tuberculin reaction in infected cattle is a delayed type hypersensitivity (DTH) reaction which is maximal at approximately 72 hours after the injection of tuberculin (Francis *et al.*, 1978). Delayed type Hypersensitivity (DTH) reactions are mediated by a population of sensitized T cells and such reactions are considered to have specific and non-specific components (Monaghan *et al.*, 1994; Thoen and Chiodin, 1993). The specific component is believed to be dependent on interaction between previously sensitized T cells expressing the CD4 phenotype, and antigen presented by macrophages in association with MHC class II molecules. The non-specific component is triggered by the release of cytokines produced by activated T cells and these cytokines are thought to be responsible for the tissue damage, which is a feature of a reaction.

2.7.1.3 Tuberculin Tests Currently in Use

There are two types of tuberculin tests in use today; both rely on the response to intradermal injections of tuberculin with an assessment of the injection site for swelling 72 hours post injection.

The Single Intradermal Tuberculin (SIDT) test, using bovine tuberculin is carried out in the skin of the neck, as is the case in Europe, or in the skin of the caudal-fold, as it is applied in North America, Australia and New Zealand (Monaghan *et al.*, 1994). In the more popular test at the skin of the neck, 0.1ml of approved bovine PPD is injected intradermally in the middle third of the neck after the hair is clipped.

The Comparative Intradermal Tuberculin (CIDT) test, using avian and bovine PPD is applied by simultaneous injection of both avian PPD (0.1ml, 2500IU) and bovine PPD (0.1ml, 5000IU) on the same side of the neck, 12-15 cm apart. A positive result is recorded when the bovine reaction is greater than the avian counterpart (Monaghan *et al.*, 1994).

2.7.1.4 Diagnostic Value

2.7.1.4.1 False Negative Reactions

Recently infected cattle fail to react to tuberculin: reactivity is usually not apparent for 30-50 days following infection (Monaghan *et al.*, 1994). In addition it has long been recognized that some cattle, including many with severe or generalized disease may not react to tuberculin (Lepper *et al.*, 1977), as it is true with stresses associated with malnutrition, concurrent infections and depressed immune responsiveness seen during the early post-partum period (Collins, 1994; Kantor, 1984). This state is referred to as anergy, and the reasons for its occurrence are poorly understood.

Desensitization (a reduction in the ability of a tuberculous animal to react for a period following injection of tuberculin), the use of tuberculin of low or reduced potency and injection of insufficient amounts of tuberculin are other problems known to occur since tuberculin was first used (Doherty *et al.*, 1995; Lepper *et al.*, 1977; Monaghan *et al.*, 1994). To overcome these problems, a period of 42-60 days is left between tests, vials of tuberculin should only be used on the day they are opened, and the use of multiple dose syringes is discouraged.

2.7.1.4.2 False Positive Reactions

Cattle, which are classified as reactors to the tuberculin test and fail to show evidence of infection at slaughter, are often termed no visible lesion (NVL) reactors. Although this problem can be greatly reduced by detailed examination of the carcass (Corner *et al.*, 1990), NVL animals can be encountered even with good abattoir techniques, attributable to non-specific reactions. The causes of such non-specific reactions include paratuberculosis with/or previous infection with *M. avium*, skin tuberculosis or exposure to environmental mycobacteria or related organisms (Monaghan, 1994). Despite the fact that there are a number of causes of non-specific reactions to tuberculin test in cattle, such reactions occur in only a small proportion of animals (see Table 4).

Table 4: Results of studies on the sensitivity of single comparative tuberculin test

Study	No. infected cattle	Reactors (%)	doubtful (%)	Sensitivity(%)*
Kerr et al. (1946b)	300	91		91
Kerr et al. (1949)	201	68	14	82
Paterson et al. (1958)	82	63.4	24.3	87.8
deJongh et al. (1969)	151	40	37	77
Leslie et al (1975)	58	62	29	91
O'Reilly et al. (1975)	91	68.8	26.2	95
O'Reilly et al. (1986)	68	62	28	90

Adapted from Monaghan *et al.*, 1994

* Sensitivity is a measure of the ability of a test to correctly identify M. bovis-infected animals and is usually expressed as a percentage (O´Reilly, 1995).

$$\text{Sensitivity (\%)} = \frac{\text{True Positives}}{\text{True positives + False Negatives}}$$

2.7.2 Post mortem Inspection

Postmortem examination of cattle and bacteriological examination of lesions are critical steps in the diagnosis of bovine tuberculosis. A tentative diagnosis of bovine tuberculosis can be made following the macroscopic detection and histopathological examination of the lesions. The sensitivity of gross post mortem examination is affected by the method employed and the anatomical sites examined. Since most tuberculous infections in cattle are acquired by inhalation (McIlroy *et al.*, 1986), between 70-90% of lesions are found in either the lymph nodes of the head or in the thoracic cavity. However, because tuberculosis is a disease affecting the reticulo-endothelial system, lesions may occur in lymph nodes in virtually any anatomical region of the body, hence a wide range of lymph nodes need to be examined. Corner et el. (1990) determined the distribution of lesions in 374 tuberculous animals in order to optimize the postmortem examination procedure (Table 5). From the data it was ascertained that 66% of tuberculous cattle had one lesion only, and 86% of these could be identified by examination of only 3 pairs of lymph nodes (mediastinal, medial retropharyngeal and bronchial) and of the lungs. Examination of 3 additional pairs of lymph nodes enabled the detection of 95% of animals with lesions (Corner, 1994; Corner *et al.*, 1990).

Table 5: The location of the most frequently affected tissues in cattle with single lesions of tuberculosis

Tissue	% of lesioned cattle detected
Medial retropharyngeal ln (left and right)	29.4
mediastinal ln (anterior and posterior)	28.2
Bronchial ln (left and right)	18.0
Lung	9.8
Mesenteric ln	2.9
Parotid ln (left and right)	2.4
Caudal cervical ln (left and right)	2.4
Superficial inguinal (left and right)	1.2
Proportion of all cattle with single lesions that were detected	94.3

ln= lymph node

Source: Corner *et al.*, 1994

2.7.3 In Vitro Cellular Assay

Works on the development of in vitro methods to measure the T-cell reactivity of cells from *M. bovis* infected cattle focussed on the lymphocyte transformation tests (LTT). The method involves incubating lymphocytes in the whole blood diluted with tissue culture media for 3-5 days, and then using radio active nucleosides to detect the level of cell proliferation (Wood and Rothel, 1994). These conditions meant that while the lymphocyte proliferation assay was useful for research purposes, it was far too complex, costly and slow for use in routine diagnosis.

2.7.4 γ-Interferon assay

The γ-interferon test is an enzyme linked immunosorbent assay (ELISA) used to detect bovine γ-interferon in plasma from infected bovine whole blood, cultured with *M. bovis* and *M. avium* PPDs (Rothel *et al.*, 1990). Wood *et al.*, (1990) and Wood (1994) claimed γ-interferon assay as a simple and rapid in vitro cellular assay for the diagnosis of bovine tuberculosis, and maybe a practical replacement for the single intradermal tuberculin test. The major advantages of the γ-interferon assay would be that it does not compromise the immune status of the animal thus making immediate re-testing of suspect animals possible, and that test results are available within as short as 24 hours.

2.7.5 Serological Diagnostic Tests

It is well established that tuberculosis in both humans and cattle induces a strong cellular response, and in a majority of infection the disease is controlled by a protective cellular immunity (Thoen and Chiodin, 1993). It also has long been frequently reported that antibody response in tuberculosis infections is low, and when present, does not have any significant protective role (Fifis *et al.*, 1994). Despite this, many investigators have attempted to produce a simple diagnostic antibody test that would replace the intradermal tuberculin test.

During the last decade, the enzyme-immunoassay was the method most frequently evaluated (Baron *et al.*, 1994; Hanna *et al.*, 1989; Hanna *et al.*, 1992; O'Reilly, 1995). Serological tests using crude mycobacterial antigens are known to have both low sensitivity and specificity, and intensive surveys have been conducted to look for species-specific epitopes on mycobacterial antigens. The report of MPB70 (22kDa) and MPB64 (24kDa) antigens as being specific to *M. bovis* (Fifis *et al.*, 1994) gave new hope that more specific diagnostic tests were possible. Although serological tests detected some tuberculous animals that were anergic to the single intradermal test, most of the findings with the identified antigens however failed to show any superiority to the existing cellular tests (Auer and Schleehauf, 1988). The weak humoral response inherent to natural infection, the wide cross-reactivity with immunodominant mycobacterial antigens and the genetically controlled diversity of individual responses to mycobacterial epitopes are largely responsible for the poor correlation observed between the serology and those obtained by bacteriology (Wood and Rothel, 1994).

2.7.6 Bacteriology

2.7.6.1 Specimens

In order to obtain the best results from the bacteriological examination of specimens, the specimens must be presented to the laboratory in the best possible condition. For the detection of the greatest number of viable mycobacteria, e.g. *M. bovis*, in stored specimens, specimens should be chilled to 4-6°C and cultured within 24-48 hours of collection; freezing is recommended for any period longer than this (Corner, 1994). Giving this condition, the main factors influencing the success of primary isolation of mycobacteria from clinical specimens are the culture media, the decontamination procedure and the incubation condition.

2.7.6.2 Culture media

Field strains of mycobacteria require enriched media for growth on primary isolation. The media used are either egg-based (e.g. Stonebrink`s medium and Löwenstein-Jensen), or agar based enriched with serum and /or blood (e.g. modified-Middlebrook7H11 medium) and tuberculous blood agar medium, also called B83 (Buxton and Fraser, 1977).

2.7.6.3 Decontamination

Digestion and decontamination of specimens is frequently necessary to release the organism from body fluids and cells, and to permit the isolation of mycobacteria from contaminated specimens (Corner, 1994). The ideal reagent used for decontamination should have a minimum toxicity for mycobacteria but maximum toxicity for other contaminating organisms. Generally, hexaadecylpyridinium chloride (HPC) at both 0.075% and 0.75%w/v, oxalic acid 5%w/v and sodium hydroxide 2-4%w/v are commonly used.

2.7.6.4 Incubation Conditions

Laboratory cultures of tubercle bacilli under optimal conditions usually remain viable for at least six months, but it is advisable to transplant cultures at regular intervals to assure viability (Karlson, 1983). Since the organism must be incubated longer than more rapidly growing bacteria, the media, must be protected against desiccation. Glycerine reduces the rate of drying, screw cap tubes or small bottles that prevent evaporation are commonly used, and culture tubes may be opened and a few drops of sterile water are added in order to maintain a moist atmosphere.

2.7.7 Biochemical Tests

Not all mycobacteria isolated from clinical material are tubercle bacilli. Some are species that normally exist as environmental saprophytes but occasionally become opportunist pathogens. The search for a simple test to identify reliably a strain such as *M. tuberculosis* and, preferably, to distinguish human from bovine variants has not been a fruitful one (Collins and Grange, 1983). There are a number of biochemical tests for *M. bovis* (Corner, 1994), the most useful being the Niacin production and Nitrate reduction tests, and susceptibility to Tiophene-2-Carboxylic acid hydrazide. As no single method is entirely reliable, combinations of tests are used.

2.7 7.1 Niacin Production Test

Although all mycobacteria produce nicotinic acid, comparative results have shown that because of a blocked metabolic pathway for conversion of free niacin to nicotinic acid mononucleotide, *M. tuberculosis* accumulates niacin and excretes it into the medium. The accumulation of Niacin in the medium caused by lack of an enzyme that converts niacin to another metabolite in the coenzyme pathway is measured by a colored end product. For this purpose a 3-4 week old pure culture, with more than 50 colonies grown on egg-based media will be selected (Baron *et al.*, 1994; Cernoch *et al.*, 1994).

2.7.7.2 Nitrate Reduction Test

The presence of the enzyme nitroreductase can be detected by the ability of a suspension of organisms to produce a colored end product from substrates that combine with nitrite, the product of nitroreductase. *M. tuberculosis* and several other species of Mycobacteria possess this enzyme, but *M. bovis* does not.

2.7.7.3 Susceptibility to Tiophene-2-carboxilic acid hydrazide (T_2H) test

The Tiophene-2-carboxilic acid hydrazide test is used to distinguish niacin-positive *M. bovis* from *M. tuberculosis* and other non-chromogenic slow growing mycobacteria. *M. bovis* is susceptible to low concentrations of T_2H (1-5μg/ml) and therefore, will not grow on the medium, whereas *M. tuberculosis* is resistant and will show growth (Cernoch *et al*., 1994)

2.7.8 Molecular Biology Approach

The advent of genetic engineering has provided alternative DNA-based strategies, which have the potential to overcome non-specific reactions. The development of agarose electrophoresis for the size separation of DNA- fragments, followed by the availability of restriction endonucleases which cleave DNA at defined sites led to the development of restriction fragment analysis of bacterial DNA. This technique produces a pattern of fragments or `finger prints`, which uniquely characterises the strain from which the DNA was isolated. Further development of the technique has provided a wealth of epidemiological information not previously available. Other DNA-based techniques such as Southern blot hybridisation also have the potential to provide a useful typing system (Butcher *et al*., 1996; Collins *et al*., 1994).

2.8 Economic Importance of Bovine Tuberculosis

Even though the determining factor for initiating programmes for the control of bovine tuberculosis was its impact on human health, the disease was also causing massive financial losses due to animal mortality and carcass and offal condemnation (Kantor and Ritacco, 1994). When suitable control measures are not taken, the effects on the economy and health progress slowly and steadily. Direct losses due to the infection become evident by a decrease in productivity, a decrease in beef production, and additional processing costs for tuberculous animals and condemnation at slaughterhouses. Among dairy cattle there also is a decrease in milk (10-18%) and in meat production (roughly 15%). The culling loss is estimated to be 30-50% of the difference between the value of a dairy or beef breeding cow and its value at slaughter (Daborn *et al*., 1996).

Studies done on the economics in Spain indicated that the loss in meat production was 10% in calves born from infected cows, 12% in milk production, 5% due to sterility and about 1.4% due to carcass condemnation (Bernues *et al*., 1997). In Argentina, the loss of milk in tuberculous cows was found to be 18% as a result of delay in the first lactation and a decrease in the number and duration of lactation, as compared to healthy animals (Kantor and Ritacco, 1994). Additionally, in view of the increasing sanitary requirements of importing countries, it is highly probable that markets will be closed for exporting countries with tuberculosis. In Cuba, the elimination of bovine tuberculosis has led to increased supplies of milk (raising actual per capita consumption of fresh milk and milk products to more than 150 lt. a year) and beef (to more than 27Kg a year) (Oritz, 1986).

In Ethiopia the economic losses due to meat condemnation in slaughtered cattle within the 5-year period (1985 to 1990) were equivalent to about 100,000 US $ (Yehualashet, 1995).

2.9 Zoonotic Importance of Bovine Tuberculosis

There are several reviews describing human tuberculosis in developed countries caused by the bovine tubercle bacillus, *M. bovis* (Collins and Grange, 1983; Daborn *et al.*, 1996; Grange and Collins, 1987; Kleeberg, 1984). These reviews indicated that there is usually an association between the number of human cases identified and the prevalence of tuberculosis in the local cattle population.

In the USA, Ravenel in 1902 and later Park in 1913 (cited by Pritchard, 1988) presented incontrovertible proof of transmission of bovine tuberculosis from cattle to man. A Federal Veterinary Control Meat Inspection Service was set up in 1906, and following the recognition that 25% of human deaths from tuberculosis were due to *M. bovis*, an eradication program began in 1917 (Pritchard, 1988).

In 1932, the Cattle Disease Committee (CDC) in Great Britain was appointed to consider measures to reduce disease, especially in dairy cows. Their report stated that bovine tuberculosis was responsible for more than 2500 human deaths annually, and for a still larger incidence of human illness (Rees and Meldrum, 1995).

Savage, 1929 (cited by Pritchard, 1988) readily established that *M. bovis* could cause all the various manifestations of tuberculosis in man, and later reviewed the relative proportions of bovine and human tubercle at different sites. Accordingly, *M. bovis* was isolated from 51% of skin lesions compared to 45% of cervical lesions, 32% of scrofuladerma cases, 27% of meningitis cases, 18% of bone and joint lesions, 17% of genito-urinary samples but only 1% of pulmonary lesions. Transmission via milk to rural and urban populations was the most important mode of spread of *M. bovis*, but skin infections were often occupational, e.g., butcher's wart, conjuctivitis in milkmaids and oral and ear infections in the families of milkers. The role of *M. bovis* in pulmonary disease and other lesions was established for Britain by Yates, Grange and Collins, (1986) who found 1.5% of all tubercle bacilli isolated from man, in the south of England, to be *M. bovis* (Yates and Grange, 1988).

By contrast, there is very little information on the prevalence of human tuberculosis caused by *M. bovis* occurring in developing countries of Africa. Information on the prevalence and distribution of bovine tuberculosis in Africa is equally scarce, but the few reports available indicate that a number of countries including Morocco, Egypt, Ethiopia, Uganda, Tanzania, Zambia, and Malawi have areas with exceptionally high levels of infection (Daborn *et al.*, 1996). As an earliest investigation in Africa, in the year 1913, Carmichael (cited by Pritchard, 1988) isolated a total of four cultures proved to be *M. bovis*, all from patients with a history of exposure to cattle in Uganda. In Zaire, *M. bovis* was isolated from five pastoralists admitted with pulmonary tuberculosis (Daborn *et al.*, 1996). The authors concluded significant public health risk.

For Ethiopia there is equally scarce epidemiological information about the impact of this zoonosis on human health. The finding of 4 bovine-types isolated from 131 milk samples taken from tuberculin positive cows, and isolation of 7 from 486 raw milk samples (Yehualashet, 1995) were the earliest signals for the danger of this particular zoonosis in Ethiopia. *M. bovis* was isolated from 1 of 247 human sputum samples in the same study.

Conversely, the transmission of *M. bovis* from man to cattle has led to explosive herd out-breaks. Spread from man to cattle has most frequently been by urinary contamination of hay or straw bedding, but it may also occur by aerogenous route or by faecal or sewage contamination of pasture, hay or bedding (Pritchard, 1988). Some workers previously have regarded man as the principal source of outbreaks of tuberculosis in cattle. Sjorgen and Hillirdal in 1978, (cited by Thoen and Steel, 1995), cited several examples of man-to-cattle transmission of the disease and, stress the danger of patients with smear positive pulmonary tuberculosis due to *M. bovis*. The reviews include that 107 of 299 patients with tuberculosis due to *M. bovis* (193 of who had pulmonary disease) were the source of infection of cattle in 128 herds, resulting in the slaughter of 1182 animals.

The source of infection of cattle in Germany has been studied extensively by Schliesser and colleagues. In the state of Hessen, where bovine tuberculosis was virtually eradicated by 1961, 12 patients with tuberculosis due to *M. bovis* infected 114 cattle in 16 herds between 1968 and 1972. One patient had open pulmonary disease but 9 had genitourinary disease and one of the latter infected 48 cows in 4 different herds. During the subsequent period 1975 to 1980, tuberculous farm workers were responsible for 5 out of 28 (18%) infected herds of cattle in the same region (Grange, 1996; Grange and Yates, 1994).

2.10 Control of Bovine Tuberculosis

2.10.1 Therapy

The advent of effective chemotherapy in the 1940s had a dramatic effect on the death rate from tuberculosis in man and proved effective prophylactically for contact of human cases. A wide variety of drugs are now used in different combinations of first and second line regimens, depending on the antibiotic-resistance pattern of isolates, the type of disease and the patient's response. Chemotherapy in man is usually required for over 6 months and the necessity for daily administration of drugs over a long period has limited its application to animals; although chemotherapy with isoniazid has been shown to be effective, for a variety of reasons it has not been incorporated into any national program (Thoen *et al.*, 1995).

2.10.2 Vaccination

The high prevalence of tuberculosis in European cattle at the end of the 19th century and the realisation of the enormous costs of the stamping-out policy led to the investigation of vaccination in Germany, England and France. Studies soon showed that live bacilli were more effective than killed bacilli or tuberculin in increasing the resistance of calves. The most widely used vaccine on the continent was `bovo vaccine`, containing live human type bacilli. The demonstration (by Griffith, 1913) that the vaccine could result in the excretion of the virulent human type bacilli in the milk cows however led to its abandonment. Shortly afterwards the attenuated bovine-type bacilli including Bacille Calmette-Guerin (BCG) were developed. Initial limited experimental trials on this BCG vaccine in cattle did indicate that it might be useful, and it was extensively used as an oral vaccine in man and cattle in many countries; however, it may also be secreted in the milk of cows. As it is necessary to vaccinate calves at birth to protect them from exposure for several months and because of vaccine-induced sensitisation to the only reliable test, vaccination therefore is regarded a hindrance to control (Pritchard, 1988).

The problem of high prevalence of tuberculosis in many developing countries in Africa, Asia and South America however has not been amenable to control by test and slaughter methods and so attention has turned once again to BCG. In Malawi, extensive controlled field trials applying BCG vaccine without either culling clinical cases or isolating reactor cattle have found no effect on tuberculin reaction, lesions at necropsy nor prevalence of *M. bovis* in lesions (Berggren, 1981).

2.10.3 Test-and -Slaughter Scheme

The strategy generally adopted worldwide for the control of bovine tuberculosis is diagnosis, herd isolation and the slaughter of infected animals to prevent transmission. Thus, effective control of bovine tuberculosis has only been achieved by the implementation of a Government-controlled scheme consisting of a combination of systematic testing and removal of infected animals, prevention of spread of infection and avoidance of introduction of disease (Bernues *et al.*, 1997; Thoen and Steel, 1995). Each country following this path has tailored its scheme to local conditions. Most countries started by introducing compensation for 'open cases' followed by a voluntary scheme for individual herds with monetary incentives. When such schemes had produced suitable areas with a pool of tuberculosis-free cattle in attested herds and when the prevalence of reactors has reduced to a suitable rate, then a compulsory area eradication plan is introduced.

3. MATERIALS AND METHODS

3.1 Study Area

The study was conducted in-and-around Addis Ababa, between March and July 1999. Administratively Addis Ababa area is divided into 6 zones, numbered 1-6, which further break into 28 'woredas', numbered 1-28 (Table 6).

Table 6: Administrative division of the Addis Ababa (study) area

Zone	Woredas	Total no. of woredas in a zone	Remark
1	3, 4, 5, 6	4	
2	20, 21, 22, 23, 24	5	
3	17, 18, 19, 28	4	
4	1, 9, 11, 12, 13, 15, 16	7	
5	2, 7, 8, 10, 14, 25	6	
6	26, 27	2	
7		1	located outside the described study area at the out skirts of town

Geographically, the area is located at an elevation of about 2,400 metres above sea level (masl). The average annual temperature is 16.1°C, and the precipitation is well above 1800 mm^3.

3.2 Study Population

According to the 1994 census, there were 58,568 heads of cattle in the area, distributed in 5167 farms. The average animal number per farm was 11.36. Composition-wise cows made up 39.6%, heifers 14.6%, bulls and oxen 15.7%, and calves and young-males make-up the balance. A large proportion of animals in these farms, 53.5%, has different levels of exotic blood. The population of cattle in different zones of the study area is presented in Table 7.

Table 7: Distribution of farms and cattle in different zones of Addis Ababa.

Zone	No.of farms	Proportion	No.of cattle	Proportion
1	166	3.2	798	1.4
2	1449	28	11492	19.6
3	1163	22.5	22554	38.5
4	997	19.3	4556	7.8
5	797	15.4	3569	6.1
6	595	11.5	15609	26.7
Total	**5,167**		**58,568**	

One hundred and sixty three private dairy holders have organized themselves into the 'Addis Ababa Dairy Producers Association'. These dairy farms make-up about 2.32% of total farms; they are distributed unevenly in different zones (Table 8). The dairy animals kept are primarily pure breed exotic cattle, which have been produced through artificial insemination.

Table 8: Number of dairy farms in Addis Ababa Region under the 'Dairy Association'

Zone	No.of Woredas/districts	No. of farms	proportion	No. of cattle	proportion
1	4	9	5.52	85	6.28
2	5	9	5.52	67	4.95
3	4	68	41.72	487	35.97
4	7	55	33.74	453	33.47
5	6	21	12.88	257	18.98
6	2	1	0.61	5	0.37
Total	**28**	**163**	**100**	**1354**	**100**

3.3 Cattle Selection

Two zones (3 and 4, Table 8) were selected from the six zones, on the ground that about 76% of the farms registered under the 'Association' are located there. Within the two zones, all farms and all animals within each farm (with the possible exception of calves under three months age and about three weeks long pre-and-post partum cows) were selected for the study. The cattle population consisted mainly of exotic breeds (Holstein-Friesian and Jersey). Five herds belonging to one national research station additionally were included, containing Boran and Friesian/Jersey-Boran crosses. The single intradermal cervical tuberculin test was performed on all the study animals.

3.4 Data Collection

Each of the farms was sequentially numbered according to the date and time of visit. The size of the farm, farm conditions and locations were recorded. Location was denoted as peri-urban or urban, depending on whether-or-not the farm was located at the periphery or in the vicinity of the city, respectively; management conditions of the farms were categorized into classes of good or poor, based on the cleanliness of the barns, watering and feeding system, herding patterns and stocking density. Clean farms with sufficient aeration, reasonable stocking density per unit area, good drainage and waste removal and individual feeding and watering practices were categorized as having good management. The owners were also interrogated for the duration of the dairy operation and for the possibility of introducing animals from other farms.

In each of the farms, animals were identified by the owners/workers. The individual animal ear tag number or the animal's name was recorded and each animal was given a sequential identification number within the herd. Breed and sex were recorded. Age was recorded for calves, yearlings and male animals and parity was registered for cows. In case of uncertainty as to the animal's age or parity, this particular information for that animal was omitted from the analysis.

Body condition scoring of all eligible animals was made using a method developed for dairy cattle (Radostits *et al.*, 1994). Briefly, animals were thoroughly inspected for their body condition. Five scores were used in which the three main scores (fat, medium and lean) were divided into different categories. Each animal was given a numerical value from 1 (lean) to 5 (fat):

Lean	1, 1.25, 1.5, 1.75, 2, 2.25,
Medium	2.5, 2.75, 3, 3.25, 3.5,
Fat	3.75, 4, 4.25, 4.5, 4.75, 5.

Anatomical structures such as the tail head, brisket and transverse processes of the lumbar vertebrae, the ribs and hips were used as references for body condition scoring.

3.5 Tuberculin Test

Cattle were restrained and the hair was clipped from the lateral side of the middle part of the neck. Each of the animals was then injected with bovine purified protein derivative (PPD), prepared from strain AN_5, produced by W.D.T., Hoyerhagen, Germany. Pre-set syringes (0.1 ml) were used to inject 50,000 I. U. of tuberculin per ml, intradermally with a short sterile needle inserted obliquely into the deeper layer of the skin on the neck as recommended. The skin fold thickness was measured pre and approximately 72 h post injection using a dial type caliper and recorded. An increase in skin fold thickness of 4 units and above (Community legislation, Directive 80/219, EEC) was taken as a positive reaction (Monaghan, *et al.*, 1994).

3.6 Bacteriology

Milk samples were collected from previously washed udders into 50-ml sterile universal tubes as previously described (Kazwala *et al.*, 1998). Nasal swabs were also taken using sterile cotton tipped applicator sticks in 20-ml centrifuge tubes (the choice of herds or animals from which milk or nasal swabs were collected was based on the preliminary tuberculin test outcome i.e., ≥ 5 units). The samples were kept in a cool box and transported to the laboratory, stayed over night at 4°C prior to processing for subsequent cultivation. Processing for cultivation was conducted in the Microflow Biological Safety Cabinet (MDH LTD. Hampshire), unless otherwise indicated.

3.6.1 Decontamination

About 30 ml of the milk sample was transferred into a sterile tube and centrifuged at 3000 g for 15 minutes at 4°C. The supernatant was discarded and the sediment re-suspended using 2 ml of sterile water. The swab was also put in a sterile tube into which 2 ml of sterile water was added. Into each tube with milk and swab, 2 ml of NaOH (2%) was added for decontamination (Corner, 1994) and left at room temperature for 15 minutes. Neutralization was effected using concentrated HCl. The neutralization process was monitored by adding a drop or two of phenol red, and was assumed achieved when the suspension color changed to light pink.

3.6.2 Cultivation

Neutralized suspensions were similarly centrifuged (as above), the supernatant discarded and about 2 ml of the milk and nasal swab sediments, pooled for individual animal, was used as an inoculum for the cultivation of mycobacterial species. Primary isolation of mycobacteria was done on Löwenstein-Jensen (LJ) media with pyruvate or glycerol. For cultivation, about 1 ml of the sediment from each sample was seeded onto the surface of each of the culture medium and incubated at 37 °C, at an angle for the first week and in upright position for up to 11 weeks; weekly visible growth was observed. Positive cultures were sub-cultured onto another set of media and incubated for another 4-6 weeks for further identification.

3.6.3 Identification

Smears from the sediment before decontamination and from cultures with evidence of growth were taken; air-dried and heat fixed by passing several times through the Bunsen flame. The smears were stained by the Ziehl-Neelsen method for microscopic examination of acid-fast bacilli as described (Cernoch *et al.*, 1994). Briefly, the heat fixed smears were stained with carbol-fuchsin, heated gently to steaming and left on the rack for about 10 minutes. The stain was poured off and the slides were washed with tap water. Decolorisation was effected by keeping the slides in 25% sulfuric acid solution and 96% ethyl alcohol for 1 minute each, in this order and washing the slides with tap water in-between. The smears were then counterstained with methylene blue solution for further 3 minutes, washed with tap water, air dried and examined by light microscope (Karl Zeiss), employing a 100× objective with oil immersion. The presence of acid fast bacilli in the smears was assumed indicative of mycobacterial infection. Further characterization was done by selected biochemical tests.

3.6.4 Characterization (Baron *et al.*, 1994)

3.6.4.1 Niacin Production Test

About 1 ml of sterile water was added with a pipette onto the surface of the medium on which visible colonies of about 4 week-old Mycobacteria existed. The surface of the medium under the colonies was slightly poked with a sterile pipette to allow niacin in the medium to dissolve in the water. The medium was left at room temperature from 30 minutes to 2 hours, then about 0.6 ml of the water was transferred to a clean screw-cap tube after which 0.5 ml of aniline dye and 0.5 ml of cyanogen bromide were added. The test tube was inspected for the development of a yellowish color change immediately; the color change is supposed to indicate positive result in comparison to both positive and negative controls.

3.6.4.2 Nitrate Reduction Test

About 1 ml of sterile water was placed in a screw-capped tube and a loop-full of a young culture of Mycobacterium was added. After addition of 2 ml NaNO3 solution (0.01M solution NaNO3 in 0.022M phosphate buffer, pH 7) the tube was well shaken, and incubated in a water bath at 37°C for 2 hours. Then step wise:

- 1 drop of 1:2 dilution of conc. HCl
- 2 drops of 0.2 per cent aqueous solution of sulfanilamide and,
- 2 drops of 0.1 per cent aqueous N-(1-naphthyl) ethylenediamine dihydrochloride were added, and the tube examined for the development of a pink to red colour in comparison with both positive and negative controls.

3.7 Data Analysis

The prevalence rate was calculated on the basis of tuberculin reactivity, dividing the number of reactors by the total number of animals tested. Similarly, herd level prevalences were computed as the number of herds with at least one reactor divided by the total number of herds tested. The within-herd prevalence was calculated by dividing the number of reactors in a herd by the total number of animals tested in that herd.

For statistical analysis a two-stage process was applied. In the first stage measures of association between each determinant and the outcome variable were calculated as an unadjusted odds ratios (OR). These were tested for statistical significance by a X^2 test for independence (Statgraphics Plus 2.1 and Microsoft Excel), and after direct standardization. In the second stage, putative risk factors were evaluated using unconditional multiple logistic regression, using SPSS. Reduction procedure module was built, by use of the stepwise backward procedure, with removal based on the likelihood-ratio statistic and $p<0.05$.

4. RESULTS

4.1 Herd Level

During the survey 93 herds from commercial `Dairy Association` and 5 herds from ´National Research´ farms were tested. Table 9 gives the number of farms selected in each zone and summarises the tuberculin test results. Overall, 47 herds (48%, CI=37.75-58.29%) contained at least one reactor-animal. Such herds were defined as positive herds (Table 10). It can be observed also that (Table 9) reactivity to tuberculin test varied significantly among farms (X^2=13.37, p=0.001) and among animals with-in farms (X^2=12.64, p=0.002) in the study zones. This difference was evident also in the subsequent multivariate analysis (Table 11).

Table 9: Number of farms studied and summarized cattle tuberculin test results

	No. of farms				No. of cattle			
Zone	Tested	Positive	% positive (95% CI)	X^2 (p-value)	Tested	Positive	% positive (95% CI)	X^2 (p-value)
3	42	15	35.7(21.6-52.0)		501	38	7.6 (5.3-10.03)	
4	46	22	47.8(32.9-63.1)		378	56	14.8 (11.3-18.8)	
Others	10	10	100 (71.5-100.0)		362	34	9.4 (6.83-13.1)	
Total	**98**	**47**	**48 (37.8-58.3)**	**13.37 (0.001)**	**1241**	**128**	**10.31 (8.7-12.14)**	**12.64 (0.002)**

Large size herds did possess more reactors compared to medium size herds, the later containing again more reactors than small size farms, i.e. 100, 68.2 and 30.1%, respectively (Table 10). This difference was statistically highly significant (X^2=25.71, p<0.001). This finding was also supported by multivariate analysis (Table 11), where herd size was found to be significantly associated (p<0.001) with tuberculin reactivity after adjusting for other confounding factors. Additional test involving regression and correlation between herd size and proportion of reactors (Fig. 1.) indicated moderately strong positive correlation (r=0.55), i.e., as herd size increases the proportion of animals within a herd showing a positive reaction also increases.

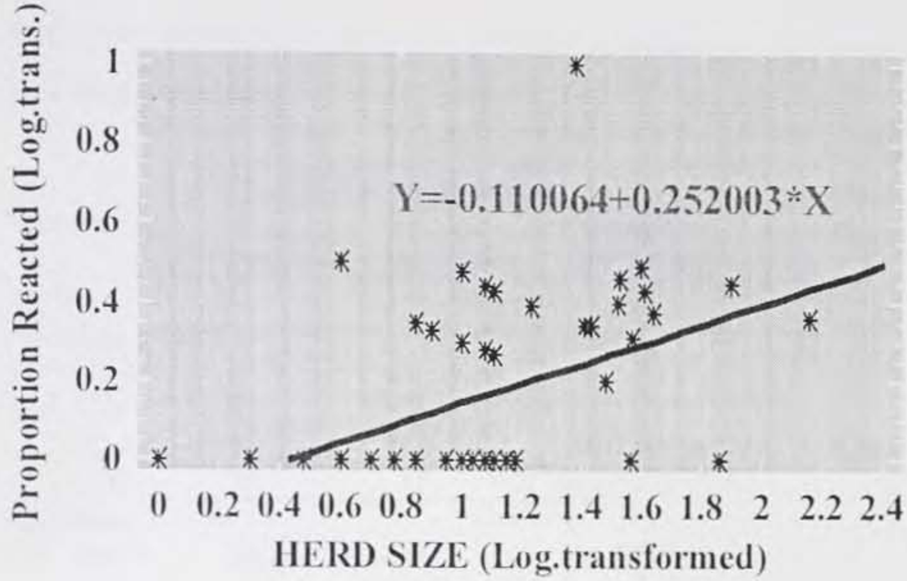

Fig. 1: Relationship between herd size and reactors proportions in a herd

Farms in which mixing of animals from different sources (purchase, grazing, mating etc.) was possible did harbor more reactors than isolated farms (63.4% vs. 36.8%). Similarly, older farms did possess a higher rate of reactors than newly or recently established farms (64.5% vs. 20%). These differences each were statistically significant (X^2=6.75, p=0.01 and X^2=13.64, p=0.001, respectively) at the univariate analysis level. Adjustments made to remove possible confounding effects of other factors in the series (Table 11) however rendered mixing of animals and farm establishment not significantly associated with herd tuberculin reactions ($p>0.1$).

Reactor rates of 54.3% and 44.4% were recorded for farms under poor and good management conditions, respectively. Likewise, reactor rates for herds located in the vicinity (urban herds) were slightly higher than for farms located at the periphery (peri-urban herds), of the city (51.4 and 46.0%, respectively). These rates in regard to farm location were not significantly different ($p>0.1$) even after adjustments have been made, but reactors rates did differ significantly between farms under poor and good management systems (p=0.02, Table 11, multivariate analysis), indicating that this factor was confounded in the initial univariate analysis (Table 10).

Table 10: Association between positive reaction to the tuberculin test in a farm and recorded variables

Variable	Tuberculin Test Positive	Tuberculin Test Negative	% positive (95% CI)	Odds Ratio (95% confidence interval)	X^2 (p-value)
Farm establishment in years					**13.64 (0.001)**
<10 *	6	24	20.0 (7.71-38.57)	1	
10-20	20	16	56.0 (38.1-72.1)	5.0 (1.65-15.19)	
>20	20	11	65.0 (45.37-80.8)	7.3 (2.28-23.16)	
Location					**0.26 (0.61)**
Peri-urban	29	34	46.0 (33.4-59.1)	1	
Urban	18	17	51.4 (34.0-69.6)	1.2 (0.54-2.84)	
Herd size					**25.71 (<0.001)**
<10 *	19	44	30.1 (19.2-43.0)	1	
10-24	15	7	68.2 (45.1-86.1)	5.0 (1.76-14.00)	
≥25	13	0	100 (75.3-100)	3.3 ϕ	
Mixing of Animals					**6.75 (0.01)**
No	21	36	36.8 (24.4-50.7)	1	
Yes	26	15	63.4 (46.9-77.8)	3.0 (1.30-6.90)	
Management condition					**0.87 (0.35)**
poor	19	16	54.3 (36.7-71.2)	1.5 (0.54-2.84)	
good	28	35	44.4 (31.9-57.5)	1	

* For farm establishment and herd size the risk for each category is compared with a base line reactor group.

ϕ Risk Ratio, Odds Ratio could not be calculated, 0 in one cell

Table 11: Results of the multiple logistic regression analysis at the herd level

Variable	Log Likelihood ratio (LR)	-2 Log LR	DF	Significance of Log LH ratio
Establishment	-43.006	3.239	2	0.1980
Herd size category +	-57.825	25.061	2	0.0000
Management condition +	-46.567	5.614	1	0.0178
Mixing	-43.760	1.507	1	0.2196
Location	-41.387	0.055	1	0.8138
Zone +	-48.222	8.925	2	0.0115

+ Variables proved significant

4.2 Animal Level

During the survey 1241 cattle were tested. Of these 128 reacted to the test, giving an overall reactors rate of 10.31% (CI=8.68-12.14). Specific reactors rates were 3.3% for calves and yearlings, 8.4% for heifers, 19.2% for animals in the 1st through 2nd parity, 11.7% for animals in the 3rd through 5th parity and 4.8% for animals in the 6th and above parity levels (Fig. 2). These differences for parity-based infection rates were highly significant (X^2=39.57, p<0.001). This significant association between parity levels and tuberculin reactivity was evident also after making adjustments for the effects of confounding factors in the multiple logistic regression analysis (Table 14), indicating that parity was significantly (p=0.03) affecting animal's reaction to tuberculin.

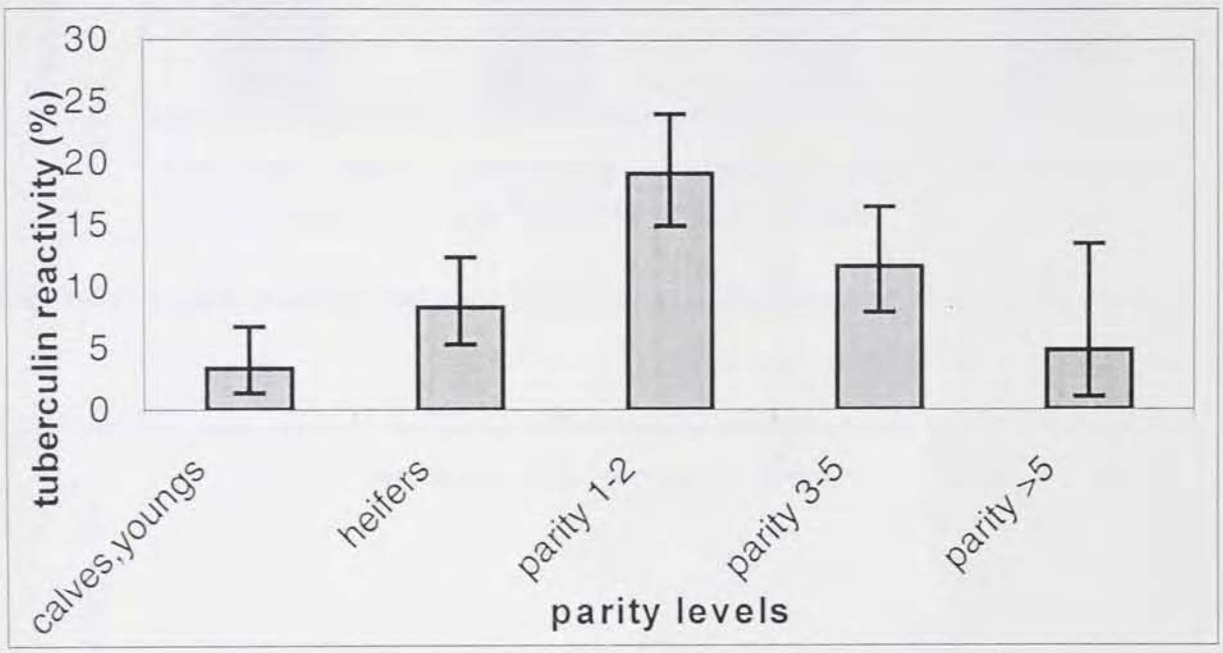

Fig. 2: Tuberculin reactivity between parity levels in 1081 female animals

The reactors rates observed for animals under different physiological conditions, i. e., open (non-milking, non-pregnant), milking, pregnant, and milking and pregnant were 8.2, 14.2, 10.0, and 12.5%, respectively (Table 13, fig. 3). This difference is not statistically significant (X^2=2.66, p>0.1), while the rate for calves, young and males (together), was significantly different (X^2=14.93, p<0.001) from the rate in adult females (Fig. 4).

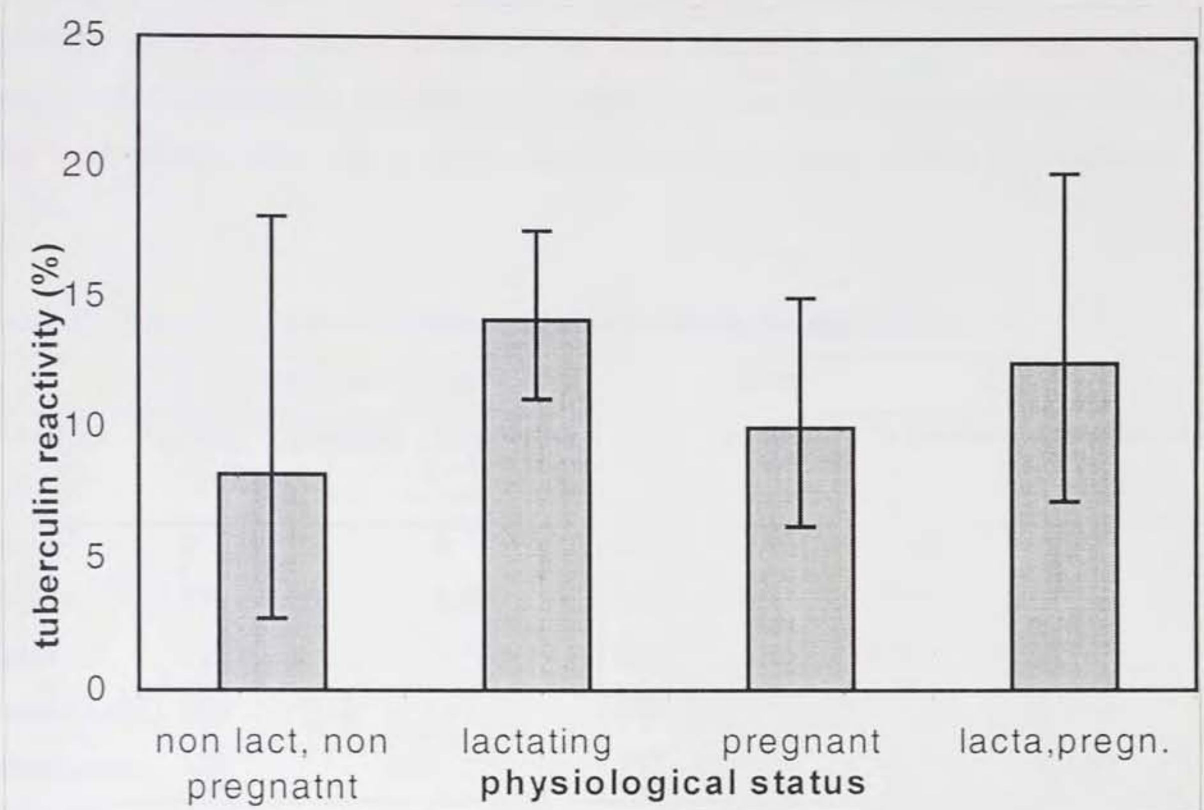

Fig. 3:Tuberculin reactivity between different physiological status of 840 adult animals

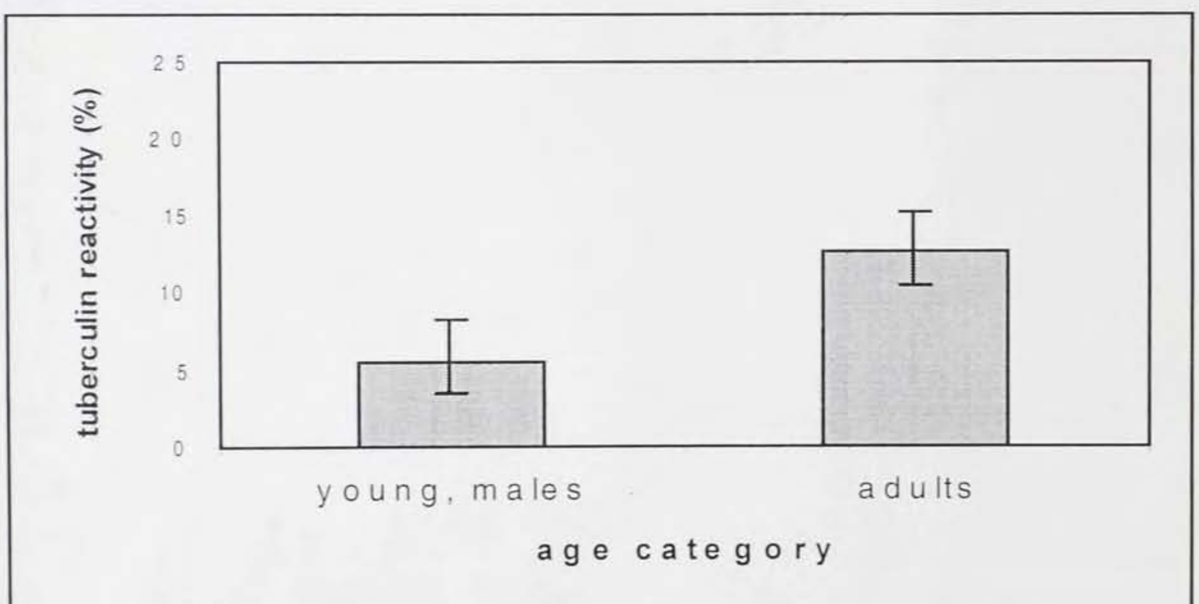

Fig. 4: Tuberculin reactivity between age categories

Breed level infection rates showed high figures for local (20.45%), followed by exotic (11.2%), and cross (5.1%) breeds. This difference is biased towards local breeds (X^2=11.23, df=2, p=0.002), due to high number of young and male animals included in the exotic and cross breeds. After standardising for these two variables, age and sex (direct standardisation, Table 12), local animals were seen to react, almost equally, to exotic animals (standardised ratio =1.16).

Table 12: Breed level infection rates after standardising for age and sex

	young and males			adults			
Breed	**total**	**positive**	**% positive**	**total**	**positive**	**% positive**	**Standard. ratio**
Local	2	0	0	42	9	21.43	
Exotic	376	30	8.00	568	76	13.38	
Cross	118	2	1.70	135	13	9.63	
Local/exotic	378			610			1.16
Local/cross	120			177			1.99

The reactors rate was slightly higher in animals in good body condition compared to poor condition (13.11% vs. 11.81%, Table 13), this difference was not statistically significant (X^2=0.13, p>0.1).

Table 13: Distribution of tuberculin test results for 1241 cattle in and around Addis according to physiological state, body condition score, parity and blood levels

Variable	Test Result Positive	Negative	%	Odds Ratio (confidence limits)	X^2 test	p-value
Physiological state					**18.50**	**0.001**
open	5	56	8.20	1.5 (0.73-4.83)		
milking	65	394	14.16	2.8 (1.72-4.67)		
pregnant	20	180	10.00	1.9 (1.02-3.59)		
milking and pregnant	15	105	12.50	2.5 (1.23-4.08)		
young and males+	22	379	5.49	1		
Parity					**38.16**	**<0.001**
young +	7	203	3.33	1		
heifers	22	241	8.37	2.7 (1.12-6.26)		
parity 1 and 2	59	248	19.22	7.0 (3.14-15.73)		
parity 3-5	28	211	11.72	3.9 (1.65-9.04)		
parity ≥6	3	59	4.84	1.5 (0.37-5.86)		
Body Score					**1.26**	**0.53**
poor	30	224	11.81	1		
medium +	56	502	10.04	0.9 (0.58-1.36)		
good	16	106	13.11	1.1 (0.59-2.16)		
Breed					**12**	**0.002**
local	9	35	20.45	4.8 (1.90-11.90)		
exotic	107	837	11.33	2.3 (1.29-4.23)		
cross +	14	239	5.53	1		

+ The risk for each category is compared with a base-line reactors group in the series

Table 14: Results of Multiple Logistic regression analysis at the animal level

Variable	Log LR	-2 Log LR	DF	Significance of Log LR
Breed	-240.767	2.936	2	0.2304
Parity/age group +	-243.764	8.929	3	0.0302
Body condition	-239.575	2.936	2	0.7590
Status	-240.144	1.690	4	0.7925

+ Risk factor proving significant

4.3 Bacteriology

Out of 46 pooled milk and nasal swab samples collected from tuberculin reactors, 4 (8.7%) were positive for acid fast bacilli. Two nasal swab samples taken from one heifer (ID 10-011) and one young bull (ID 96-011) were similarly positive, indicating 6.1% (n=33) culture positive rate. Additionally, 2 milk samples (n=12) taken from lactating cows showed visible growth which proved to be *Mycobacterium* (Table 15). Other 16 samples were overgrown by contaminants and had to be discarded.

Two of the positive cultures showed rapid growth and were too contaminated to get a pure colony. Likewise, two other positive cultures failed to show a workable size of colonies in the subsequent subculture and were discarded. Biochemical testing of the remaining four positive cultures using Niacin production and Nitrate reduction tests in parallel, gave synchronous negative results (Table 15), proving that *M. bovis* was the most probable agent involved.

Table 15: Results of biochemical tests of the 8 acid-fast bacilli positive cultures and of controls

Sample ID	Specimen	Growth at	Niacin test	Nitrate test	Remarks
4-002	Milk & swab	7th week	negative	negative	
4-005	Milk & swab	11th week	negative	negative	
10-011	Swab only	11th week	Not tested	Not tested	Growth failed*
11-023	Milk & swab	8th week	negative	negative	
19-003	Milk & swab	11th week	negative	negative	
96-011	Swab only	7th week	Not tested	Not tested	Growth failed*
33170	Milk only	2nd week	Not tested	Not tested	contaminated
33180	Milk only	3rd week	Not tested	Not tested	contaminated
----ϕ	Bovine type	reference	negative	negative	negative control.
M-TB 1927	EHNRI strain	,,	positive	positive	2(+)
M-TB 1978	,, ,,	,,	positive	positive	4(+)

* Workable size of colonies could not be obtained in the sub-culture

ϕ Identity not described

5. DISCUSSION

Some reports exist for government farms tested for tuberculosis in Addis Ababa and elsewhere in Ethiopia (Yehualashet, 1995), but there have been no reports on the tuberculosis status so far in the emerging privately owned dairy farms in the country. The present survey was the first attempt to address this continuously growing body of privately owned commercial dairy farms. Tuberculosis had to be suspected as it was already known that bovine tuberculosis was present at a high prevalence in some important areas of the country, e.g., A. A., Debrezeit, Modjo, Diredawa, etc (Haile mariam, 1975; Yehualashet, 1995).

The overall tuberculin reactor's rate in this study of 10.31% is slightly lower than previous reports (Kiros, 1998; Yehualashet, 1995). The lower prevalence rate may be due to the fact that the earlier investigations have come from samples from unusually high prevalence farms (large size government farms instead of small or medium size private farms). Considering the sensitivity and specificity of 72% and 98%, respectively, reported for the single cervical intradermal tuberculin test (Francis *et al.,* 1978), the adjusted prevalence (=true prevalence) of the disease is estimated at 12%.

Proportions of animals giving a positive reaction varied significantly between investigated zones (Table 9). This is in agreement with previous reports (Cook *et al.,* 1996; Morris *et al.,* 1994) who stated that the large body of data arising from tuberculosis control programs in cattle shows substantial variation in both incidence and prevalence rates between geographical regions and between farms within regions. This probably indicates a difference in the exposure of cattle to agents capable of stimulating a positive reaction i. e. TB infection or a difference in the response of cattle to such exposure.

As herd size increased, so did the risk of cattle within the herd showing a positive reaction (RR=3.3). The proportion of reactors also varied correspondingly with herd size, showing a moderately strong relationship, with a correlation coefficient (r) of 0.55 and a coefficient of determination (r^2) of 30.65 (Fig. 1). This result is consistent with previous reports (Barwinek and Taylor, 1996; Cook *et al.,* 1996) and may arise from the fact that introducing tubercular infection into a negative herd may increase with herd size. Because of increased contact, lateral spread of infection within a herd may be favoured increasing the probability of infection than is usually the case with small size herds.

There are numerous reports documenting that poor housing and other poor managerial inputs predispose to tubercular infection (e.g. Barwinek and Taylor, 1996; Griffin *et al.*, 1993; Marangon *et al.*, 1998). There is also some evidence that animals´ resistance to tuberculosis is reduced by a shortage of feed and/or an in-balanced diet, attributable to a deficiency of protein, minerals and vitamins in the diet (Griffin *et al.*, 1993). Accordingly in the current study herds under poor management conditions had higher rates of reactors than those under good management (p=0.02, Table 11).

In contrast to observations by e.g. Marangon et el., (1998), introduction (mixing) of animals did not seem to be associated with increased risk of tuberculosis, although a high proportion of farmers in the study area share bulls and purchase in-heifers from a common source. This study thus indicated that there was a high potential for contact between cattle in different herds, both in infected and non-infected herds; however this factor was not found a significant risk factor for tuberculosis presence in the study area.

By univariate analysis, older farms were identified to possess a higher rate of reactors than newly or recently established farms (OR=7.3, Table 10). This may lead to the conclusion that bovine tuberculosis in the region is not a recent problem, since bovine tuberculosis establishes itself when animals are confined (intensive farming) and the disease maintains itself in infected farms unless control action is put in place (Alhaji, 1976; Griffin *et al.*, 1993; Marangan *et al.*, 1998). The possibility that animals from the older farms might have been involved in the establishment of new ones, through unmonitored purchase of in-heifers, might have led to the establishment of the disease in the latter ones; in the multiple logistic regression analysis, reactors rates between the two groups did no more differ (p>0.1, Table 11), indicating that the factor was initially confounded.

Reactors rates for farms located in the vicinity of the city were slightly higher than those located at the periphery although, this difference was not statistically significant (OR=1.24, CI 0.54-2.84). It is likely that once confined, as is usually the case with intensive farming system, animals remain susceptible, irrespective of the location.

The survey revealed a very low reactor rate for the young and the few male animals ($X^2=14.93$, $p<0.001$, Fig. 4). This result is in consent with many works (e.g. Alhaji, 1976; Berggren, 1981) and was expected, as these very young animals had only been exposed to the infection for a relatively short time. It therefore can be assumed that at young age, only very few cattle become infected. Similarly, in agreement with the work of Cook et al. (1996), lower reactor rates were observed also for older animals (Table 13, Fig. 2). It would have been expected that a larger number of older animals would have reacted to the tuberculin test. It is however possible that these animals have lived with the disease for some times; as the disease is frequently progressive in cattle (Alhaji, 1976; Morris *et al.*, 1994; Pritchard, 1988), the presence of progressive lesions provides sufficient repeated antigenic stimulation and leads to the temporary depression of skin reactivity (Corner, 1994; Lepper *et al.*, 1977). This situation is frequently observed with tuberculin testing in short period intervals (Doherty *et al.*, 1995; Francis *et al.*, 1978).

The physiological status like lactation and/or pregnancy was not seen to be associated with the test outcome (Fig. 3), when young and male animals were excluded from the analysis ($X^2=3.33$, $p>0.1$). This is in agreement with a previous report of Cook et al. (1996), but contradicts with other investigations (Francis *et al.*, 1978; Wood *et al.*, 1991), who claim that pregnant animals show lower reactivity as a result of stress-induced immune suppression. In this study, in absolute terms, lactating cows did react more frequently to the test than pregnant ones, but paradoxically, reproductively inert cows (non-pregnant, non-lactating) were seen to react at a lower rate compared to the pregnant animals (Table 13, Fig. 3).

In agreement with a previous report (Cook *et al.*, 1996), body condition scores were not seen to have an effect on tuberculin reactivity ($X^2=0.13$, $p>0.10$, Table 13). This finding is in contrast to the established fact that poor nutrition predisposes to tubercular infection (Collin, 1994; Griffin *et al.*, 1993; Marangan *et al.*, 1998); malnutrition is also implicated as an important factor in anergy (Francis *et al.*, 1978; Kantor *et al.*, 1984; Wood *et al.*, 1991). It can be suggested that the risk associated with an increased susceptibility due to malnutrition could be offset by a lower reactivity in malnourished cattle.

Breed based analysis indicated no significant superiority of the local, autochthonous cattle (Table 12). This contradicts with earlier reports (Barwinek and Taylor, 1996; Kiros, 1998; Yehualashet, 1995). Previous works (Bedard *et al.*, 1993; Marangon *et al.*, 1998) reported not only higher prevalence rates in local Zebu cattle, but prevalence in cross-bred cattle also was lower than in pure breeds; these results perfectly agree with the current report. Comparison, made between crosses, local cattle (under similar managerial system), and exotic animals, after standardizing for age and sex (direct standardization, Table 12), support this finding, where local, autochthonous cattle were found to react 2.0 and 1.16 times more likely to tuberculin than crosses and exotic animals irrespective of age and sex. The question remains whether differences in the tuberculosis infection rates between cattle breeds are rather due to the production systems in which they are kept than due to genetic reasons.

In this study 8.7% of 46 pooled milk and nasal swabs, and 6.1% of 33 nasal swab samples (8.79% in total, including 2 positive milk samples) did contain tubercle bacilli. This isolation rate is relatively high in comparison to other works (Kazwala *et al.*, 1998; Yehualashet, 1995) and may be attributed to the fact that milk and nasal swab samples were pooled samples in most cases and, that samples were taken from animals reacting strongly to the tuberculin test, i.e. ≥5 mm increase in skin fold thickness.

Although the number of *M. bovis* positive samples is low in absolute terms, it indicates that the habit of pooling milk in the Addis Ababa area does pose a great public health danger to milk consumers. Kleeberg (1984) has indicated that one cow can excrete enough viable bacilli to contaminate the milk of up to hundred cows, when their milk is pooled. The practice of feeding calves this pooled milk raw will also facilitate the perpetuation of the infection in a given farm.

The isolation of *Mycobacterium* from 6.1% of the nasal swabs taken is lower than findings in other reports (e.g. McIlroy *et al.*, 1986). It is a known fact though that swabs are not good material for the recovery of mycobacteria (Cernoch *et al.*, 1994); however, isolation from the two reactors would suggest that excretion of the organism is common. This figure could be expected to increase, perhaps dramatically, by sequential sampling, a technique consistently recommended for the detection of tuberculosis in human cases where morning pooled samples are taken over several days.

6. CONCLUSION

The results of the study support the hypothesis that in the urban and peri-urban dairy production systems of Addis Ababa, bovine tuberculosis is associated with classical risk factors of herd size, herd management, cattle movements and persistence within infected herds. Given this situation, the success of tuberculosis eradication from cattle herds at this stage seems rather unrealistic. Surveillance should therefore be strengthened firstly towards these aspects.

The strategy generally adopted for the control of bovine tuberculosis is diagnosis, herd isolation and slaughter of infected animals to prevent transmission. It is frequently reported that diagnosis with tuberculinisation failed to show consistent relationships between results of the tuberculin tests and the post-mortem findings. This has led to the conclusion that the intradermal tuberculin test is basically a herd test, and that it is of much lower value in countries with different systems of herd management. Unfortunately, in this study it was not possible to make post-mortem examinations of the reactors to exclude non-specific reactions; an evaluation of the test under local conditions therefore seems the foremost and immediate important step to come up with a working standard of the test in Ethiopia.

It was not possible to obtain data on the level of mycobacterial infection in dairy farming families, but human tuberculosis is on the increase in African countries, running concurrent with the increasing prevalence of HIV-infection. A proper assessment of the contribution of bovine mycobacterioses in Ethiopia to the overall number of human infections will therefore require further work in the field. The public health implications of *M. bovis* infections, which are acquired mainly through consumption of infected milk and aerosol transmission however, should be taken seriously. The fact that milk from all cows on a farm is pooled before being sold to consumers exacerbates the danger of the zoonosis to the general public.

7. REFERENCES

Alhaji, I. (1976): Bovine tuberculosis: a general review with special reference to Nigeria. *Vet. Bull.* **46** (11), 829-839.

Auer, L.A., Schleehauf, S. M. (1988): Antibodies to mycobacteria in cattle not infected with *M. bovis*. *Vet. Microbiol.* **18**, 51-61.

Baron, E.J., Peterson, L.R., Finegold, S.M. (1994): *Diagnostic Microbiology*. 9th ed. American Society for Microbiology: Washington D.C. pp. 591-633.

Barwinek, F., Taylor, N. M. (1996): Assessment of the socio-economic importance of tuberculosis in Turkey and possible strategies for control or eradication. Turkish-German Animal Health Information Project. Ankara. Deutsche Gesselschaft für Technische Zusamenarbeit (GTZ), Eschborn, Germany.

Bedard, B.G., Martin, S.W., Chinombo, D. (1993): A prevalence study of bovine tuberculosis and brucellosis in Malawi. *Prev. Vet. Med.* **16,** 193-205.

Berggren, S. A. (1981): Field experiment with BCG Vaccine in Malawi. *Br. Vet. J.* **137**, 88.

Bernues, A., Manrique, E., Maza, M.T. (1997): Economic evaluation of bovine brucellosis and tuberculosis eradication programs in a mountain area of Spain. *Prev. Vet. Med.* **30**, 137-149.

Berrada, J. and Barajas-Rojas, J.A. (1995): Control of bovine tuberculosis in developing countries. In: Thoen, C.O. and Steel, J.H. (eds.). *M. bovis* infection in animals and humans. Ames: Iowa State University Press. pp. 159-166

Buddle, B.M., Keen, D., Thompson, A., Jowett, G., McCarthy, A.R., Heslop, J., Lisle, G.W., Stanford, J. L. (1995): Protection of cattle from bovine tuberculosis by vaccination with BCG by the respiratory or subcutaneous route, but not by vaccination with killed *Mycobacterium vaccae*. *Res Vet. Sci.* **59**, 10-16.

Butcher, P D., Hutchinson, N.A., Doran, T.J., Dale, J.W. (1996): The application of molecular techniques to the diagnosis and epidemiology of mycobacterial diseases. *J. Appl. Bacteriol.* **81**, 53S-71S.

Buxton, A., Fraser, G. (1977): *Mycobacterium*. In: Animal Microbiology. vol. I, chapter 21, Oxford: Blackwell Scientific Publications, pp. 229-235

Cernoch, P.L., Enns, R.K., Sanbolle, M.A. and Wallace, R.J. (1994): Laboratory Diagnosis of the Mycobacterioses. Coord. ed., Weissfeld, A. S., American Society for Microbiology, Washington, D.C.

Collins, C.H., Grange, J.M. (1983): A review: The bovine tubercle bacillus. *J. of App. Bacteriol.* **55**, 13-29.

Collins, D.M., Radford, A.J., Lisle, G.W. and Jacobe, H.B. (1994): Diagnosis and epidemiology of bovine tuberculosis using molecular biological approaches. *Vet. Microbiol.* **40**, 83-94.

Collins, F.M. (1994): The immune response to mycobacterial infection: Development of new vaccines. *Vet. Microbiol.* **40**, 95-110.

Colston, M.J. (1996): The cellular and molecular basis of immunity against mycobacterial diseases. *J. App. Bacteriol.* **81**, 33S-39S.

Cook, A.J. C., Tuchili, L.M., Buve, A., Foster, S.D., Faussets, P.G., Pandel, G.S., McAdam, K.P.J. (1996): Human and bovine tuberculosis in the Monze district of Zambia. *Br. Vet. J.* **152**, 37-46.

Corner, L.A. (1994). Postmortem diagnosis of *M. bovis* infection in cattle. *Vet. Microbiol.* **40**, 53-56.

Corner, L. A., Melville, L., McCubbin, K., Small, K.J., McCormick, B.S., Wood, P.R., Rothel, J.S. (1990): Efficiency of meat inspection procedures for the detection of tuberculosis lesions in cattle. *Aust. Vet. J.* **67**(11), 389-392.

Daborn, C.J., Grange, J.M., Kazwala, R.R. (1996): The bovine tuberculosis cycle-an African perspective. *J. App. Bacteriol.* **81**, 27S-32S.

Doherty, M.L., Monaghan, M.L., Bassett, H.F., Quinn, P.J. (1995): Effect of a recent injection of PPD on diagnostic tests for tuberculosis in cattle infected with *M. bovis*. *Res Vet. Sci.* **58**, 217-221.

Fifis, T., Rothel, J.S., Wood P.R. (1994): Soluble *M.bovis* protein antigens: Studies on their purification and immunological evaluation. *Vet. Microbiol.* **40**, 65-81.

Francis, J., Choi, C.L., Frost A.J. (1973): The diagnosis of tuberculosis in cattle with special refernce to bovine PPD tuberculin. *Aus. Vet. J.* **49**, 246-251.

Francis, J., Seiller, R.J., Wilkie, I.W., O'Boyle, D., Lumlden, M.J. and Frost, A.J. (1978): The sensitivity and specificity of various tuberculin tests using bovine PPD and other tuberculins. *Vet. Record.* **103**, 420-435.

Goff, S.L. (1996): Effect of dexamethasone treatment of tuberculous cattle on results of the γ-interferon test for M. bovis. *Vet. Immunol. Immunopathol.* **53**, 39-47.

Grange, J.M. (1995): Human aspects of *M. bovis* infection. In: Thoen, C.O., Steel, J.H. (eds.). *M. bovis* infection in animals and humans. Ames: Iowa State University Press. pp. 29-46.

Grange, J.M. (1996): The biology of the genus *Mycobacterium. J. App. Bacteriol.* **81** 1S-9S.

Grange, J.M., Collins, C.H. (1987): Bovine tubercle bacilli and disease in animals and man. *Epidemiol. Inf.* **92**, 221-234.

Grange, J.M., Yates, M.D. (1994): Zoonotic aspects of *M. bovis* infection. *Vet. Microbiol.* **40**, 137-151.

Griffin, J.M., Hahesy, T., Lynch, K., Salman, M.D., McCarthy, J., Hurley, T. (1993): The association of cattle husbandry practices, environmental factors and farmer characteristics with the occurrence of chronic bovine tuberculosis in dairy herds in the Republic of Ireland. *Prev. Vet. Med.* **17**, 145-160.

Haile mariam, S. (1975): A brief analysis of the activities of the Meat Inspection and Quarantine Division Department of Veterinary Service: Ministry of Agriculture (MOA), Addis Ababa, Ethiopia.

Haagsma, J., Angus, R.D. (1995): Tuberculin production. In: Thoen, C.O. and Steel, J.H., (eds.). M. bovis infection in animals and humans. Ames: Iowa State University Press. pp. 73-84.

Hanna, J., Neill, S.D., O'Brien, J.J. (1989): Use of PPD and phosphatide antigens in an ELISA to detect the serological response in experimental bovine tuberculosis. *Res. Vet. Sci.* **47**, 43-47.

Hanna, J., Neill, S.D., O'Brien, J.J. (1992): ELISA tests for antibodies in experimental bovine tuberculosis. *Vet. Microbiol.* **31**, 243-249.

Jiwa, S.F.H., Kazwala, R.R., Aboud, A.A.O., Kalaye, W.J. (1997): Bovine tuberculosis in the Lake Victoria zone of Tanzania and its possible consequences for human health in the HIV/ AIDS era. *Vet. Res. Comm.* **21**, 533-539.

Kantor, I.N. (1995): The Americas: Regional and country status reports. In: Thoen, C.O. and Steel, J.H., (eds.). *M. bovis* infection in animals and humans. Ames: Iowa State University Press. pp. 173-194

Kantor, I.N. and Ritacco, V. (1994): Bovine tuberculosis in Latin America and the Caribbean: current status, control and eradication programmes. *Vet. Microbiol.* **40**, 5-14.

Kantor, I.N., Odeon, A.C., Stefan, P. E., Auza, M.J., Madrid, C.R., Marchevsky, N. (1984): Sensitivity of the cervical and the caudal fold tuberculin tests with *M. bovis* in infected cattle of Argentina. *Rev. sci. tech. Off. int. Epiz.* **3**(1), 137-150.

Karlson, A.G. (1983): In: Merchant, P., and Packer, R.A. (eds.). Veterinary Bacteriology and Virology. 7th ed. Ames: Iowa State University Press. pp. 441-465.

Kazwala, R.R., Daborn, C.J. Kusiluka, L.J.M., Jiwa, S.F.H., Sharp, J.M., Kambarage, D.M. (1998): Isolation of *Mycobacterium* species from raw milk of pastoral cattle of the Southern Highlands of Tanzania. *Trop. Ani. Prod.* **30**, 233-239.

Kebede, B. (1997): Looking ahead for sustainable livestock development. Ethiopian Society for Animal Production (ESAP) Proceedings. Addis Ababa, Ethiopia.

Kiros, T. (1998): Epidemiology and zoonotic importance of bovine tuberculosis in selected sites of Eastern Shoa, Ethiopia. Berlin: Freie Universität Berlin and Addis Ababa University. MSc thesis.

Kleeberg, H.H. (1984): Human tuberculosis of bovine origin in relation to public health. *Rev. Sci. tech. Off. Int. Epiz.* **3**(1), 11-32.

Lepper, A.W.D., Pearson, C.W., Corner, L.A. (1977): Anergy to tuberculin in beef cattle. *Aus. Vet. J.* **53**, 214-216.

Marangan, S., Martini, M., Pozza, M.D., Neto, J.F. (1998): A case-control study on bovine tuberculosis in the Veneto region (Italy). *Prev. Vet. Med.* **34**, 87-95

McIlroy, S.G., Neill, S.M., McCracken, R.M. (1986): Pulmonary lesions and *M. bovis* excretion from the respiratory tract of tuberculin reacting cattle. *Vet. Record.* **118**, 718-721

Meslin, F.X., Cosivi, O. (1995): WHO. In: Thoen, C.O. and Steel, J.H. (eds.). *M. bovis* infection in animals and humans. Ames: Iowa State University Press. pp XXII-XXV.

Monaghan. M.L., Doherty, M.L., Collins, J.D., Kazda, J.F.; Quinn, P.J. (1994): The tuberculin test. *Vet. Microbiol.* **40**, 111-124.

Morris, R.S., Pfeiffer, D.U., Jackson, R. (1994): The epidemiology of *M. bovis* infections. *Vet. Microbiol.* **40**, 153-177.

Neill, S.D., Pollock, J.M., Bryson, D.B., Hanna, J. (1994): Pathogenesis of *M. bovis* infection in cattle. *Vet. Microbio.* **40**, 41-52

O'Reilly, L.M. (1995): Tuberculin skin tests: sensitivity and specificity. In: Thoen, C.O.and Steel, J.H. (eds.). *M. bovis* infection in animals and humans. Ames: Iowa State University Press. pp. 85-91.

Oritz, R.E. (1986): Cuba: Eradication of bovine tuberculosis. *World Animal Review.* **59**, 34-37.

Pino, J.A. (1991): The Tropics and the world demand for animal protein. In: Ristic, M. and McIntyrl, I. (eds.). Disease of Cattle in the Tropics. Martinus Nishoff Publishers. pp. 297-307

Pittler, D.R., Steel, J.H. (1995): Germany: Regional and country status reports. In: Thoen C.O. and Steel, J.H. (eds.). *M. bovis* infection in animals and humans. Ames: Iowa State University Press.

Pollock, J.M., Anderson, P. (1997): Predominant recognition of the ESAT-6 protein in the first phase of infection with *M. bovis* in cattle. *Infec. Immun.* **65**(7), 2587-2592.

Pollock, J.M., Pollock, D.A., Campbell, D.G., Girvin, R.M., Crockard, A.D., Neill, S.D. and Mackie, D.P. (1996): Dynamic changes in circulating and antigen-responsive T-cell sub-populations post-*M. bovis* infection in cattle. *Immunol.* **87**, 236-241.

Pritchard, D.G. (1988): A century of bovine tuberculosis 1888-1988: conquest and controversy. *J. Comp. Path.* **99**, 357-386

Pritchard, D.G., Francis, D.A., Gripp, R., Harding, R.B., Jones, E.P., Mintern, C., McGovern, P.T. (1975): An abattoir survey of bovine tuberculosis in the Karamoja region of Uganda. *Br. Vet. J.,* **131**, 120-127

Radostits, O.M., Leslie, K.E., Fetrow, J. (1994): Herd Health: Food Animal Production Medicine. 2nd ed. Philadelphia: W.B. Saunders Company. pp 296.

Rees, W.H., Meldrum, K.C. (1995): Great Britain: Regional and country status reports. In: Thoen, C.O. and Steel, J.H. (eds.). *M. bovis* infection in animals and humans. Ames: Iowa State University Press. pp. 250-256.

Reichel, M.P., Oloya, J. (1994): An evaluation of diagnostic tests for tuberculosis and brucellosis in cattle in Uganda. Proceedings of the 4th workshop on Veterinary Epidemiology. Uganda: Freie Universität Berlin and Makerere University, pp. 33-45.

Roberts, C.A., Boylston, A., Budkley, L., Chamberlain, A.C., Murphy, E.M. (1998): Rib lesions and tuberculosis: the palaeopathological evidence. *Tubercle and Lung Disease*. **79**(1), 55-60.

Rothel, J.S., Jones, S.L., Corner, L.A., Cox, J.C., Wood, P.R. (1990): A sandwich enzyme

Nader, A., DeKantor, I. N, Ritacto V., Augier , J., Romanian, F. (1988): Assessment of a PPD tuberculin produced from the BCG strains of *M. bovis* for use in cattle. *Rev. sci. tech. Off. Int Epzi.* **7**: 301-309

Neill, S .D., Hanna, J. O'Brein, J. J., McCraken R. M (1989): Transmission of tuberculosis from experimentally infected cattle to in-contact calves. *Vet. Rec.* **124**: 269-271

-- Neill, S. D., Pollock, J. M., Bryson, D. B., Hanna, J. (1994): Pathogenesis of *M. bovis* infections in cattle. *Vet. Microbiol.* **40**: 41-52

Newell, D. G., Hewinson, R. G. (1995): Control of bovine tuberculosis by vaccination. *Vet. Rec.* **136**: 459 - 463

Nicholson, M. J. and Butterworth, M. H. (1986): A guide to condition scoring of Zebu cattle. ILCA, Addis Ababa, Ethiopia.

Nolan, A., Wilesmith, J. W. (1994): Tuberculosis in badgers (*Meles Meles*). *Vet. Microbiol.* **40**: 179-191

O' Reilly, L. M. and Costelo, E. (1988): Bovine tuberculosis. *Iri. Vet. News*: **10** (8): 17-25

O' Reilly, L. M., Dabron, C. J. (1995): The epidemology of *Mycobacterium bovis* infection in animals and in man. *Tubercle and lung disease*. **76**: 1-46

Orme, I. M. (1997): Progress in the development of new vaccine against tuberculosis. *Int. J. Tuberc. Lung Dis.* 1 (2): 95-100

Ortega, C., De Blas, N., Frankena, K., Noordhuizen, J. (1996): Win episcope 1.0 statistical software. Wageningen Agricultural University.

Peterson, E. M., Lu, R. Floyd, C., Nakasone, A., Friedly, G., De La Maza, L. M. (1989): Direct identification of *M. tuberculosis, M. avium* and *M. intracellulare* from amplified primary cultures in BACTEC media using DNA probes. *J. Clin. Microbiol.* **27**: 1543-1547

Pollock, J. M., Andersen, P. (1997a): Predominant recognition of *ESAT-6* protein in first phase of infection with *M. bovis* in cattle. *Infec. Immun.* **65** (7): 1-6

Pollock, J. M., Andersen, P. (1997b): The potential of the *ESAT-6* antigen secreted by virulent Mycobacterium for specific diagnosis of tuberculosis. *J. Inf. dis.* **175** (5): 1251-

Pollock, J. M., Pollock, D. A., Campbell, D. G., Grivin, R. M., Crockard, A. D., Neill, S. D., Mackie D. P. (1996): Dynamic changes in circulating and antigen-responsive T-cell sub-population post infection of *M. bovis* in cattle. *Immunol.* **87**: 236-241

Pritchard, D. G. (1988): A century of bovine tuberculosis 1888-1988: Conquest and Controversy. *J. Comp. Pathol.* **99**: 356-399 immunoassay for bovine interferon-gamma and its use for the detection of tuberculosis in cattle. *Aust. Vet. J.* **67**, 134-137.

Sanousi, S.M., Omer, E.E. (1985): Bovine tuberculosis in Benghazi Cow Project (Libya). *Int. J. Zoon.* **12**, 203-206.

Stanford, J.L., Stanford, C. A. (1996): Immunotherapy with M. vaccae and the treatment of Tuberculosis. *J. Appl. Bacteriol.* **81**, 81S-86S

Tegegne, A., Gebre wold, A. (1997): Prospects for peri-urban dairy development in Ethiopia. Ethiopian Society for Animal Production (ESAP) proceedings.

Thoen, C.O. and Bloom, B.R. (1995): Pathogenesis of *M. bovis*. In: Thoen, C.O. and Steel, J.H. (eds.). *M. bovis* infection in animals and humans. Ames: Iowa State University Press. pp. 3-14.

Thoen, C.O., Chiodin, R. (1993): *Mycobacterium*. In: Gyles, C.L. and Thoen, C.O. (eds.). Pathogenesis of bacterial infections in animals. 2nd ed. Ames: Iowa State University Press. pp. 44-56

Thoen, C.O., Steel, J.H. (1995): *M. bovis* infection in animals and humans. Ames: Iowa State University Press.

Thoen, C.O., Huchzermeyer, H., Himes, E.M. (1995): Laboratory Diagnosis of bovine tuberculosis. In: Thoen, C.O. and Steel, J.H. (eds.) *M. bovis* infection in animals and humans. Ames: Iowa State University Press. pp. 63-74.

Thoen, C.O., Schliesser, T., Kormendy, B. (1995): Tuberculosis in captive wild animals. In: Thoen, C.O. and Steel, J.H. (eds.). *M. bovis* infection in animals and humans. Ames: Iowa State University Press. 93-104.

Tweddle, N.E., Livingstone, P. (1994): Bovine tuberculosis control and eradication programs in Australia and New Zealand. *Vet. Microbiol.* **40**, 23-29.

Wood, P.R., Rothel, J.S. (1994): In vitro immunodiagnostic assays for bovine tuberculosis. *Vet. Microbiol.* **40**, 125-135.

Wood, P.R., Corner, L.A., Plackett, P. (1990): Development of a simple, rapid in vitro cellular assay for bovine tuberculosis based on the production of gamma interferon. *Res. Vet. Sci.* **49**, 46-49.

Wood, P.R., Corner, L.A., Rothel, J.S., Baldock, C., Jones, S.L., Cousins, D.B., McCormick, B.S., Francis, B.R., Creeper, J., Tweddle, N.E. (1991): Field comparison of the interferon-gamma assay and the intradermal tuberculin test for the diagnosis of bovine tuberculosis. *Aust. Vet. J.* **68**(9), 286-290.

Yates, M.D., Grange, J.M. (1988): Incidence and nature of human tuberculosis due to bovine tubercle bacilli in South-East England: 1977-1987. *Epidemiol. Inf.* **101**, 225-229.

Yehualashet, T. (1995): Ethiopia: Regional and country status reports. In: Thoen, C.O. and Stee, J.H. (eds.). *M. bovis* infection in animals and humans. Ames: Iowa State University Press. pp. 273-278.

8. ANNEX

Annex 1. Tuberculosis Survey: Questionnaires Formats

8.1 Questionnaires for Dairy Holders

8.1.1 General Information

Date ________

Farm ID. No. _______ Owner_______

Sex___ Age_________

Address ___________

Location	1. Intraurban________		2. Peri urban________
Farm type	1. dairy____________	2. Beef ____________	3. Mixed_____
Production System	1. Intensive_________	2. Semi intensive__________	3. Land less__
Herd size	1. ≤ 10______	2. 10-24___________	3. ≥25_______
Management cond.	0. Poor______	1. __________	

8.1.2 Social Factors

How long have you been in the business? 1. <10 years__2. 10-20 years 3. >20 years__

How did you start the business?

1. inherited_________2. bought the enterprise___________3. bought animals__________

What are your reasons to run the enterprise?

1. to produce milk for home consumption

2. " " market

3. to do fattening

To whom do you sell the milk? 1. consumers________2. DDE______3.Intermediate caters_

Is the farm open for visitors from out side 1. Yes_______2. No_

Do animals in your farm have a chance to get mixed with animals from other farms?

1. Yes_______ 2. No_______

If yes, when? 1. when grazingout __2. when being-watered______3. during medical care

Do animals enter into your herd by purchase? 1. Yes______2. No__

8.1.3 On Tuberculosis

What breed of animals do you keep? 1. Local ______2. Cross ______3. Exotic_____

Do you know that bovine TB. can be transmitted to man through milk or beef?

1. yes_ 2. No_

If yes,

Do you test animals for TB. when you buy? 1. Yes_______2. No_______

How do you sell or consume the milk you produce?

1. sell or consume raw

2. boil

3. pasteurize

Have your animals ever been tested for TB.? 1. Yes_______ 2. No_______

If yes, what was the out come? 1. positive____2. negative___

What did you do with the reactors_________

Have you ever had in your herd, animal with long-standing cough or body wastage?

1. yes_______ 2. no__

What do you do with test positive animals in your herd?____________________

Annex 2: Bovine tuberculosis survey; Cattle Tuberculin test results record sheet

ID	Breed	Sex	Age	BCS	Status	Mesbef	Mesaft	Diff	DX	Remark

BSC, body condition score; Mesbef, skin fold thickness before tuberculin injection; Mesaft, skin thickness 72 h after tuberculin injection; Diff, increase in skin fold thickness, DX; interpretation of results

Annex 3: Bovine TB survey; Herd tuberculin test results record sheet

Farm ID No.	Zone	Herd size	No. positive	% positive	Remark

Annex 4: Bovine TB. Survey; Bacteriological test (culture) result record sheet

Animal ID	Age	Breed	BCS	Status	Tuberculin reaction in mm	Sample taken	Week checked	Remark

Annex 5: Bovine TB. Survey: Data Collection and Record Sheet

1. Region________________ Date______________
2. Location: Zone _______ Woreda __________ Kabale ______
3. Herd ID. No.___________ Herd size__________
4. Herd test Result 1. SIDT___________
5. No. of Positive reactors ______
6. Culture result . 6.1. ________ 6.2. ________
7. Biochemical Tests 7.1. ________ 7.2. ________
8. Probable Aetiology 8.1.________ 8.2. ________ 8.3. ________

Annex 6: Bovine Tuberculosis survey: CODE BOOK

Variable	Code description	Missing value
Farm ID. No.	001-200	999
Case ID. No.	001-200/1-2000	9999
Location	Peri urban, 0; urban, 1	
Breed	Local = 1, Exotic= 2, Cross=3	
Sex	Male =1, Female = 0	
Age/parity	young, 0; Heifers, 1; parity 1 and 2, 2; parity 3-5, 3; parity ≥6, 4	
Body Condition	poor=1, medium=2, good=3.	9
Test Results		
Culture	slow growers=1, rapid growers=2,	9
Biochemical	Niacin +ve=1, Nitrate +ve=2,	9
Aetiology	M. bovis=1, M. tb=2, M. avium=3,	others=4

Annex 7: List of Reagents

I. Reagents for LJ media

Bacto-asparagine	3.6 g
Mono potassium phosphate	2.4 g
Magnesium sulfate	2.4 g
Magnesium citrate	0.6 g
Potato flour	30.0 g
Glycerol*	12.0 ml
Malachite green	0.4 g
Whole egg	1000.0 ml
Distilled water	600.0 ml

*replaced by 0.4% pyruvate for M. bovis

II. Reagents for Decontamination procedure- -

1. Sodium hydroxide (2%)
   - Sodium hydroxide 4.0 g
   - Distilled water 200.0 ml

2. 0.1 N Hydrochloric acid
3. Phenol red solution (0.005-0.01%)
   - All the three are autoclaved and stored at 4 °C

III. Reagents for Ziehl-Neelsen Acid-Fast Stain

1. Carbolfuchsin Stain
   - Basic fuchsin 0.3 g
   - Ethyl alcohol (95%) 10.0 ml
   - Phenol (melted crystal) 5.0 ml

2. Acid-Alcohol
   - Hydrochloric acid (concentrated) 3 0 ml
   - Ethyl alcohol (95%) 97.0 ml

3. Methylene blue (Counter stain)
   - Methylene Blue (90% dye content) 0.3 g
   - Ethyl alcohol (95%) 30.0 ml
   - Potassium hydroxide 80.01%) 100.0 ml

IV. Reagents for Niacin Production test

1.	4% aniline:	96 ml ethyl alcohol (95%) + 4 ml colourless aniline Stored in a brown bottle at 4°C.
2.	10% Cyanogen bromide.	5 gm Cyanogen bromide in 50 ml distilled water. Stored in a brown bottle at 4°C.

V. Reagents for Nitrate Reduction test

1. 0.01M NaNO3 in 0.022M Phosphate buffer

- $NaNO_3$ = 0.085g
- KH_2PO_4 = 0.117g
- $Na_2HPO_4.12H_2O$ = 0.485g
- Distilled H_2O = 100 ml.

2.*	Concentrated HCl:	10 ml conc. HCl in 10 ml dist. water.
3.*	0.2% Sulfanilamide:	0.2 gm sulfanilamide in 100 ml dist. Water.
4.*	0.1% ethylene diamine dihydrochloride:	0.1 gm n-(1-naphthyl) ethylenediamine dihydrochloride in 100 ml dist. water

* 2-4 kept in the refrigerator

Annex 8: Map of Addis Ababa showing the 'Woredas' and study sites

9. CURRICULUM VITAE

Personal Data

Name	Asseged Bogale
Date of Birth	05/07/1966
Place of Birth	Borana, Ethiopia
Marital Status	Single
Nationality	Ethiopian
Address	Addis Ababa University, F. V. M., P. O. Box 887, Debrezeit, Ethiopia.

Academic Background

Year	Study Level	Institute	Award
1998-Present	Postgraduate Study	Freie Universität Berlin, Germany	
1986-1991	Higher Education	Faculty of Veterinary Medicine, A. A. U., Ethiopia	Degree of Doctor of Veterinary Medicine (DVM)
1982-85	Secondary	Yavello Senior Secondary School	
1975-1981	Elementary	Hidilola Elementary School	

Professional Career

1999-Present	Research on Bovine Tuberculosis, Diagnostic and Epidemiological aspects.
1996-97	Lecturer, Department of Physiology, Pharmacology and Biochemistry, F. V. M., Addis Ababa University.
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1994-95	Head Veterinarian, Southern Range lands Development Project, Oromia Agricultural Development Office

1991-93	Team leader, Animal Health Services, South Omo Zone Agricultural Department

Communication

1999	On-going Bovine tuberculosis: A Cross Sectional Study in and around Addis Ababa (M Sc. Thesis)
1991	Epidemiological Study of Major Skin Diseases of Cattle in Southern Range lands (DVM, thesis paper)
1990	Problems Associated with Introduction of Cross breeds to Ethiopian Peasant Sector (Seminar Paper)

Other Skills

Language

English	Fluent, both Writing and Speaking
Deutsch	Communication

Two other local Ethiopian languages

Computer Skill

Microsoft Word

Microsoft Excel

Statgraphics Plus 2.1

10. SIGNED DECLARATION SHEET

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

Name Asseged Bogale

Signature

Date submitted 15/11/99

This thesis has been submitted for examination with our approval as University advisors.

Dr. A. Lübke-Becker

Dr. Tadele Kiros Tadele K.