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EPIDEMIOLOGY OF TUBERCULOSIS IN CAMELS SLAUGHTERED AT AKAKI ABATTOIR, CENTRAL ETHIOPIA

PhD DISSERTATION

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EPIDEMIOLOGY OF TUBERCULOSIS IN CAMELS SLAUGHTERED AT AKAKI ABATTOIR, CENTRAL ETHIOPIA

A dissertation submitted to the College of Veterinary Medicine of Addis Ababa University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Veterinary Public Health

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DEDICATION

This paper is dedicated to my late father, Jibril Yousuf who passed away while I was to pursue my PhD study, whom I remember every time with deep sorrow. This paper is also dedicated to my mother who shaped me with patience and strength in all aspects of my life; I wish Allah give her long age and healthy life!! The dissertation should also be dedicated to my beloved husband and my lovely children.

STATEMENT OF THE AUTHOR

First, I declare that this dissertation is my *bonafide* work and that all sources of materials used for this dissertation have been duly acknowledged. This dissertation has been submitted in partial fulfillment of the requirements for a PhD degree at Addis Ababa University, College of Veterinary Medicine and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this dissertation is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

I was born on July 15, 1978 in Dire Dawa City, Eastern Ethiopia. I attended my Elementary and Secondary school education at Aba-Yezid, Abadir Islamic school, Legehare elementary school and at Dire Dawa Comprehensive Secondary School, Dire Dawa, respectively. I then joined the Addis Ababa University, College of Veterinary Medicine and graduated with the Doctor of Veterinary Medicine (DVM) degree in July 2000. After two years of clinical service as Clinician at Harari Agricultural Bureau in Harar town, I joined the College of Veterinary Medicine as academic staff in the Department of Clinical Studies with the rank of Assistant lecturer in December, 2002. After two years of service, I was promoted to the rank of lecturer and specialized in my host institution, the College of Veterinary Medicine, and graduated with Masters Degree in Tropical Veterinary Epidemiology in July, 2007. I am now with the rank of Assistant Professor in the Department of Clinical Studies and instructor in both undergraduate and postgraduate masters classes. I married to Dr. Ahmed Issa and I am the mother of two beautiful daughters and two handsome boys.

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LIST OF ABBREVIATIONS

AFB	Acid Fast Bacilli
ALIPB	Aklilu Lemma Institute of Patho-Biology
APC	Antigen Presenting Cell
AUC	Area under the Curve
BCG	Bacillus Calmette Guerien
BCS	Body Condition Score
BTB	Bovine Tuberculosis
CaMLN	Caudal Mediastinal Lymph Node
CBPP	Contagious Bovine Pleuropneumonia
CBN	Conformal Bayesian Network
CD4/8	Cluster of Differentiation 4/8
CFP	Culture Filtrate Protein
CMI	Cell Mediated Immunity
CSA	Central StatisticalAgency
CrMLN	Cranial Mediastinal Lymph Node
CVMA	College of Veterinary Medicine and Agriculture
DNA	Deoxyribo-Nucleuc Acid
dNTP	di-Nitro Tri-Phosphate
DR	Direct Repeat
ELISA	Enzyme Linked ImmunoSorbent Assay

LIST OF ABBREVIATIONS (*Continued*….)

ESAT	Early Secreted antigen target
EU	European Union
HIV/AIDS	Human Immuno-deficiency Virus/Aquired Immuno-Deficiency Syndrome
HPC	Hexadecyl Pyridinium Chloride
IFN	Interferon
IL	Interleukien
INH	Iso-Nicotinic acid Hydrazide
IS	Insersion sequence
KBBN	Knowledge Based Bayesian Network
LN	Lymph node
MDR-TB	Multi-Drug Resistance Tuberculosis
MIRU	Mycobacterial Interspersed Repeatitive Unit
Mph/kph	Meter per hour/Kilometer per hour
MTB	*Mycobacterium tuberculosis*
MTBC	*Mycobacterium Tuberculosis Complex*
MVA	Modified Vaccine Ankara
NK	Natural Killer cell
NMSA	National Metreological Service Agency
NTM	Non-Tuberculous Mycobacteria
NWC	New World Camelids

LIST OF ABBREVIATIONS (*Continued*….)

OIE	World Organization for Animal Health
OR	Odds Ratio
OWC	Old World Camelids
OPADC	Oromiya Pastoralist Areas Development Commission
PLN	Parotid Lymph Node
PCR	Polymerase Chain Reaction
PPD	Purified Protein Derivatives
RD	Region of Difference
RPLN	Retro-Pharyngeal Lymph Node
ROC	Reciever Operating Characteristics
REA	Restriction Endonuclease Analysis
RNA	Ribo-Nucleuc Acid
Rpm	Revolution per minute
SICCT	Single Intradermal Comparative Cervical Tuberculin
SPSS	Statistical Package for Social Sciences
SMLN	Sub-Mandibular Lymph Node
TB	Tuberculosis
TCH	Thiophen Carboxylic acid Hydrazide
TrBLN	Tracheo-Broncheal Lymph Node
Th	Helper T cell

LIST OF ABBREVIATIONS (*Continued*….)

TNF	Tumour Necrotic factor
TST	Tuberculin Skin Test
UK	United Kingdom
UNDP	United Nations Development Program
USA	United States of America
WHO	World Health Organization
ZN	Zehiel Neelson

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ABSTRACT

Camels are important sources of milk and meat for the pastoral communities of East Africa as they are adapted to the harsh environment in this region. However, these animals are facing different health constraints including tuberculosis (TB). TB can also be transmitted to pastoralists from camels since their milk is consumed raw and as the pastoralists live in close physical contact with potentially infected camels. However, little data is available on the epidemiology of TB in camels in this region. In addition, the diagnostic performance of the single intra-dermal comparative cervical tuberculin test (SICCT) for diagnosis of TB in camels has not been evaluated in Ethiopia. This study was undertaken between February, 2014 and July, 2016 to investigate the epidemiology of TB and to evaluate the performance of SICCT in camels slaughtered at Akaki abattoir, central Ethiopia. In order to achieve these objectives, a cross-sectional study design was used to recruit 2070 camels for epidemiological studies while the evaluation of SICCT was conducted on 168 camels. Gross pathology was used as a gold standard to define the disease status of each camel. Methodologically, SICCT, post mortem examination, bacteriological culturing and molecular typing were employed.

The prevalence of TB in camels slaughtered at Akaki Abattoir was 7.54% (95%CI: 6.45% -8.7%). Statistically significant association (χ^2=26.2, p=0.000) was observed between body condition of the camels and lesion prevalence of TB. However, no significant association (p>0.05) was observed between prevalence and either age, sex, or origin of the camels. Multivariate logistic regression analysis revealed that poor body condition was significantly associated [p= 0.000, (Adjusted OR=2.55, 95%CI: 1.743 - 3.734)] as a risk factor for TB in camels. Relatively higher frequency of lesion was detected in female, poor body conditioned, and Borena camels with a proportion of 87.2%, 46.2%, and 84%, respectively. Anatomically, lesion was frequently found in the sub-mandilbular (54.8%) and retro-pharyngeal (16.94%) lymph nodes. Moreover, severe lesion was observed in sub-mandibular (2.47±0.24), retro-pharyngeal (0.35±0.2) and trachea-broncheal (0.35±0.2) lymph nodes.

The result of the validation of SICCT showed that at the cut-off value of ≥3mm, the SICCT had optimum performance. At this cut-off value, the sensitivity and specificity of SICCT were 60.7% and 85%, respectively. Moreover, at a cut-off value ≥3mm, the receiver operating characteristics (ROC) analysis revealed that area under the ROC curve was 0.729 (0.615-0.842) which was statistically significant (p=0.000) for sensitivity and specificity evaluation.

Of the total 168 camels tissues cultured, 36 camels tissues were culture positive for mycobacteria with the isolation rate of 21.4% (36/168). A total of 80 isolates were harvested from the different lymph node tissues of 36 culture positive camels. Identification of the isolates using multiplex polymerase chain reaction (PCR) revealed that 48.8% (39/80) were members of the *Mycobacterium tuberculosis* complex (MTBC) while the rest were non-tuberculous mycobacteria (NTM). Spoligotyping of the 39 isolates indicated that majority of the isolates (94.9%) identified as orphan strains with no shared spoligotype international type (SIT) in the Spoligotype database while two isolates were identified as SIT-1088 and SIT-118. The lineage of the strains based on Conformal Bayesian Network (CBN) analysis revealed that the dominant lineages were Euro-American (84.6%) and Indo-Oceanic (12.8%) lineages while only one strain was identified as *M. africanum* lineage (2.6%). The result of the present study suggested the use of ≥3mm cutoff point for the SICCT to diagnose TB in dromedary camels of Ethiopia. Strains of *M. tuberculosis* identified in camels in the present study could help to elucidate the potential role of camels in the epidemiology of tuberculosis in humans and warrants further investigation as to the transmission dynamics of the disease between pastoralists and their camels which would be helpful to design disease control programs.

Key words: *Akaki abattoir, central Ethiopia, comparative tuberculin test, dromedary camels, epidemiology, gross pathology, mycobacterial culture, molecular characterization, tuberculosis.*

1. INTRODUCTION

Pastoral production system accounts for the livelihood of 50-100 million people in developing countries and 60% of this population lives in more than 21 African countries confined to the most arid regions of the continent (Sheik-Mohammed and Valema, 1999; UNDP, 2007). In Eastern Africa, Ethiopia has the largest pastoralist population (7-8 millions) representing around 20 ethnic groups (Markakis, 2004). Pastoralists depend on livestock for their livelihood, moving seasonally from place to place in search of water and pasture for their animals (Nori, 2005). In these communities, animal products such as milk consumed raw and this habit combined with close physical contact and sharing the same dwelling with their animals create a potential public health concern for transmission of zoonotic diseases such as tuberculosis (TB).

The dromedary camels have an estimated world population of 18 million across the arid and semi-arid environments of African and Asian countries. In Africa, dromedary population of about 15 million accounts for about 74% of the world and of these, 60% are found in East African countries (Somalia, 6.2 million; Sudan, 2.8 million; Ethiopia, 1.7 million; Kenya, 0.9 million) (Rhodes, *et al.*, 2015). The threat posed by increasing and prolonged periods of drought particularly in arid and semi arid areas of Africa has forced pastoralists to undergo adaptive strategies such as herd diversification, where emphasis on camel husbandry is becoming a priority. Increasing numbers of pastoralists in Ethiopia are herding camels in response to less predictable weather patterns, because camels are more drought hardy than any other species of animals.

The one-humped camel, or dromedary camel (*Camelus dromedarius*) is one of the world's hardiest domesticated animals. Camels are the back bone of the pastoral economy and subsistence for vast majority of pastoralists. These animals ensure adverse climatic conditions in the arid areas and are the most efficient animals in converting fodder into energy for work and products like milk and meat. They are vital source of

transport, meat, milk and income for pastoralists in the Sahel, East Africa, the Middle East and South Asia (Fassi-Fehri, 1987). Camel production is practiced by pastoral communities under diverse constraints in dry and marginal areas. The dominance of other ruminant species over camels perhaps might have masked the potential contributions of these animals to the national and household economy. In contrast to cattle and small ruminants, camels have received very limited attention from policy makers in Ethiopia. Camels have been marginalized as a species due to factors that include the underestimation of the population size, the under-rating of their economic significance, and the fact that they are disproportionately owned by marginalized pastoralists (Aklilu and Catley, 2011). Scarce research information on disease reveals that camels may be either carriers of, susceptible to or suffering from a vast array of infectious and parasitic diseases (Megersa, 2010). Infectious and parasitic diseases appear to be the major constraints that are hampering the potential performances of the animals.

Tuberculosis affects Old World Camelids (OWC) including Dromedaries and Bacterian camles (Mustefa, 1987; Rhodes *et al.*, 2015). Tuberculosis is also a serious emerging disease in the steadily increasing NWC population of the United Kingdom (UK) (Twomey *et al.*, 2010a). Tuberculosis is prevalent in camels; Elmossalami *et al*., (1971) indicated a higher TB prevalence of 13% in camels in Kazakistan and a more recent study in Ethiopian abattoirs indicated a similar prevalence of 10% based on the identification of gross lesions in apparently healthy dromedaries (Mamo *et al.,* 2011). Camel TB has been described also in Pakistan (Zubair *et al*., 2004), Nigeria (Abubaker *et al.*, 2014) and Ethiopia (Mamo *et al.*, 2009, 2011; Gumi *et al.*, 2012; Zerom *et al.*, 2013; Kassaye *et al.*, 2013; Beye *et al.*, 2014).

Of the MTBC, *M. tuberculosis*, *M. bovis*, *M. pinnipedi*, *M. caprae*, and *M. microti* have been isolated from camelids (Oevermann *et al*., 2004; Pate *et al.*, 2006; Waters *et al.*, 2006; Twomey *et al*., 2007; Lyashchenko *et al*., 2007; OIE, 2008; Ryan *et al*., 2008; Garcia-Bocanegra *et al*., 2010). Non-tuberculous mycobacteria (NTBC) such as *M.*

kansasii, M. aquae, M. fortuitum and *M. smegmatis* have also been isolated from OWCs as causative agents of camel TB (Elmossalami *et al.,* 1971; Kinne *et al.,* 2006; Mamo *et al.,* 2011; Zerom *et al.,* 2013). Strains of *M. tuberculosis* have also been identified from camels in southern Ethiopia (Gumi *et al.,* 2012), from camels in Eastern Ethiopia (Zerom *et al.,* 2013), from goats in Afar by (Mamo *et al.,* 2012), (Mulugeta *et al.,* 2013) from pigs in central Ethiopia.

Rationale of the study

Tuberculosis (TB) is chronic granulomatous disease caused by a group of phylogenetically closely related group of bacteria collectively known as the *Mycobacterium tuberculosis* complex (MTBC) (WHO, 2013). TB is one of infectious diseases affecting millions of people world wide. Ethiopia has high incidence rate of TB infection and the disease is one of major public health problems in the country. The country is one among the world's 22 countries with high TB burden (WHO, 2014). In Ethiopia, pastoralist areas are well known for high TB prevalence where the pastoralists keep large number of livestock as a means of livelihood and survival strategy in the arid and semi-arid regions of the country. Camels are the back bone of majority of pastoralists in the country where the habit of sharing the same dwelling and consumption of raw camel products that may favour the transmission of zoonotic diseases like TB. There is little published information on the epidemiology of TB specifically relating to camelids (Rhodes *et al.,* 2015). However, recently few studies have been conducted on the epidemiology of tuberculosis in dromedary camels in Ethiopia (Mamo *et al.,* 2009, 2011; Gumi *et al.,* 2012; Kassaye *et al.,* 2013; Zerom *et al.,* 2013; Beye *et al.,* 2014).

Although diagnosis of TB in live animals commonly based on tuberculin skin test and the single intradermal comparative cervical tuberculin test (SICCT) considered as the OIE recommended test for the international trade of live camels, the test was not validated in this animal species and other non-bovids. Therefore, validation of the

SICCT to decet tuberculosis in dromedary camels of Ethiopia would be of practical significance to the existing local conditions. Strain diversity in MTBC has important phenotypic significance. Different MTBC strains and lineages differ in their growth, immunogenicity, disease severity, transmission, gene expression and metabic profiles (Gehre *et al.,* 2013). Thus identifying circulating strains of MTBC in camels originating from pastoralist livestock production systems of borena and fentale districts would provide an important insight as to the potential role of camels in the epidemiology of TB and clinical significance of the disease in camel health and production. It is therefore, of paramount importance to undertake detailed epidemiological investigation of tuberculosis in dromedary camels of Ethiopia and molecular characterization of *Mycobacterium tuberculosis* complex strains circulating in camels. This study is therefore, aimed with the following objectives.

General objective

- To investigate the epidemiology of tuberculosis in camels slaughtered at Akaki municipality abattoir, central Ethiopia.

Specific objectives

- To estimate the prevalence of TB in camels slaughtered at Akaki municipality abattoir using gross post mortem lesion and SICCT,
- To evaluate the diagnostic test performance of SICCT in detecting tuberculosis in camels,
- To characterize isolates of mycobacteria particularly *Mycobacterium tuberculosis* complex strains that can cause TB lesions in tissues of camels.

2. LITERATURE REVIEW

2.1. *Genus Mycobacterium*

2.1.1. General Description and Taxonomy

The genus *Mycobacterium* belongs to the kingdom bacteria, phylum Actinobacteria, order Actinomycetales and family Mycobacteriacae (Quinn *et al*., 2004). The generic name, *Mycobacterium* was introduced by Lehman and Neuman in 1896. The organism was named so because of the mould like pellicular growth of these organisms in liquid medium. 'Myco' means fungus and 'bacterium' means bacteria (Bhatia and Ichpujani, 1994). Sometimes, Mycobacterium occurs as branching filamentous forms but these easily fragment into rods. The rods tend to stain irregularly and often have a beaded appearance. The mycobacteria are most closely related to the genera Rhodococcus and Nocardia and all the three genera have similar cell wall type but comparatively Mycobacteria have characteristics of slow growth rate (Quinn *et al.*, 2011).

The genus *Mycobacterium* includes *Mycobacterium tuberculosis* complex and *Mycobacterium avium* complex, other pathogenic mycobacteria and numerous species of saprophytic microorganisms present in soil and water. Mycobacteria are obligate aerobes, non-spore forming and non-motile bacilli, and are 0.6-1.0 × 1.0-10 μm in size. Their high cell wall lipid content excludes standard aniline dyes, so that once stained with special staining procedures, mycobacteria are resistant to decolorization even by acid alcohol. This property is termed acid fastness, so that mycobacteria are commonly referred to as acid-fast bacilli. In contrast, these microorganisms are not readily stained with the gram staining method and are considered weakly gram-positive (Gyles *et al.*, 2010).

2.1.2. Morphology and biochemical composition

Mycobacteria are aerobic, obligate pathogens, non-spore-forming, non-motile, rod shaped acid-fast and non-flagellate bacilli. Individual species differ in size; the rods of *Mycobacterium bovis* are slender and up to 4μm in length. Pathogenic species grow slowly on solid media, colonies visible after incubated for 3 to 6 weeks (Quinn *et al.*, 2011). Although mycobacteria are cytochemically gram-positive, the high lipid and mycolic acid content of their cell walls prevents uptake of the dyes employed in the gram stain. They do not stained readily, once stained they resist decolorization by acid or alcohol and are therefore called "acid-fast" bacilli.

The cell wall lipids bind carbolfuchsin which is not removed by the acid-alcohol decolorizer used in the Ziehl-Neelsen (ZN) staining method. Bacilli that stain red by this method are called acid-fast or ZN-positive. Lipid-rich walls render *Mycobacteria* hydrophobic and resistant to adverse environmental influences. The high lipid content of this pathogen accounts for many of its unique clinical characteristics (Quinn *et al.*, 2011). Mycobacterial cell walls have a complex structure and contain a high proportion of lipids approximately 30% to 40% of the total weight, a significant number which are responsible for their resistance to humoral defense mechanisms, disinfectants, acids and alkalis (Rastogi *et al*., 2001).

2.1.3. Staining Characteristics

The tubercle bacilli cannot be stained by the staining methods that are effective with other bacteria, as there is a resistance to penetration of dyes into the cell as a result of large amount of lipid. The cell may be stained by Ziehl Neelson (Acid-fast staining) and Kinyoun stain (Harada *et al*., 1974; Freeman, 1979). Acid fast staining of Mycobacteria in the form of beadings is obtained by means of a carbolfuchsin solutio n (Ziehl Neelsen stain) prepared from pararosaniline or from certain kinds of basic fuchs in.

Acid-fast staining of mycobacteria in rod form results by using a carbolfuchsin prepared from rosaniline or from other basic fuchsins included new fuchsin. The kinyoun stain is a method of staining acid fast microorganisms, specifically *Mycobacterium* and *Nocardi a*. The procedure for kinyoun staining is similar to the Ziehl-Neelsen stain. The kinyoun staining method uses carbolfuchsin as a primary stain, followed by decolorization with an acid-alcohol solution and methylene blue as a counterstain. Kinyoun carbolfuschsin has a greater concentration of phenol and basic fuchsin and does not require heating in order to stain properly. When viewed under a microscope, a kinyoun stained slide will show acid-fast organisms as red and non acid-fast organisms as blue (Harada *et al*., 1974).

2.1.4. Growth requirements and culture media

The optimum temperature for growth is 37^0C. Mycobacteria are strict aerobes that growth best on complex organic media such as Lowenstein-Jensens (LJ), which contains whole eggs and potato flour. A dye, malachite green, inhibits contaminants. Oleic acid-albumin media, such as Middlebrook's 7H10 agar, are also used for isolation, often in combination with Lowenstein-Jensens (Dwight *et al*., 2002). Growth rates for mycobacteria are slow, with generation times ranging from 2 to more than 20 hours. Based on different generation times, mycobacteria can be divided into slow and rapid growers. Slow growers require more than 7 days to form visible colonies on solid medium, whereas rapid growers form colonies within 7 days. The luxuriant growth of *M. tuberculosis* on glycerol containing media, giving the characteristics "rough, tough and buff" colonies, is known as eugonic. *M. bovis* has sparse, thin growth on glycerol containing media which is called dysgonic. However, *M. bovis* grows well on pyruvate containing media without glycerol (Quinn *et al*., 2004).

2.2. Overview of *Mycobacterium tuberculosis* complex

Mycobacterium tuberculosis complex consists of closely related organisms: *Mycobacterium tuberculosis, Mycobacterium africanum, Mycobacterium bovis, Mycobacterium caprae, Mycobacterium microti, Mycobacterium pinnipedii, the dassie bacillus, and Mycobacterium canettii* (Brosch *et al.*, 2002; Cousins *et al*., 2003; Akos *et al.*, 2009). They are characterized by 99.9% similarity at the nucleotide level and identical 16S rRNA sequences but differ widely in terms of their host tropisms, phenotypes, and pathogenicity (Sreevtsan *et al.*, 1997).

2.2.1. The evolution and population structure of M. tuberculosis Complex

The MTBC is defined as a complex of seven distinct bacterial species named *M. tuberculosis, M. canettii*, *M. africanum, M. pinnipedii, M. microti, M. caprae* and *M. bovis*. Although strains of the MTBC show a remarkable sequence similarity of at least 99.9% (Smith *et al.*, 2006), the different bacterial species show a certain host tropism. E.g., *M. bovis* most commonly affects cattle, *M. tuberculosis* affects humans, *M. microti* is most frequently isolated from voles (Brosch *et al.*, 2002; Smith *et al.*, 2005, 2006;). Nevertheless, spill over to other hosts has been observed for most of the bacteria (Kubica *et al.*, 2003; Smith *et al.*, 2006; Xavier *et al.*, 2007). The different clades of the MTBC and the phylogenetically informative large genomic deletions (RDs) that allow their differentiation (Brosch *et al.*, 2002) can be seen in the figure below (Figure 1). It has been suggested that the MTBC should rather be considered as a group of different "ecotypes", adapted to distinct hosts (Smith *et al.*, 2005). Generally, ecotypes can be described as the entirety of all individuals that occupy a specific ecological niche and that can be identified as a clustered group using specific genomic markers (Cohan, 2001).

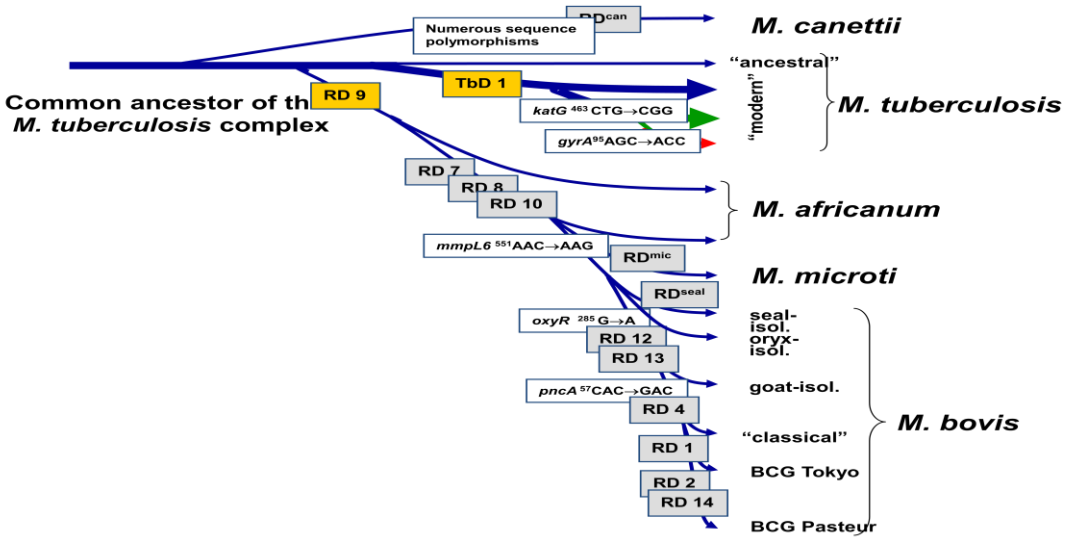


Figure 1: Evolutionary scenario of the MTBC phylogentically informative large genomic deletions (Source: Brosch *et al.*, 2002).

The identification of ecotypes of the MTBC and their defining molecular traits is still ongoing. In fact, host adaptation of MTBC strains may not necessarily be restricted to the species level but could be more specific (Smith *et al.*, 2005; Mostowy *et al.*, 2005). Indeed, in a recent study, Gagneux *et al.*, suggested that a variable host pathogen compatibility between distinct strains of *M. tuberculosis* and human hosts of different ethnicity (Gagneux *et al.*, 2006). Except for *M. canettii* (Gutierrez *et al.*, 2005), there is little evidence for horizontal gene transfer between other ecotypes of the MTBC (Smith *et al.*, 2006). Therefore, the population structure of MTBC can be considered as highly clonal if *M. canettii* is excluded. The clonality of the MTBC and its ecotypes can be most evidently shown by the matching phylogenetic trees that have been constructed using different molecular markers (Smith *et al.*, 2006; Gagneux and Small, 2007). Also, the observation of linkage disequilibrium between 12 independent microsatellite loci in strains of *M. tuberculosis* has suggested a clonal population structure (Supply *et al.*, 2003). The reason for the strict clonality of the MTBC could be due to an infrequent encounter of the different ecotypes (Smith *et al.*, 2005), mutations in the recA gene or other barriers to recombination (Smith *et al.*, 2006).

Because of the smaller genome size of *M. bovis* as compared to *M. tuberculosis* and because no significant chromosomal regions are present in *M. bovis* but absent in all strains of *M. tuberculosis*, it can be inferred that *M. bovis* emerged from an *M. tuberculosis*-like ancestor. Therefore, it has been suggested that tuberculosis disease was first present in humans prior to animals (Brosch *et al.,* 2002). This hypothesis has been a matter of controversial discussions (Smith *et al.,* 2006); however, recent work has added new evidence to this theory. In two independent studies, analysis of the VNTR and SNP profiles, respectively, of an extensive collection of MTBC strains allowed to differentiate two major clades of the MTBC. The first clade exclusively comprised strains of the human pathogen *M. tuberculosis*. However, the second clade comprised strains of *M. tuberculosis* and in addition all strains of *M. africanum*, *M. pinnipedii*, *M. microti, M. caprae* and *M. bovis* (Wirth *et al.,* 2008; Hershberg *et al.,* 2008). It was thus suggested that the last common ancestor of both clonal groups already constituted a human host adapted pathogen (Smith *et al.,* 2006; Wirth *et al.,* 2008; Hershberg *et al.,* 2008). Using Bayesian statistics, it was estimated the age of the MTBC at 40,000 years. The calculated time of the MTBC emergence would thus coincide with the expansion of ‘‘modern’’ human populations out of Africa (Wirth *et al.,* 2008).

2.2.2. Phylogeny of human adapted strains of M. tuberculosis complex

The whole genome analysis of MTBC consist seven human-adapted lineages (lineage 1-7) each associated with specific global geographic locations (Comas *et al.,* 2013). The lineages grouped as modern lineage with a deletion gene in the genomic region known as tuberculosis deletion-1 (TbD1) and others known as ancestoral strainswithout this deletion (Brosch *et al.,* 2002). The TbD1 deleted modern clad consist lineages 2, 3, and 4. Among these, lineage 2 (East-Asian lineage includes the Beijing family) and lineage 4 (Euro-American) diversified more recently than the remaining MTBC strains. Lineage 2 predominates in East Asia, but is also present in Central Asia, Russia, and South-Africa. Lineage 4 mostly occurs in Asia, Europe, Africa, and America.

The ancesteral strains lineage 1 and modern strain lineage 3 shows a more restricted geographical distribution limited to East Africa, Central-South- and South-East Asia. The most geographically restricted lineages are lineage 5, 6, and 7 which are all associated with specific regions of Africa. Lineages 5 and 6 are also known as *M. africanum* West Africa 1 and West Africa 2, respectively and almost excusively occur in West Africa and/or inrecent immigrants from those regions (De Jong *et al.,* 2010). Similarly, the recently discovered lineage 7 is confined to Ethiopia and recent immigrants from the country although the reasons why these lineages limited to specific regions are unknown (Firdessa *et al.,* 2013).

In addition, Africa is the only region of theworld that contains all the seven main human adapted MTBC lineages and harbours the largest diversity of these lineages (Gagneux and Small, 2007). Modern human (*Homo sapiens*) from Africa are known to be phylogenetically 'ancesteral' and harbor most of the known human genetic diversity (Tishkoff *et al.,* 2009). Human adapted MTBC exhibits a phylo-geographic population structure with different lineages associated with different human populations (Gagneux *et al.,* 2006).

Different MTBC strains and lineages differ in their growth rates, immunogenicity, disease severity, disease oresentation, disease transmission and also differ in their gene expression and metabolic profiles (Gehre *et al.,* 2013; Rose *et al.,* 2013). It was reported that significant differences in mycolic acid profiles between different MTBC strains and lineages (Portevin et al., 2014). The virulence of the MTBC is directly linked to transmission, unlike many other pathogens where transmission occurs independently of disease virulence (Ebert and Bull, 2003). The direct link between virulence and transmission in TB infection is because in TB, latently infected individuals are considered as non-infectious (Barry *et al.,* 2009) and individuals harbouring the largest number of acid fast bacilli in their sputum are considered the most infectious (Behr *et al.,* 1999).

Virulence in TB is the general result of the ability of the bacteria to survive in face of the host immune responses, their capacity to caiuse lung damage, to survive the aerosolization process outside the host, successful transmission and infect a new host. Different studies have found increased virulence associated with reduced and delayed infamatory responses. Modern MTBC strains are more globally wide-spread and more virulence than other lineages. Hence, modern strains are associated with a delyed inflammatory response (i.e. higher virulence) that might be linked to the global success of these strains (Wang *et al.,* 2010; Chen *et al.,* 2014). MTBC lineage variation has an effect on disease severity (Stavrum *et al.,* 2014). Epidemiological studies have supported that strains from 'modern' lineages are more transmissible than other MTBC strains (Buu *et al.,* 2012).

2.2.3. Mycobacterium bovis and Bovine Tuberculosis

Mycobacterium bovis (*M. bovis*) is slow-growing (16 to 20 hours generation time), aerobic facultative intracellular bacterium with a dysgonic colony shape when cultured on Löwenstein-Jensen medium (Kubica *et al.,* 2006) and the causative agent of tuberculosis in cattle (known as bovine TB). Related to *M. tuberculosis*-the bacterium which causes tuberculosis in humans, *M. bovis* can jump the species barrier and cause tuberculosis in humans. As all *Mycobacterium* spp., *M. bovis* has an unusual cell wall surface structure characterized by the dominant presence of mycolic acids and a wide array of lipids (Glickman and Jacobs, 2001). This waxy lipid envelope confers an extreme hydrophobicity, which renders the bacteria acid and alcohol fast, a feature that can be exploited to identify mycobacteria via the Ziehl-Neelsen staining technique (Steingart *et al.,* 2006). The mycobacterial surface lipids also have a potent biologic activity and are thought to play a crucial role in pathogenesis (Glickman and Jacobs, 2001).

Mycobacterium bovis can be identified on the basis of specific biochemical and metabolic properties. E.g., *M. bovis* requires pyruvate as a growth supplement, is negative for niacin accumulation and nitrate reduction, shows microaerophilic growth on Lebek medium and is generally resistant to pyrazinamide. In contrast, *M. tuberculosis* does not require pyruvate as a growth supplement, is positive for niacin accumulation and nitrate reduction, shows aerophilic growth on Lebek medium, and is usually not mono-resistant to pyrazinamide (Cole, 2002; Kubica *et al.,* 2006). However, the unequivocal validity of these characteristics is challenged by several studies (Kubica *et al.,* 2006; Niemann *et al.,* 2000). Different molecular markers and techniques have been discovered and developed in the past that allow the unambiguous identification and differentiation of *Mycobacterium* spp. and the members of the MTBC (Durr *et al.,* 2000; Mostrom *et al.,* 2002; Huard *et al.,* 2006; Pinsky and Banaei, 2008).

Bovine tuberculosis (BTB) is a chronic, generally respiratory disease, which is clinically difficult to diagnose although emaciation, loss of appetite, chronic cough and other signs of pneumonia could be symptoms developing at relatively late stages of the infection in cattle (Ayele *et al.,* 2004). Especially in developing countries, clinical forms of many other chronic, emaciating diseases, like African Trypanosomiasis, chronic contagious bovine pleuro-pneumonia (CBPP) or chronic multi-parasitism, are difficult to be distinguished from BTB. BTB pathology is characterized by the formation of granulomatous lesions, which can within the course of the disease regress or exhibit extensive necrosis, calcify or liquefy and subsequently lead to cavity formation (Cassidy, 2006; Hope and Villarreal-Ramos, 2008). During meat inspection procedures on cattle carcasses in slaughterhouses, tuberculous lesions are primarily found in the upper and lower respiratory tract and associated lymph nodes (Cassidy, 2006). However, the bacteria can also develop a systemic infection, disseminate within its host and affect other organs (Coetzer and Tustin, 2004).

Currently, *M. bovis* in humans is becoming increasingly important in developing countries, as humans and animals are sharing the same micro-environment and dwelling premises, especially in rural areas. At present, due to the association of Mycobacteria with the HIV/AIDS pandemic and in view of the high prevalence of HIV/AIDS in the developing world and susceptibility of AIDS patients to tuberculosis in general, the situation changing is most likely (Amanfu, 2006). In developing countries and Ethiopia is one of those where BTB is considered as prevalent disease in cattle population, its zoonotic implication has also significantly indicated as increasing trend to be of public health hazards (Ameni *et al.*, 2003a,b; Regassa, 2005; Amanfu, 2006; WHO, 2006; Shitaye *et al.*, 2007).

2.2.4. Epidemiology of Bovine tuberculosis

Bovine tuberculosis (BTB) is an endemic disease of cattle in Ethiopia and has been reported from different regions of the country on the bases of tuberculin tests and abattoir meat inspection with prevalence ranging from 1.5% in Addis Ababa to 78.7% in Debre Zeit dairy farms (Regassa, 1999). A preliminary study to characterize mycobacteria infecting cattle from two different management systems in central Ethiopia showed approximately 27% of isolates from grazing cattle were *M. tuberculosis*, while cattle in a more intensive production system were exclusively infected with *M. bovis*. The practice of local farmers discharging chewed tobacco directly into the mouths of pastured cattle was identified as a potential route of human-to-cattle transmission of *M. tuberculosis* (Ameni *et al*., 2011). In a study conducted in and around Addis Ababa, the overall tuberculin reactor's is 10.31% (Bogale *et al.*, 2000).

In Ethiopia, as indicated by tuberculin test and slaughter house data, TB disease prevalence of 10% was reported in cattle from the Selalle region north of Addis Ababa and in animals in Holeta in the central highlands. These regions are major dairy farming

areas that supply mainly unpasteurized milk to the urban and peri-urban areas of Addis Ababa (Ameni *et al*., 2007a). Mycobacteria isolated from 52 cattle from these regions of these 30 animals were from the same intensive production farm in Holeta and 22 were grazing cattle from 15 different farms in the Selalle region (Ameni *et al*., 2007a; Berg *et al*., 2009; Biffa *et al*., 2009). Despite the documented endemicity of BTB in Ethiopian cattle, information on the disease epidemiology in animals and humans is largely unknown (Bogale *et al*., 2000; Ameni *et al*., 2003a,b; Bogale *et al*., 2004). Due to inadequate disease surveillance system, lack of diagnostic facilities, inadequate financial resources and qualified manpower, and lack of prioritization from government, there is no national data on the distribution of bovine tuberculosis (Bogale *et al*., 2004).

2.2.4.1. Prevalence

The study conducted in an integrated extensive production system in the Ethiopian highlands indicated the prevalence of BTB ranging from 3.4% (Regassa, 2001) to 22.6% and the studies that conducted in small holder production system show the prevalence of BTB as 4.2% (Gemta, 2000) and 16.2% (Regassa, 2005). A study aimed at describing the magnitude and distribution of gross lesions compatible with bovine tuberculosis (BTB) in Ethiopian slaughter cattle in five abattoirs (four municipal and one export) located in various cattle husbandry systems in Ethiopia showed the highest and lowest prevalence of TB being recorded in Adama 24.7% and Yabello abattoirs 4.2%, respectively. Cattle whose origin was from Addis Ababa and its surrounding areas had higher prevalence of TB infection 23.9% with tuberculous lesions than those from elsewhere, with lowest prevalence 3.4% recorded in cattle originating from south-eastern region. Cattle maintained in dairy farms had high degree of exposure to the infection than those maintained in other types of management system where tuberculous lesions found to be more prevalent in dairy farms 23.9% followed by small scale fattening 15.7%, large-scale fattening 4.8% and pastoral/agro-pastoral production system 7.5%. TB prevalence showed a marked variation between categories of age, breed, class

of animals, abattoir, geographic origin and husbandry system. It was higher in old animals than middle age group; in exotic than local breed; in calves than other classes of animals (Biffa *et al.,* 2009).

Post mortem examination of 3322 carcasses showed 337 (10.1%) presented lesions suggestive of BTB with 69 (20.5%) having generalized and 268 (79.5%) localized type. The results showed that old cattle tend to develop lesions at a relatively higher frequency than adults. Similarly, exotic cattle (Holstein) were found to be more affected than cross-bred and local zebu cattle. There was also variation in occurrence of tuberculous lesions across the different types/classes of cattle, with considerably higher prevalence recorded in calves. On the other hand, there was no variation in the distribution of tuberculous lesions between categories of sex and body condition score (Biffa *et al.,* 2009).

The study conducted in Gondar town and Dembia districts of North Gondar zone of Amhara National Regional state showed out of the total 311 cattle subject to comparative intradermal tuberculin test, overall prevalence of BTB was 7.1% positive. In the outdoor and indoor management system, a prevalence of BTB 4.5% and 8.1% were found, respectively. Furthermore, prevalence of BTB at herd level 15.6% and 37.8% were obtained, respectively. The prevalence of BTB was 7.2% and 6.7% in Gondar and Dembia district, respectively but there was no significant difference in prevalence of BTB between districts. The prevalence of BTB in 4-7 years age group was affected significantly higher than that of 1-4 and more than 7 year age group. Similarly, there was significant difference in prevalence of BTB in fat, medium, lean and emaciated body condition (Mohammed *et al*., 2012).

The study conducted on 2231 small ruminants in four districts, namely, Amibara, Dubti, Afambo, and Chifra of the Afar pastoral region of Ethiopia using comparative intradermal tuberculin skin test, postmortem examination, mycobacteriological culture and molecular typing methods showed the overall animal prevalence of TB in small ruminants as 0.5% at ≥4 mm and 3.8% ≥2 mm cut-off points. The herd prevalence was

20% and 47% at ≥4 mm and ≥2 mm cut-off points, respectively. The overall animal prevalence of *Mycobacterium avium* complex infection was 2.8% and 6.8% at ≥4 mm and ≥2 mm cut-off points, respectively. Mycobacteriological culture and molecular characterization of isolates from tissue lesions of tuberculin reactor goats resulted in isolation of *Mycobacterium tuberculosis* and non-tuberculosis Mycobacteria as causative agents of tuberculosis and tuberculosis-like diseases in goats, respectively. It was suggested that the isolation of *Mycobacterium tuberculosis* in goat as a potential transmission of the causative agent from human and warrants further investigation in the role of small ruminants in epidemiology of human tuberculosis in the region (Mamo *et al.,* 2012).

2.2.4.2. Source of infection and mode of transmission

The source of the pathogenic mycobacteria is usually infected animals. However, wild animal reservoirs of *M. bovis* occur such as badgers in Europe, brush-tailed opossums in New Zealand, Cape buffalo in East Africa and deer in Europe and America (Quinn *et al*., 2004). Organisms are excreted in the exhaled air, in sputum, feces (from intestinal lesions and swallowed sputum from pulmonary lesions), milk, urine, vaginal and uterine discharges, discharges from open peripheral lymph nodes and semen. While most wildlife and feral animals are unimportant as sources for infection to cattle, in some areas of the world certain wildlife species appear to be a significant maintenance host and reservoir for infection in cattle (Radostits *et al*., 2007).

Transmission between animals is mostly thought to occur by inhalation of contaminated aerosol (Kaneene and Pfeiffer, 2006). Evidence for a generally respiratory route of infection has first come from studies of the tuberculous lesion distribution in cattle. Most commonly, the upper and lower respiratory tract and associated lymph nodes are affected by lesions and the minimum dose required to establish disease in artificially infected cattle appeared to be 1000 times less for the respiratory route than for the oral

route (Cassidy, 2006). However, infection can also occur via the gastro-intestinal tract when animals ingest contaminated food, water or milk. In this case, lesion distribution is characterized by the presence of mesenteric lymph node lesions (Kaneene and Pfeiffer, 2006).

2.2.4.3. Risk factors

Bovine tuberculosis can infect most mammalian species although bovids and especially cattle are the main hosts. Bovine TB affects a broad range of mammalian hosts including humans, cattle, deer, llamas, pigs, domestic cats, wild carnivores (fox, coyotes, badgers), possums, mustelids and rodents; it rarely affects equids or sheep (Delahay *et al.*, 2002). Housing predisposes to the disease, as does high stocking intensity and a large number of animals on a farm so that the disease is more common and serious where these forms of husbandry are practiced. The closer the animals are in contact the greater is the chance that the disease will be transmitted. In spite of the low overall incidence in countries where cattle are at pasture all the year round, individual herds with 60-70% morbidity may be encountered. Amongst beef cattle the degree of infection is usually much lower because of the open range conditions under which they are kept. However, individual beef herds may suffer a high morbidity if infected animals are introduced and large numbers of animals have to drink from stagnant water holes, especially during dry seasons (Radostits *et al*., 2007).

Zebu (*Bos indicus*) type cattle are thought to be much more resistant to tuberculosis than European cattle and the effects on these cattle are much less severe but under intensive feedlot conditions a morbidity rate of 60% and a depression of weight gain can be experienced in tuberculous Zebu cattle. In pigs, disease levels reflect those in the local cattle population from which the infection derives either by the ingestion of dairy products or by grazing over the same pasture as cattle. The lower relative prevalence in pigs is due to a number of factors, particularly the tendency of the disease to remain

localized in this species and the early age of slaughter. Prevalence is higher in older pigs. When the disease is common among dairy cattle in an area, 10-20% of the local pigs are likely to be infected. A high prevalence has been observed in pigs bedded on shavings infected with *M. bovis*. The causative organism is moderately resistant to heat, desiccation, and many disinfectants. It is readily destroyed by direct sunlight unless it is in a moist environment. In warm, moist, protected positions, it may remain viable for weeks (Radostits *et al*., 2007).

Commonly found risk factors for tuberculosis disease in cattle are close contact of animals such as in intensive livestock production systems (Ayele *et al.,* 2004; Ameni *et al.,* 2006; Cleaveland *et al.,* 2007), increasing herd size (Cleaveland *et al.,* 2007) and increasing age (Kazwala *et al.,* 2001; Oloya *et al.,* 2006; Munyeme *et al.,* 2008a). Another important risk factor is the contact or proximity of cattle and wildlife reservoirs of *M. bovis*, which obstruct disease eradication schemes in a number of countries. E.g., in the UK the Eurasian badger (*Meles meles*) represents a maintenance host for *M. bovis* (Cheeseman *et al.,* 1989). White-tailed deer (*Odocoileus virginianus*) has been identified as a disease reservoir in Michigan, USA (O'Brien *et al.,* 2006), the brush tail possum (*Trichosurus vulpecula*) is a disease reservoir in New Zealand (Coleman and Cooke, 2001; Porphyre *et al.,* 2008) and the African buffalo (*Syncerus caffer*) has been identified as a main reservoir host for *M. bovis* in southern Africa (Michel *et al.,* 2006). Recent work also indicates an important influence of host genetics on disease susceptibility. In this respect, a report from Ethiopia described lower disease prevalence in African cattle breeds as compared to exotic animals (Ameni *et al.,* 2006; Ameni *et al.,* 2007a,b); but also differences between distinct zebu breeds have been described in Chad (Diguimbaye-Djaibe *et al.,* 2006). The main risk factors for *M. bovis* infections in humans are poverty, malnutrition, HIV infection, the consumption of raw milk and close contact to livestock (Ayele *et al.,* 2004).

Bovine tuberculosis (BTB) has been largely eradicated in the industrialized world with the exception of few countries in which the presence of wildlife reservoir obstructs

disease elimination even though extensive control measures are applied (Kaneene and Pfiefer, 2006). However, it is well known that BTB is present in many developing countries (Cosivi *et al.,* 1998). Nevertheless, due to the absence of disease surveillance and control, little accurate information is available on the prevalence and distribution of *M. bovis* infections in Sub-Saharan Africa.

BTB is primarily of economic importance as it can have considerable direct effect on milk and meat production and animal reproduction. Moreover, national and international trade and other economic sectors may be indirectly affected by the disease (Zinsstag *et al.,* 2006). BTB can also infect wildlife and thus have unpredictable consequences for entire ecosystems. E.g., in the southern region of the Kruger National Park in South Africa, 38% of the buffalos are infected with strains of *M. bovis,* originally introduced from domesticated cattle. Carnivores such as lions, cheetah and leopards, feeding on infected animals are frequent spillover hosts (Renwick *et al.,* 2006). Moreover, wildlife reservoirs of *M. bovis* hamper disease eradication schemes in several countries (Bengis *et al.,* 2002; Michel *et al.,* 2006). BTB also bears a zoonotic potential and it is of public health concern (Thoen *et al.,* 2006).

The problems associated with BTB are of particular importance for many countries in Africa and especially the arid and semi-arid zones, where more than 50% of all African cattle, sheep and goats are raised (Otte and Chilonda, 2002) and where the livelihood of millions of people is based on livestock farming (WHO, 2006). Literature indicated that there are estimates of 556 million poor livestock keepers in the world with 30% of them living in sub-Saharan Africa and being most severely affected by the consequences of BTB (WHO, 2006; Thornton *et al.,* 2002). However, data on disease prevalence in cattle or wildlife or the frequency of zoonotic transmission is generally scarce. This is mostly due to the absence of disease surveillance, insufficient laboratory capacity and the lack of veterinary expertise and may lead to a general underestimation of the disease prevalence in these regions (Ayele *et al.,* 2004).

In a study in Ethiopia, mesenteric lymph node lesions were more often found in grazing animals compared to animals kept indoors (Ameni *et al.,* 2006). Thus, infection via the gastro-intestinal tract may be more important in cattle subjected to extensive livestock production systems as they are commonly observed in Africa. Because close contact between animals is relatively rare in extensive production systems, aerosol transmission of *M. bovis* is most likely occurring at water points, or when animals are gathering at night for protection from predators or during the daytime under trees when seeking shelter from the sun (Ayele *et al.,* 2004). Different routes of transmission may take place if tuberculosis infection becomes systemic and other organs such as the urinary tract or the mammary glands become involved. Especially the latter can be responsible for early infections in calves (Kaneene and Pfiefer, 2006). Moreover, consumption of contaminated milk represents the most important route of zoonotic tuberculosis transmission although disease transmission can be easily prevented by milk pasteurization (Ayele *et al.,* 2004; Cosivi *et al.,* 1998).

2.2.5. Epidemiology of Tuberculosis in Dromedary Camels

After the first description of TB in OWCs in the Egyptian Official Gazette in 1888 only sporadic reports were documented until Mason in 1912 published his pathological observations on a series of 20 cases detected during a year's surveillance at a Cairo abattoir. This was followed by a further study of camels slaughtered between 1910 and 1916 which suggested a prevalence of 1.6-5.4% and affecting chiefly old animals (Mason, 1917). In a brief review (Mustafa, 1987) mentioned that TB was more commonly observed in farmed camels and those in close proximity to cattle but appeared to be rare among pastoral camels, suggesting that close contact facilitates transmission between domesticated animals. In 1991 TB was reported as rare in Somalia, a country which at that time had one of the largest populations of OWC in the world (Abdurahman and Bornstein, 1991). The prevalence of TB in dromedaries is also

thought to be rare in Dubai, where only four cases have been documented in a 25 year observation period (Wernery *et al.,* 2007).

In contrast Elmossalami *et al*., (1971) indicated in his study in Kazakhstan suggesting a higher TB prevalence of 13% in camels and a more recent study in Ethiopian abattoirs suggested a similar prevalence of 10% based on the identification of gross lesions in 906 apparently healthy dromedaries (Mamo *et al.,* 2011). Camel TB has been described also in Pakistan (Zubair *et al*., 2004), Nigeria (Abubaker *et al.,* 2014) and Ethiopia (Mamo *et al.,* 2009, 2011; Gumi *et al.,* 2012; Zerom *et al.,* 2013; Kassaye *et al.*, 2013; Beye *et al.*, 2014). *M. bovis*, *M. tuberculosis* and non-tuberculous mycobacteria (NTBC) such as *M. kansasii, M. aquae, M. fortuitum* and *M. smegmatis* have all been isolated from OWCs as causative agents of camel TB (Elmossalami *et al.,* 1971; Kinne *et al.,* 2006; Mamo *et al.,* 2011; Zerom *et al.,* 2013).

Camelids are susceptible to TB, and DNA typing of *M. bovis* isolates from infected camelids to date has largely reflected the locally predominant genotypes of the bacterium found in the neighbouring cattle and wildlife. As there is currently no routine surveillance testing regime for TB in camelids; by the time it has been detected by clinical or post-mortem examination, the disease can be advanced and with severe pathology. Although TB is notifiable in camelids in GB there is no compulsory scheme for regular TB testing of camelid herds. In the absence of an active disease surveillance regime, initial detection of TB in camelids primarily relies on the submission by private veterinary surgeons of carcasses or tissue samples for post-mortem examination at government veterinary laboratories, either as routine casework or as suspected clinical cases of TB that die on farm or are euthanized due to a deteriorating body condition (Rhodes *et al.,* 2015).

Clinical signs in infected camelids tend to be vague or non-existent. Subtle changes in behaviour may be detected by observant owners. In some there is a short period of illness terminating with respiratory symptoms. Other signs such as weight loss, loss of

appetite, exercise intolerance or an intermittent dry cough are not consistent. Some camelids remain in good body condition until sudden death. As there is no routine surveillance for camelids, it is for the owner or their veterinary surgeon to arrange a post-mortem examination for any dead or moribund animals. The respiratory system and associated lymph nodes are most frequently affected. The lung lesions may be so extensive that it is surprising that severe pathology did not prove fatal earlier. The lesions are white or creamy and caseous. There may be miliary lesions or multiple foci in the lungs, and in more advanced cases these lesions coalesce to give large areas of caseous necrosis, often involving whole lobes. The lymph nodes of healthy camelids can be small and difficult to find. By contrast, tuberculous lymph nodes are often massively enlarged and contain multiple white, cream or yellow-tinged caseous foci and in severe cases the whole node may be replaced by one large caseous lesion (Rhodes *et al.,* 2015).

Infection of camelids with *M. microti* (originally identified as a pathogen of voles and shews) but probably more wide-spread than originally envisaged (Kremer *et al.,* 1999), has also been described in Great Britain (GB) as well as other countries, resulting in tuberculous lesions that are indistinguishable from those caused by *M. bovis*. Non-MTBC mycobacteria may also cause disease in camelids but reports are rare. In the European Union (EU) and other countries, the single intradermal comparative tuberculin skin test, using tuberculin purified protein derivatives (PPD) extracted from *M. bovis* (PPDB) and *M. avium* (PPDA), remains the primary official TB test for camelids. Although highly specific, this test appears to have low sensitivity in South American camelids (Rhodes *et al.,* 2015). One positive consequence of the tuberculin skin test is the boosting effect of specific antibodies (in infected animals), known as an anamnestic antibody response, which was described in an experimental *M. bovis* infection of llamas in 1998. Thus, the sensitivity of TB-specific antibody tests can be enhanced by a prior skin test, whether the animal is skin-test-positive or not. One negative impact of the lack of reliability in interpretation of the recommended skin test is that testing in OWCs may be discontinued, resulting in reduced disease surveillance and so posing severe limitations upon any programme to control camelid TB (Rhodes *et al.*, 2015).

TB in OWCs of eastern Ethiopia was studied for the first time in 2009 in order to describe its prevalence and to isolate *M. bovis* from infected camels as the causative organism (Mamo *et al.,* 2009). A study on 276 pastoral camels slaughtered between December 2006 and May 2007 at an abattoir east of Addis Ababa showed 14 camels have lesions typical of TB infection, providing an estimate of 5% prevalence in pastoral camels in this area. However, of these 14 lesioned camels, only four had AFB-positive tissue impression smears, and only one of these four samples was PCR-positive for MTBC (Mamo *et al.,* 2009). Another study of 906 apparently healthy camels from two further pastoral regions of eastern and southern Ethiopia showed 91 camels to have suspicious TB lesions, providing an estimated prevalence of 10%. Acid fast Bacilli (AFB) positive mycobacterial isolates were cultured from 31 (34%) of these lesioned camels, of which 21 provided a positive PCR signal for the genus Mycobacterium, but only two were confirmed as MTBC and identified as *M. bovis* (Mamo *et al.,* 2011). The apparently poor recovery of a positive causative agent (i.e. *M. bovis*) from the majority of lesioned camels in these two studies in Ethiopia could be related to a non-optimal culture for non-MTBC mycobacteria, and also reflect the diversity of mycobacteria causing mycobacterial diseases in camels. More recent 16S rDNA sequencing of these non-MTBC mycobacteria has revealed *M. terrae* complex, *M. flavescens, M. brasiliensis, M. chelonae* and *M. avium* as causative agents (Mamo *et al.,* 2011unpublished data).

Other abattoir studies have similarly highlighted the potential for involvement of non-MTBC as well as MTBC in camel mycobacterioses in Ethiopia. A detailed post mortem examination of 293 OWCs from eastern Ethiopia showed an estimated prevalence of 12.3% (36/293), with the occurrence of TB lesions significantly associated with female dromedaries. Mycobacteria were isolated from 61% (22/36) of those gross lesions investigated, and molecular characterization of the isolates showed just three to be *M. tuberculosis,* and the majority of the isolates from this study (15/22) to be non-MTBC (Zerom *et al.,* 2013). Gumi *et al.,* (2012) examined 694 camels slaughtered at Filtu and Addis Ababa abattoirs (mainly camels from southern Ethiopia) and isolated three AFB-

positive isolates of which one was *M. tuberculosis* and the other two were non-MTBC. The latter group of organisms have similarly been identified as a significant cause of mycobacterial infections in Ethiopian cattle, with one study describing 30% (53/171) of isolates as containing 11 non-MTBC species (Berg *et al.,* 2009). These findings not only suggest the importance of non-MTBC in OWC TB in Ethiopia, but also highlight a potential role for OWCs in the transmission of *M. tuberculosis* in humans (Rhodes *et al.*, 2015).

2.2.6. Pathogenesis of Mycobacterium tuberculosis Complex

Tuberculosis spreads in the body by two stages, the primary complex and post primary dissemination. The primary complex consists of the lesion at the point of entry and in the local lymph node. A lesion at the point of entry is common when infection is by inhalation. When infection occurs via the alimentary tract, a lesion at the site of entry is unusual, although tonsillar and intestinal ulcers may occur. More commonly the only observable lesion is in the pharyngeal or mesenteric lymph nodes.

A visible primary focus develops within 8 days of entry being effected by the bacteria. Calcification of the lesions commences about 2 weeks later. The developing necrotic focus is soon surrounded by granulation tissue, monocytes, and plasma cells and the pathognomonic 'tubercle' is established. Bacteria pass from this primary focus, which is in the respiratory tract 90-95% of cases in cattle, to a regional lymph node and cause the development of a similar lesion there. The lesions in the lungs in cattle occur in the caudal lobes in 90% of cases. In calves fed tuberculous milk, the primary focus is likely to be in the pharyngeal or mesenteric lymph nodes, with hepatic lesions as the major manifestation of post-primary spread. Post primary dissemination from the primary complex may take the form of acute miliary tuberculosis, discrete nodular lesions in various organs, or chronic organ tuberculosis caused by endogenous or exogenous reinfection of tissues rendered allergic to tuberculoprotein. In the latter case there may

be no involvement of the local lymph node. Depending upon the sites of localization of infection, clinical signs vary but, because the disease is always progressive, there is the constant underlying toxemia which causes weakness, debility, and the eventual death of the animal (Radostits *et al*., 2007).

TB infection begins when the mycobacteria reach the pulmonary alveoli, where they invade and replicate within endosomes of alveolar macrophages. The primary site of infection in the lungs, known as the "Ghon focus", is generally located in either the upper part of the lower lobe, or the lower part of the upper lobe. Tuberculosis of the lungs may also occur via infection from the blood stream which is known as a 'Simon focus' and is typically found in the top of the lung. This hematogenous transmission can also spread infection to more distant sites, such as peripheral lymph nodes, the kidneys, the brain, and the bones. All parts of the body can be affected by the disease, though for unknown reasons it rarely affects the heart, skeletal muscles, pancreas, or thyroid (Kumar *et al.,* 2007).

Tuberculosis is classified as one of the granulomatous inflammatory diseases. Macrophages, T lymphocytes, B lymphocytes, and fibroblasts are among the cells that aggregate to form granulomas, with lymphocytes surrounding the infected macrophages. The granuloma prevents dissemination of the mycobacteria and provides a local environment for interaction of cells of the immune system. Bacteria inside the granuloma can become dormant, resulting in latent infection. Another feature of the granulomas is the development of abnormal cell death (necrosis) in the center of tubercles. To the naked eye, this has the texture of soft, white cheese and is termed caseous necrosis (Crowley and Leonard, 2010).

If TB bacteria gain entry to the bloodstream from an area of damaged tissue, they can spread throughout the body and set up many foci of infection, all appearing as tiny, white tubercles in the tissues. This severe form of TB disease, most common in children and those with HIV, is called miliary tuberculosis. People with this disseminated TB

have a high fatality rate of about 30% even with treatment (Jacob *et al*., 2009). In many people, the infection waxes and wanes. Tissue destruction and necrosis are often balanced by healing and fibrosis. Affected tissue is replaced by scarring and cavities filled with caseous necrotic material. During active disease, some of these cavities are joined to the air passages bronchi and this material can be coughed up which contains living bacteria, and so can spread the infection. Treatment with appropriate antibiotics kills bacteria and allows healing to take place. Upon cure, affected areas are eventually replaced by scar tissue (Crowley and Leonard, 2010).

2.2.7. Immunity to Mycobacterial Infection

2.2.7.1. Innate immunity

Based on the predominance of tuberculous lesions in the respiratory tract and associated lymph nodes of diseased cattle, it was early believed that BTB is transmitted from animals to animals through inhalation of infectious aerosols. Further evidence for this route of transmission has come from a number of other studies (Cassidy, 2006). Bacteria entering the respiratory tract, passing the mucociliary layer and gaining access to the alveolar space are thought to be phagocytized by macrophages, which may constitute the main cellular host for Mycobacteria *in vivo* (Hope and Villarreal-Ramos, 2008). Following uptake into the phagosome, macrophages attempt to kill the bacteria by phago-lysosome fusion and acidification. However, mycobacteria are able to prevent lysosomal delivery by manipulating the host cell signal transduction pathways using an array of bacterial effector lipids and proteins (Nguyen and Pieters, 2005).

Mycobacteria are therefore believed to reside and multiply primarily within the phagosomes before they eventually destroy the phagocytes. However, this assumption has been challenged by a recent study describing the translocation of *M. tuberculosis* from phago-lysosomes to the cytosol of myeloid cells, from the second day after

phagocytosis (Van der *et al.,* 2007). Although infected macrophages are not believed to act as the main antigen presenting cells (APC) they can trigger an immune response through the secretion of pro-inflammatory cytokines (e.g. TNF-α) and chemokines, leading to the recruitment of other phagocytes and lymphocytes to the lung (Hope and Villarreal-Ramos, 2008; Thoen and Barletta, 2006). The most important APCs in tuberculosis infection are probably dendritic cells, which also play a major role in modulating the host immune response. Mycobacteria may get access to the lung tissue through M-like cells in the bronchi. From there, they can penetrate to the underlying lymphoid tissues, get phagocytized by dendritic cells and transported to the draining lymph nodes, where immune responses are initiated (Hope and Villarreal-Ramos, 2008).

Interestingly, activation of bovine dendritic cells enhances their ability to kill ingested *M. bovis* to a lesser extent and significant numbers of live bacilli are able to persist. Dendritic cells may therefore constitute a reservoir for pathogenic mycobacteria. However, in a non-activated state, dendritic cells are more potent in killing ingested bacteria than macrophages. Natural Killer (NK) cells and γδ T-cells are crucial in the early innate immunity against mycobacterial infections. They release IFN-γ when activated through the interaction with dendritic cells and thus contribute to the Th1 based immune response. Conversely, NK and γδ T-cells also play a role in fully activating dendritic cells (Hope and Villarreal-Ramos, 2008).

2.2.7.2. Adaptive immunity

Cell Mediated Immune Response

The interaction of *M. bovis* with bovine dendritic cells leads to cell maturation and increases expression of surface molecules involved in T-cell interactions. Moreover, altered cytokine profiles and especially the secretion of IL-12 and TNF-α are observed upon infection of bovine dendritic cells with *M. bovis* (Hope *et al.,* 2004). The secretion of these cytokines and mycobacterial antigen presentation on the surface of dendritic

cells help triggering an adaptive cell mediated immune (CMI) response. This CMI response is commonly known as the Th1-type immune response and characterized by the secretion of high levels of IFN-γ and IL-2 by Th1 CD4 T-cells (Thoen and Barletta, 2006). The production of IFN-γ and IL-2 by Th1 cells can activate macrophages in order to become highly microbicidal. Following activation, macrophages may be able to kill most of the mycobacteria within the phagosome by releasing increased amounts of hydrolytic enzymes, reactive oxygen intermediates and reactive nitrogen intermediates (including nitric oxide) (Hope and Villarreal-Ramos, 2008; Thoen and Barletta, 2006).

Cell-mediated immune (CMI) responses, including specific T lymphocytes and activated mononuclear macrophages, are important in host resistance to virulent acid-fast bacilli. The acquired cell-mediated immune response to *M. tuberculosis* infections is complex and involves T cells. The CD4 T cells secrete gamma interferon, which limits bacterial growth and may enhance clearance of the bacillus. The CD8 T cells directly destroy intracellular bacteria by releasing antimicrobial protein granulysin. Gamma delta T cells may influence the cellular response by promoting an influx of monocytes and lymphocytes and limiting inflammatory cells that may cause tissue damage. Various cytokines (gamma interferon) activate tuberculostatic macrophage functions and limit the replication of mycobacteria; however, mechanism involved in killing of mycobacteria is unclear. The importance of active oxygen metabolites, such as superoxide anion or singlet oxygen, has been investigated, but convincing evidence is not available regarding the significance of these components in protection of the host. Reactive nitrogen intermediates (nitrogen oxide) produced by peritoneal macrophages have been proposed to be important in killing (Gyles *et al*., 2004).

Control of infection is ultimately dependent on the induction of strong CMI response and granuloma formation, in which CD4 but also CD8 T-cells play a pivotal role. If the immune response against mycobacterial infection is strong enough to contain bacterial growth, active disease does not develop although infection may never be fully cleared. If the balance between the host's defenses and the persisting mycobacteria is tipped in

favor of the pathogen, active disease occurs and the granuloma formation progresses (Stewart *et al.,* 2003). At a late stage, BTB granulomas are characterized by extensive necrosis which can lead to liquefaction and cavity formation. Rapture of these cavities into the bronchi consequently allows aerosol spread of the bacteria (Thoen and Barletta, 2006). It was observed that microscopic lesions were observed in experimentally infected cows as early as seven days after inoculation of *M. bovis.* Gross lesions were detected in the upper respiratory tract, in the lungs and the lymph nodes draining these areas at 14 days post-infection (Cassidy, 2006). At the slaughterhouse, granulomatous lesions are most often detected in lymph nodes (especially mediastinal or bronchial). This is due to the fact that fluids in an animal together with activated macrophages eventually pass through the lymph nodes where the pathogens are filtered (Thoen and Barletta, 2006).

Although necessary for protection, the CMI response in tuberculosis infection can also contribute to the immuno-pathogenesis of tuberculosis. The Th2-type immune response functions as a regulatory element to counteract and down regulate the pro-inflammatory CMI response, elicited by IFN-γ producing cells (Hope and Villarreal-Ramos, 2008). It is also associated with an increased humoral immune reaction and is mainly triggered by CD4 Th2 cells and the secretion of IL-4. It is believed that Th1-type immune responses wane towards late disease stages. In contrast, humoral Th2-type immune responses increase as disease and pathology progresses and bacterial load increases (De la Rua-Domenech *et al.,* 2006). Although the factors that trigger the transition from a Th1- to a Th2- type immune response are not well known, it is thought that the CMI response is most relevant to determine protection; in contrast, the humoral response is considered detrimental (Thoen and Barletta, 2006). TB in cattle is characterized by an early Th1 type CMI response, whilst humoral immune responses develop as disease progresses. CMI responses can wane and animals become anergic, and SICCT as well as IFN-γ tests have been shown to give false negative results in such disease stages (De la Rua-Domenech *et al.,* 2006; Pollock *et al.,* 2005; Welsh *et al.,* 2005). Anergic animals are thought to be heavily diseased and highly infective (Pollock *et al.,* 2005).

Antibody Immune Response

Antibody responses during tuberculosis were analyzed by an enzyme-linked immunosorbent assay with a panel of 10 protein antigens of *Mycobacterium tuberculosis*. It was shown that serum IgG antibodies were produced against a variety of *M. tuberculosis* antigens and that the vast majority of sera from tuberculosis patients contained antibodies against one or more *M. tuberculosis* antigens. The number and the species of serologically reactive antigens varied greatly from individual to individual. In a given serum, the level of specific antibodies also varied with the antigen irrespective of the total number of antigens recognized by that particular serum (Konstantin *et al*., 2013).

2.2.8. Diagnosis of Tuberculosis

Diagnosis of tuberculosis infection in animals is often based on clinical signs, necropsy findings and specific immune response. In camelids, this strategy is difficult to conduct because of the lack of adequate tests for live animals (Alvarez *et al.*, 2011). A definitive diagnosis can be made only at post-mortem examination by demonstration of typical gross lesions, followed by histopathology and confirmatory bacterial culture. Because of the chronic nature of the disease and the multiplicity of signs caused by the variable localization of the infection, the disease occurs in a particular area it must be considered in the differential diagnosis of many other diseases. The diagnosis of tuberculosis in live animals is mainly based on the tuberculin skin test and demonstration of the organism in exudates or excretions from lesions of slaughtered animals (Radostits *et al*., 2007).

2.2.8.1. Identification of the agent

The presence of *M. bovis* in clinical and post-mortem specimens may be demonstrated by examination of stained smears or tissue sections and confirmed by cultivation of the organism on primary isolation medium. According to OIE standards (2009), sample collection containers should be clean and preferably sterile where containers contaminated by environmental mycobacteria may result in the failure to identify *M. bovis* infection due to rapid growth of environmental mycobacteria.

Microscopic examination

The mycobacteria cell wall contains a waxy coat known as mycolic acid which retains the primary staining dye, basic fuchsin. The acid-fast stain is used to differentiate microorganisms which resist decolonization with alcohol solution as their cell wall contains a waxy coat. The procedure of acid- fast stain takes suspicious organ sample from camel that died due to tuberculosis and stain with Ziehl-Neelsen (ZN). The sample are treated with concentrated carbon fuchsin and heat at interval until steam rise without boiling the fluid, wash in water, decolorize by flooding the slide with acid-alcohol for 1-2 minutes and wash in water, counter stain with methylene blue or malachite green for 30-60 seconds wash in water, blot dry and examined under oil immersion, the acid-fast mycobacteria retain the carbon fuchsin and appear as bright red or pink with blue or green back ground (OIE, 2009).

Mycobacterium bovis can be demonstrated microscopically on direct smears from clinical samples and on prepared tissue materials. The acid fastness of *M. bovis* will be demonstrated with the classic Ziehl-Neelsen stain. The presumptive diagnosis of mycobacteriosis can be made if the tissue has characteristics histological lesions (caseeous necrosis, mineralization, epitheliod cells, multinucleated giant cells and macrophages) (OIE, 2009).

Mycobacteriological Culture

Culture from lung and lymph node using Lowenstein-Jensen (LJ) medium slants after decontamination and concentration of the sample (OIE, 2009) is the gold standard method for the diagnosis of tuberculosis. The specimens are sectioned using sterile blades, minced with scissors and homogenized with a sterile mortar and pestle, stomacher or blender under a biological safety cabinet; followed by decontamination with either detergent such as 0.375–0.75% hexadecyl pyridinium chloride (HPC), an alkali (2–4% sodium hydroxide) or an acid (5% oxalic acid). The alkali or acid mixture is shaken for 10–15 minutes at room temperature and then neutralised. Neutralisation is not required when using HPC. The suspension is centrifuged, the supernatant is discarded, and the sediment is used for culture and microscopic examination.

It is recommended that as a minimum, pooled lymph node samples from the head and thorax be cultured when no visible lesions are detected in tuberculin or interferon test positive animals at post-mortem examination (OIE, 2009). For primary isolation, the sediment is usually inoculated on to a set of solid egg-based media, such as Lowenstein–Jensen, Coletsos base or Stonebrinks; these media should contain either pyruvate or pyruvate and glycerol. An agar-based medium such as Middlebrook 7H10 or 7H11 or blood based agar medium may also be used.

Cultures are incubated for a minimum of 8 weeks (and preferably for 10–12 weeks) at 37°C with or without CO_2. The media should be in tightly closed tubes to avoid desiccation. Slopes are examined for macroscopic growth at intervals during the incubation period. When growth is visible, smears are prepared and stained by Ziehl-Neelsen technique. Growth of *M. bovis* generally occurs within 3-6 weeks of incubation depending on the media used (OIE, 2009). Characteristic growth patterns and colonial morphology can provide a presumptive diagnosis of *M. bovis;* however every isolate needs to be confirmed. It is necessary to distinguish *M. bovis* from the other members of the '*M. tuberculosis* complex', i.e. *M. tuberculosis* (the primary cause of tuberculosis in

humans), *M. africanum* (occupies an intermediate phenotypic position between *M. tuberculosis* and *M. bovis*), *M. microti* (the 'vole bacillus', a rarely encountered organism), *M. pinnipedii* and *M. caprae*. *Mycobacterium tuberculosis* may infect cattle and sensitise cattle to bovine tuberculin without causing typical lesions. Sometimes *M. avium* or other environmental mycobacteria may be isolated from tuberculosis-like lesions in cattle. In such cases, a careful identification is needed, and mixed infection with *M. bovis* should be excluded (OIE, 2009).

Isolates can be identified by determining traditional cultural and biochemical properties. On a suitable pyruvate-based solid medium, colonies of *M. bovis* are smooth and off-white (buff) in colour. The organism grows slowly at 37°C, but does not grow at 22°C or 45°C. *Mycobacterium bovis* is sensitive to thiophen-2-carboxylic acid hydrazide (TCH) and to isonicotinic acid hydrazide (INH). This can be tested for by growth on 7H10/7H11 Middlebrook agar medium or on egg-containing media. The egg medium should be prepared without pyruvate because it inhibits INH and could have a similar effect on TCH (which is an analogue of INH) and thus give false-positive (resistant) results. *Mycobacterium bovis* strains are also sensitive to para-amino salicylic acid and streptomycin. Effective drug concentrations are different for egg-based and agar based media. Results for niacin production and nitrate reduction are negative in *M. bovis*. In the Amidase test, *M. bovis* is positive for urease and negative for nicotinamidase and pyrazinamidase. It is a micro-aerophillic and non-chromogenic bacterium (OIE, 2009).

Nucleic acid recognition methods (Molecular techniques)

Rapid identification of isolates to the level of *M. tuberculosis* complex can be made by Gen Probe TB complex DNA probe or polymerase chain reaction (PCR) targeting 16S–23S rRNA, the insertion sequences IS*6110* and IS*1081*, and genes coding for *M.tuberculosis* complex specific proteins, such as MPB70 and the 38 kDa antigen-b have been used. Specific identification of an isolate as *M. bovis* can be made using PCR targeting a mutation at nucleotide positions 285 in the *oxy*R gene,169 in the *pncA* gene,

675/756/1311/1410 and 1450 of the *gyr*B gene and presence/absence of RDs (Regions of Difference) (Niemann *et al.,* 2000; Parsons *et al.,* 2002). Alternatively, molecular typing techniques such as spoligotyping will identify *M. bovis* isolates and provide some molecular-typing information on the isolate that is of epidemiological value (Kamerbeek *et al.,* 1997).

PCR has been widely evaluated for the detection of *M. tuberculosis* complex in clinical samples (mainly sputum) in human patients and has recently been used for the diagnosis of tuberculosis in animals. A number of commercially available kits and various 'in-house' methods have been evaluated for the detection of the *M. tuberculosis* complex in fresh and fixed tissues. Various primers can be used, as described above. Amplification products have been analysed by hybridisation with probes or by gel electrophoresis (Durr *et al.,* 2000).

Commercial kits and the in-house methods, in fresh, frozen or boric acid-preserved tissues, have shown variable and less than satisfactory results in inter-laboratory comparisons. False-positive and false negative results, particularly in specimens containing low numbers of bacilli, have reduced the reliability of this test. Variability in results has been attributed to the low copy number of the target sequence per bacillus combined with a low number of bacilli. Variability has also been attributed to decontamination methods, DNA extraction procedures, techniques for the elimination of polymerase enzyme inhibitors, internal and external controls and procedures for the prevention of cross-contamination. Improvement in the reliability of PCR as a practical test for the detection of *M. tuberculosis* complex in fresh clinical specimens will require the development of standardized and robust procedures. Cross contamination is the greatest problem with this type of application and this is why proper controls have to be set up with each amplification. However, PCR is now being used on a routine basis in some laboratories to detect the *M. tuberculosis* group in paraffin embedded tissues. Although direct PCR can produce a rapid result, it is recommended that culture be used in parallel to confirm a viable *M. bovis* infection. A variety of DNA-fingerprinting

techniques has been developed to distinguish the *M. tuberculosis* complex isolates for epidemiological purposes. These methods can distinguish between different strains of *M. bovis* and will enable patterns of origin, transmission and spread of *M. bovis* to be described (Durr *et al.,* 2000).

The most widely used method is spoligotyping (from 'spacer oligotyping'), which allows the differentiation of strains inside each species belonging to the *M. tuberculosis* complex, including *M. bovis*, and can also distinguish *M. bovis* from *M. tuberculosis* (Kamerbeek *et al.,* 1997). The use of a standard nomenclature for the spoligotypes according to the database Mbovis.org (http://www.mbovis.org) is encouraged to allow international comparison of profiles. Other techniques include restriction endonuclease analysis (REA) and restriction fragment length polymorphism (RFLP) using IS*6110* probe (especially where there are >3–4 copies of IS*6110* in the isolate), the direct repeat (DR) region probe, the PGRS (polymorphic GC repeat sequence) probe (Skuce *et al.,* 2002) and the pUCD probes (O'Brian *et al.,* 2000). The mycobacterial interspersed repetitive units (MIRU)-variable number tandem repeat (VNTR) typing has also been developed to increase the discrimination of the *M. tuberculosis* complex species (Frothingham and Meeker, 1998; Supply *et al.,* 2000). Often a combination of techniques may be used to gain the maximum discrimination between strains (Cousins *et al.,* 1999). The genome of *M. bovis* has been sequenced and this information has contributed to improved methods of genetic fingerprinting and to the development of PCR assays that define the subspecies of the *M. tuberculosis* complex.

2.2.8.2. Methods for molecular epidemiological investigations

The different genotyping techniques used for the molecular characterization of MTBC strains have been reviewed extensively (Mostrom *et al.,* 2002; Harris, 2006; Van Soolingen, 2001; Kanduma *et al.,* 2003; Haddad *et al.,* 2004). Molecular typing of bacterial strains can offer general insights into the population structure of pathogens. In

the case of MTBC, a high degree of genetic homogeneity of strains isolated from patients from a given setting indicates recent transmission of tuberculosis. Conversely, a heterogenic population structure, especially in a low prevalence area, could suggest that tuberculosis reactivation may be more frequent. Moreover, molecular epidemiological tools can help to identify transmission chains, risk factors for tuberculosis infections (Harris, 2006) and they have been useful for the detection of laboratory cross-contaminations (Haddad *et al.*, 2004).

Spacer oligotyping (Spoligotyping)

Spoligotyping makes use of the variability of the MTBC chromosomal direct repeat (DR) locus for strain differentiation (Kamerbeek *et al.*, 1997). The DR region is composed of multiple well conserved direct repeats of 36 bp which are separated by distinct, non-repetitive spacer sequences of similar size (Hermans *et al.*, 1991) (Figure 2). In the standard spoligotyping scheme, a PCR with primers complementary to the DR-sequence is used to amplify all spacer sequences of a given strain. One of the two primers is labeled with a biotin marker. The PCR products are denatured and hybridized to a standard set of 43 oligonucleotides covalently linked to a membrane.

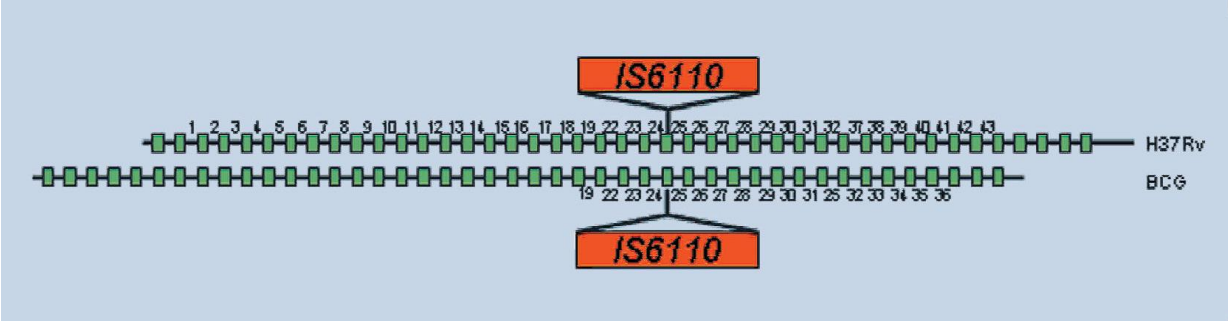


Figure 2: The structure of the DR locus in the genome of *M. tuberculo*sis H37Rv and *M. bovis* BCG P3. The green rectangles depicts the 36 bp Direct Repeat (DR) (Source: Marco *et al.*, 2004)

These oligonucleotides correspond to 37 spacers from *M. tuberculosis* H37Rv and 6 additional spacers from *M. bovis* BCG P3. If any of these spacers are also present in an investigated strain, they will be amplified during the PCR and hybridized to the spacers on the membrane. The successful hybridization can be visualized by incubation with Streptavidin peroxidase (which binds to the biotin molecule), subsequent addition of a chemiluminescent Streptavidin peroxidase substrate and exposure to a light sensitive film (Kamerbeek *et al.*, 1997).

Ninety-four (1-94) variable spacers have been identified, pieces of DNA of the variable spacers have been synthesised and 43 of them have been introduced vertically onto the membrane used for traditional spoligotyping. The conserved direct repeats (DR) serve as targets for the primers and thus are the basis for amplification. These products can be added to the membrane of a species or samples containing a species of an unknown genotype and belonging to the *M. tuberculosis* complex after PCR has been carried out. After hybridization and detection, the spoligo-pattern can be read from the horizontal axis of the various clinical isolates belonging to the *M. tuberculosis* complex, such as *M. tuberculosis*, *M. bovis subsp. caprae comb. nov*, *M. bovis* BCG, *M. bovis*, *M. canettii*, *M. microti* and *M. africanum* (Marco *et al.*, 2004) (Figure 3).

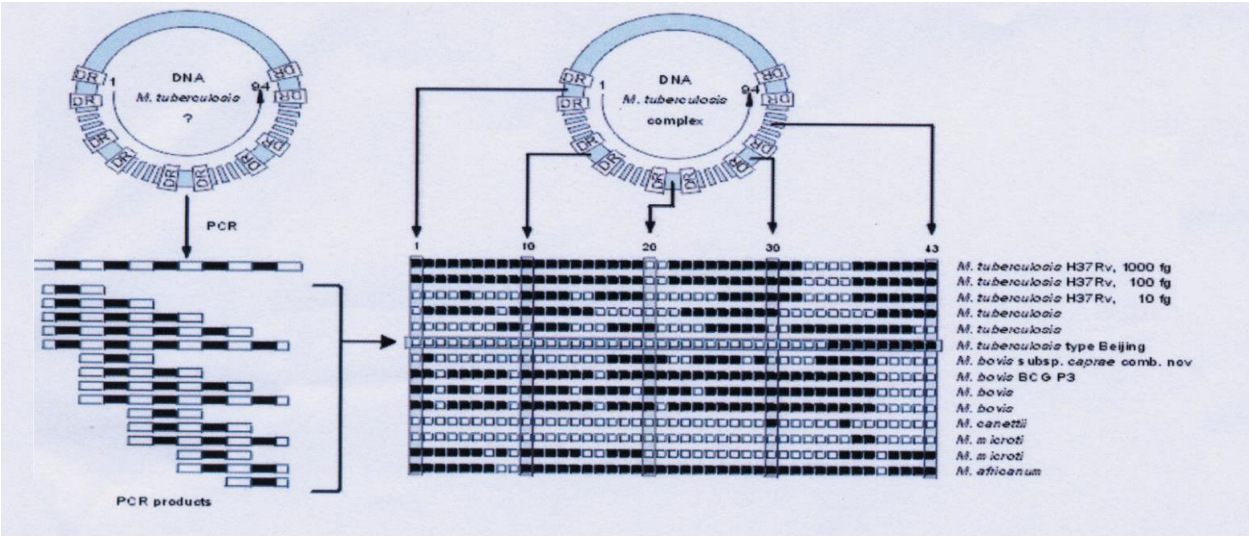


Figure 3: The DNA sequence of the DR region for the reference species *M. tuberculosis* H37Rv and *M. bovis* BCG P3 (Source: Marco *et al.*, 2004).

Compared to other molecular typing techniques, spoligotyping is not very discriminatory although its resolving power could be increased by inclusion of additional spacers in an extended spoligotyping protocol (Van der Zanden *et al.*, 2002). The technique has been proven to be useful for a first assessment of the MTBC strain diversity in many population surveys (Cousins *et al.*, 1999; Costello *et al.*, 1999; Allix *et al.*, 2006).

Spoligotyping also allows the differentiation of MTBC members; e.g., *M. bovis* strains characteristically lack spacers 3, 9, 16 and 39-43 (Smith *et al.*, 2006). Moreover, strain groups and families can be readily identified and extensive databases (www.*Mbovis*.org and SpolDB4) (Brudey *et al.*, 2006) have facilitated the comparison of strains isolated in different countries. It is assumed that the DR locus has a unidirectional evolution (spacers can only be lost and not reacquired) and spoligotyping can therefore be particularly useful for phylogenetic analyses (Smith *et al.*, 2005).

Large sequence polymorphism (LSP) analysis

Large sequence polymorphism (LSPs) generally refers to large genomic deletions or insertions. Large genomic deletions (also called region of difference, RD) are widely used for the phylogenetic analyses of MTBC but are not appropriate for molecular epidemiological studies, due to a low mutation rate (Brosch *et al.*, 2002; Smith *et al.*, 2006; Gagneux and Small, 2007). A major drawback of spoligotyping and VNTR typing is the possible occurrence of homoplasies; that is, the development of identical molecular types in unrelated strains. Such events are very unlikely to occur for large genomic deletions unless an LSP is associated with repetitive and transposable elements. Moreover, because of the suspected absence of horizontal gene transfer in MTBC, large genomic deletions cannot be reacquired and the direction of evolution can be determined without the use of an outgroup. Large genomic deletions can be discovered through comparative genomic hybridization using bacterial genomic DNA and oligonucleotide microarrays. After specific LSPs or RDs have been identified, simple PCR protocols to

rapidly assess their presence or absence in population surveys can be developed (Smith *et al.,* 2006).

2.2.8.3. The tuberculin Skin test: Delayed hypersensitivity test

The standard method for detection of bovine tuberculosis is the tuberculin test (the OIE prescribed test for international trade), which involves the intradermal injection of bovine tuberculin purified protein derivative (PPD) and the subsequent detection of swelling (delayed hypersensitivity) at the site of injection 72 hours later. This may be performed using bovine tuberculin alone or as a comparative test using avian and bovine tuberculins. The tuberculin test is usually performed on the mid-neck, but the test can also be performed in the caudal fold of the tail. The skin of the neck is more sensitive to tuberculin than the skin of the caudal fold. To compensate for this difference, higher doses of tuberculin may be used in the caudal fold.

Delayed hypersensitivity may not develop for a period of 3-6 weeks following infection. Thus, if a herd/animal is suspected to have been in contact very recently with infected animals, delaying testing should be considered in order to reduce the probability of false-negatives. As the sensitivity of the test is less than 100%, it is unlikely that eradication of tuberculosis from a herd will be achieved with only a single tuberculin test. It should be recognised that when used in chronically infected animals with severe pathology, the tuberculin test may be unresponsive. The tuberculin test has not been well validated in most non-bovid and non-cervid species. The comparative intradermal tuberculin test is used to differentiate between animals infected with *M. bovis* and those responding to bovine tuberculin as a result of exposure to other mycobacteria. This sensitization can be attributed to the antigenic cross-reactivity among mycobacterial species and related genera. The test involves the intradermal injection of bovine tuberculin and avian tuberculin into different sites, usually on the same side of the neck, and measuring the response 3 days later (OIE, 2009).

Intradermal tuberculin skin test (TST) is the international choice method for field diagnosis of bovine TB in live animals and the World Organisation for Animal Health (OIE) recommended difference between the increase in skin thickness for the test to be positive should be at least 4mm after 72 hours (OIE, 2009). However, the performance of TST is affected by environmental and host factors; and the nature of the tuberculin used (De la Rua-Domenech *et al.,* 2006; Ameni *et al.,* 2008; Marcotty, 2009; Humblet *et al.,* 2010). A perfect cut-off point in a specific geographic area or country may not be useful in another environment or another country (De la Rua-Domenech *et al.,* 2006; Strain *et al.,* 2011) and the ability of the test to accurately predict true positive disease status depends on its sensitivity, specificity and prevalence of the disease in the population tested (De la Rua-Domenech *et al.,* 2006).

The OIE recommended cut-off value was established mainly in developed countries for *Bos taurus* cattle and different cut-off values are applied according to a particular country's disease status and objective of its disease control programme. Severe interpretations have been used in Chad, Ethiopia and Tanzania (Kazwala *et al.,* 2001; Delafosse 2002; Ameni *et al.,* 2008; Ngandolo *et al.,* 2009) and in regions or herds where *M. bovis* infection had been confirmed based on the discretion of the veterinarian. TST together with slaughter of positive reactors to examine for TB lesions; culture of suspected TB specimens and other modern diagnostic techniques (eg: gamma-Interferon, ESAT-6 tests, serologic and fluorescence polarization assays) have been compared and are being validated for maximum diagnosis of bovine TB in cattle in various environmental conditions (Pollock *et al.,* 2003; De la Rua-Domenech *et al.,* 2006; Thom, 2006; Buddle *et al.,* 2009).

The potency of tuberculins must be estimated by biological methods based on comparison with standard tuberculins, and potency is expressed in International Units (IU). In several countries, bovine tuberculin is considered to be of acceptable potency if its estimated potency guarantees per bovine dose at least 2000 IU (±25%) in cattle. In cattle with diminished allergic sensitivity, a higher dose of bovine tuberculin is needed,

and in national eradication campaigns, doses of up to 5000 IU are recommended. The volume of each injection dose must not exceed 0.2 ml (OIE, 2009).

Test procedure *(OIE standard)*

A correct injection technique is important. The injection sites must be clipped and cleaned. A fold of skin within each clipped area is measured with callipers and the site marked prior to injection. A short needle, bevel edge outwards and graduated syringe charged with tuberculin attached, is inserted obliquely into the deeper layers of the skin. The dose of tuberculin is then injected. A multi-dose syringe or multiple injection gun may be used provided that delivery of the volume and safety are assured. The dose of tuberculin injected must be no lower than 2000 International Units (IU) of bovine or avian tuberculin. A correct injection is confirmed by palpating a small pea-like swelling at each site of injection. The distance between the two injections should be approximately 12–15cm. In animals in which there is no room to separate the sites sufficiently on one side of the neck, one injection must be made on each side of the neck at identical sites in the centre of the middle third of the neck. The skin-fold thickness of each injection site is re-measured 72 hours after injection. The same person should measure the skin before injection and when the test is read.

A number of alternative methods of interpreting the skin test responses have been adopted, recognizing that false-positive reactions may be caused by sensitisation by other mycobacteria and by local inflammation. It is important to recognise that there is a balance between sensitivity and specificity and achieving high concurrent values may not be possible. Appropriate policies need to be in place depending on disease prevalence and according to risk (e.g. where a wildlife reservoir is present). The interpretation is based on observation and the recorded increases in skin-fold thickness. In the single intradermal test (which requires a single injection of bovine tuberculin), the reaction is commonly considered to be negative if only limited swelling is observed, with an increase of no more than 2 mm and without clinical signs, such as diffuse or

extensive oedema, exudation, necrosis, pain or inflammation of the lymphatic ducts in that region or of the lymph nodes. The reaction is considered to be inconclusive if none of these clinical signs is observed and if the increase in skin-fold thickness is more than 2mm and less than 4mm. The reaction is considered to be positive if clinical signs, as mentioned above, are observed or if there is an increase of 4mm or more in skin-fold thickness.

Moreover, in *M. bovis*-infected herds, any palpable or visible swelling should be considered to be positive. Sometimes a more stringent interpretation is used, particularly in a high risk population or incontact animals. Animals that are inconclusive by the single intradermal test should be subjected to another test after an interval of 42 days to allow desensitisation to wane (in some areas 60 days for cattle and 120 days for deer are used). Animals that are not negative to this second test should be deemed to be positive to the test. Animals that are positive to the single intradermal test may be subjected to a comparative intradermal test or blood test. Any re-test should be performed in accordance with the local or national control programmes standard.

In the interpretation of the intradermal comparative test, a reaction is usually considered to be positive if the increase in skin thickness at the bovine site of injection is more than 4 mm greater than the reaction shown at the site of the avian injection. The reaction is considered to be inconclusive if the increase in skin thickness at the bovine site of injection is from 1 to 4 mm greater than the avian reaction. The reaction is considered to be negative if the increase in skin thickness at the bovine site of injection is less than or equal to the increase in the skin reaction at the avian site of injection.

In the caudal fold test, a short needle, bevel edge outwards, is inserted obliquely into the deeper layers of the skin on the lateral aspect of the caudal fold, midway along the fold and midway between the hairline and the ventral aspect of the fold. The standard interpretation is that any palpable or visible change is deemed to be a reaction. A modified interpretation is also in use: a positive test is any palpable or visible swelling at

the site of the injection that has a caudal fold thickness difference of 4 mm when compared with the thickness of the opposite caudal fold. If an animal has only one caudal fold, it is considered to be test positive if the caudal fold thickness is 8 mm or more.

Today, the two most widely used tests for the diagnosis of BTB are the single intradermal comparative cervical tuberculin (SICCT) test and the Bovigam® test (Prionics) (de la Rua-Domenech et al., 2006). Both are based on the detection of the early CMI response in tuberculosis infection. Cell mediated immune (CMI) response based diagnostic tests allow a relatively early detection of *M. bovis* infection in cattle. At late disease stages, the CMI response can wane as opposed to a generally increasing humoral immune response and SICCT or Bovigam® tests can give false negative results (de la Rua-Domenech *et al.,* 2006; Pollock *et al.,* 2005; Welsh *et al.,* 2005). Therefore, late stage diseased animals are thought to be more accurately detected by serological tests, which are based on the detection of antibodies for *M. bovis* specific antigens (Plackett *et al.,* 1989). Importantly, there is evidence that such skin test anergic animals are heavily diseased, shed higher amounts of bacteria and may be one cause of persistent and severe herd breakdowns (Pollock *et al.,* 2005; De la Rua-Domenech *et al.,* 2006; Palmer and Waters, 2006). Also, in countries without BTB control programs such animals are likely to be more prevalent and may contribute to a larger extent to the spread of the disease than animals at an early disease stage (Pollock *et al.,* 2005).

The Bovigam® test measures the *in vitro* IFN-γ production of whole blood cells after exposure to PPD-B and PPD-A for 16-24 hours (De la Rua-Domenech *et al.,* 2006; Gormley *et al.,* 2006). IFN-γ production is quantified by a sandwich enzyme-linked immunosorbent assay (ELISA). A large disadvantage of the Bovigam® test is the requirement to quickly process the blood samples soon after collection; however, the method appeared to be somewhat more sensitive than SICCT (De la Rua-Domenech *et al.,* 2006; Gormley *et al.,* 2006).

Some ELISA based serological tests for the diagnosis of BTB have been developed (De la Rua-Domenech *et al.,* 2006; Amadori *et al.,* 2002). However, the currently available serological tests generally show a low sensitivity and could not yet replace the CMI response based diagnostic tests. More recently, a fluorescence polarization assay (FPA) for the detection of antibodies against MPB70 has been assessed for the diagnosis of BTB in naturally infected cattle. Although test sensitivity was relatively low (62%), specificity was nearly 100% (Jolley *et al.,* 2007)

2.2.8.4. Post Mortem Examination

Gross Pathology

The pathology of TB in OWCs (old world camelids) was described nearly 100 years ago in cases detected in an Egyptian abattoir (Mason, 1917)**.** Pathological reports on NWCs have also emerged in recent years (Ryan *et al.,* 2008). The organs most frequently affected in both groups of camelids are the lungs and associated thoracic lymph nodes, where typical caseos necrotic lesions can be particularly extensive. Mason (1912) mentioned that all cases in OWCs had lesions at these sites, 60% of cases exclusively involving these sites; other affected tissues included the liver, spleen, kidney, trachea and pericardium. A similar distribution of lesions has been reported in NWCs (Twomey *et al.,* 2007). Intestinal and cutaneous lesions have also been observed in llamas (Twomey *et al.,* 2010a).

Gross lesions consists of multiple confluent, yellowish, caseous nodules (diameters up to 10 cm) with friable centers in the lungs, livers, spleen, bronchial lymph nodes, hepatic lymph nodes, mediastinal lymph nodes, and mesenteric lymph nodes. Similar nodules of a smaller size can be observed in the adjacent serosa. By examination of cut sections, these nodules are yellowish and firm with an onion skin-like structure and a partially mineralized center. Additional findings included hydrothorax, ascites, hepatic lipidosis,

splenomegaly, pulmonary edema and congestion, esophageal petechiae, intestinal hemorrhages, and cervical subcutaneous edema (Kamerbeek *et al*., 1997). The severity of pathological descriptions varies depending on how animals were selected for necropsy. Thus, animals selected following clinical disease often have severe pathology, whereas infected animals identified in ante-mortem immunological tests, and culled prior to development of clinical signs, are likely to have less severe gross lesions (Wernery *et al.*, 2007).

Microscopic Lesions (Histopathology)

Post-mortem examination was carried out on the slaughtered camels. Tissues specimens are taken from the inspected tubercules and fixed in 10% neutral formalin. Processed routinely for embedding in paraffin and sectioned at 4-5 µ thick. Sections are stained with haematoxylin and eosin and examined microscopically and for the detection of acid fast bacilli in tissues, sections are stained with Ziehl-Neelsen stain.

Histologically, the caseous nodules presented as granulomas composed of large numbers of closely packed, epithelioid macrophages are mixed with various numbers of lymphocytes, plasma cells, and neutrophils. The larger granulomas showed central necrosis with foci of mineralization and fibrous capsules of various thicknesses. Epithelioid macrophages contained eosinophilic, fine granular cytoplasms. Ziehl-Neelsen and Fite-Faraco staining revealed abundant acid-fast bacilli (AFB) within the epithelioid macrophages throughout all layers of the granulomas. They appeared as irregular long, straight, or curved structures without branches (Kremer *et al*., 1999).

2.2.9. Treatment, Control and Vaccination against Tuberculosis

Bovine TB is extremely difficult to treat and animals are culled rather than having any treatment even if attempted. If the cow is treated, it can take as long as nine months and involves using various antibiotics at different intervals as a result of its resistant nature. This can be a highly expensive option when dealing with an infected cow (http://www.netvt.co.uk). Treatment of infected animals is therefore not usually attempted, although there are some reports of anti-TB drugs being used in captive wild animals (Thoen *et al.*, 2009?).

Treatment of human tuberculosis can be grouped into two, latent TB infection (LTBI) and active TB infection (ATBI). Treatment of latent TB Infection greatly reduces the risk that TB infection will progress to TB disease. Every effort should be made to begin appropriate treatment and to ensure completion of the entire course of treatment for latent TB infection. TB disease can be treated by taking several drugs for 6 to 9 months. It is very important that people who have TB disease finish the medicine, taking the drugs exactly as prescribed. If they stop taking the drugs too soon, they can become sick again; if they do not take the drugs correctly, the bacteria that are still alive may become resistant to those drugs. First line treatment regimens in humans are Isoniazid, rifampin, ethambutol and pyrazinamide (Julie and Gerberding, 2003). TB that is resistant to these drugs is harder and more expensive to treat.

Albert Calmette and Camille Guérin achieved the first genuine success in immunization against tuberculosis in 1906, using attenuated bovine strain tuberculosis. *M. bovis* bacillus Calmette-Guérin (BCG), the only licensed TB vaccine, is a live attenuated strain of *M. bovis* which was passaged by Calmette and Guérin almost one hundred years ago. BCG affords highly variable protection against pulmonary disease, which accounts for the major burden of global TB mortality and morbidity throughout the world. Furthermore, revaccinating with BCG during adolescence in a population vaccinated with BCG at birth does not improve protective efficacy. BCG is currently

administered in mass immunization campaigns to neonates in high risk populations as part of the World Health Organization (WHO) Expanded Programme on Immunization (EPI). The most new vaccine strategies being developed incorporate BCG, either by genetically engineering BCG to be more immunogenic, or by developing a subunit booster vaccine which is designed to be given after BCG vaccination (McShane, 2011).

Tuberculosis prevention and control efforts primarily rely on the vaccination of infants and calves, the detection and appropriate treatment of active cases. The development of a cattle vaccine against *M. bovis* infection has been considered a major priority for the control of BTB in the UK (Krebs *et al.*, 1998). Provided an effective vaccine is available, cattle vaccination may be the most cost effective BTB control strategy and therefore also especially useful for interventions and disease control in developing countries (Hope and Villarreal-Ramos, 2008). The variability in BCG efficacy has been attributed to several factors such as the vaccine strain itself (Behr *et al.*, 1999), the regionally predominant infecting strains (Abebe and Bjune, 2006) or host genetics (Hope and Villarreal-Ramos, 2008). Moreover, it was shown that exposure to environmental NTM can influence the protective efficacy of BCG (Brandt *et al.*, 2002).

In camels, after the diagnosis of TB in two Bactrian camels kept in a zoo, prophylactic treatment of the remaining 17 camels was attempted using isoniazid incorporated into pelleted feed at a dose of 2.4 mg/kg, fed *ad libitum* (Bush *et al*., 1990). However, possibly due to isoniazid toxicity, several camels died, exhibiting signs of bone marrow suppression. National control programmes are often based on intradermal tuberculin testing, but because of the limitations of this test in camelids, these programmes are unlikely to be successful; however, a combination of ante mortem could improve the sensitivity of herd testing. Control depends on the removal of infected animals and prevention of further introduction of infection into the herd, but the disease will not be eradicated until infection is controlled in reservoir hosts, such as in wildlife (Thoen *et al*., 2006). Vaccines are not yet available for camelids.

2.2.10. Public Health Significance of Bovine TB

World Health Organization (WHO) defines zoonosis as 'those diseases and infections which are naturally transmitted between vertebrate animals and man.' Human population encounters animal disease with varying frequency depending on their occupation, geographical location and the prevailing culture of the country. Weather living in urban or rural environment, animals constantly may have close contact with human on farm (food producing animals), at area of residence (dogs, cats, cage birds), through leisure activities (horse, wild life) or by virtue of the occupation of individual as veterinarians or animal nurses. This close contact can result in the occurrence and transmission of zoonotic disease, which is naturally transmitted between vertebrate animal and man. Zoonotic tuberculosis is an infectious disease of domestic animals that can be transmitted from animal to human through consumption of raw milk and meat from infected animals and directly through erogenous route (Quinn *et al*., 2011).

Tuberculosis is one of the major global reportable zoonotic diseases, killing approximately 1.5 to 2 million people every year. Although *M. tuberculosis* is responsible for most human cases, bovine TB caused by *M. bovis*, is an important zoonosis that can spread to people through ingestion of raw milk and sometimes by inhalation of infectious droplets (Thoen *et al*., 2006). Outbreaks of bovine TB are therefore of considerable concern to public health officials and personnel responsible for the health of animals in zoos, animal parks and private herds. The number of *M. bovis* associated human TB cases has significantly declined in developed countries as a result of eradication programmes and pasteurization of milk (Thoen *et al*., 2006). Nevertheless, *M. bovis* still represents a zoonotic risk for people who are in close contact with infected animals; for example, a case of cutaneous TB in a veterinary surgeon was associated with contact with an infected Alpaca (Twomey *et al*., 2010b). Camel milk is also a potential source of infection, particularly as it is commonly consumed raw without boiling, and *M. bovis* was isolated from pooled milk samples from camels in Russia (Donchenko *et al*., 2011).

Human tuberculosis of animal origin (zoonotic TB) is an important public health concern in developing countries. Human TB of animal origin caused by *M. bovis* is becoming increasingly prevalent. The lack of both control and diagnostic measures and pasteurization of milk, coupled with a high prevalence and incidence of HIV/AIDS in human population. Concomitantly, TB is a major opportunistic infection in HIV-infected persons, and the world health organizations (WHO) estimated that 70% (6 million) of the people co-infected with TB and HIV (WHO, 2009).

Infections of humans with *M. bovis* are rare in most countries that successfully apply disease control measures. However, *M. bovis* may be frequent in countries where the disease is enzootic in cattle. In a recent study in the San Diego region of California, USA, *M. bovis* infections accounted for 45% of the tuberculosis cases in children (Rodwell *et al.*, 2008); most of these children were of Hispanic origin with ties to Mexico, where BTB is prevalent. The poor, especially in developing countries, are thought to be at highest risk to contract zoonotic tuberculosis (WHO, 2006) and also the observed higher susceptibility of HIV-infected persons to *M. bovis* infections is of major concern (Lobue, 2006).

2.3. Description of Camels: Nature's True Nomads

Domesticated representatives of the family *Camelidae* are an important part of the national livestock populations of numerous countries in arid and semi-arid regions of Asia and Africa, as well as the high mountains of the Andes in South America. The family *Camelidae* comprises two genera: 1) the genus *Camelus* (Linnaeus, 1758), includes two species. The first species is *Camelus dromedarius*, the dromedary or one-humped camel, with approximately 80% in Africa and 20% in Asia. The second species is *Camelus bactrianus* (Linnaeus), the bactrian or two-humped camel, which are in their natural habitat in Asia. 2) The genus *Lama* comprises *Lama glama* (the llama), *Lama pacos* (the alpaca), *Lama guanicoe* (the guanaco), and *Vicugna vicugna* (the vicugna).

Only the *Lama glama* and *Lama pacos* have been domesticated. They are raised in herds in the Andes at altitudes above 2,500 m (Fassi-Fehri, 1987).

Camels have lived in some of the most desolate corners of our planet, and not only do they live, they thrive. Most large animals are unable to survive in these kinds of desolate places because of their large requirements for resources such as food and water. Camels are able to use this to their advantage as a survival strategy. By living in deserts, mountains, and other arid places, camels are able to avoid predators, and others who would compete for resources. Camels are only able to do this because of their amazing ability to efficiently use the resources their environments provide. A camel can travel long distances which allow them to take advantage of the maximum number of resources. They can withstand a massive amount of dehydration which allows them to survive not only between watering holes, but sometimes between seasons. When at a watering hole, camels are able to gorge themselves and rehydrate quickly. For food, camels are omnivorous. They can eat almost anything be it vegetation, meat, or bone, salty or sweets. But it is their temperament that is truly endeared the camel to man. Docile and sweet under a caring hand, but stubborn and angry if ill treated, the camel wins both your heart and your respect (Denise Chan and Neil Carey, 2007).

Camel meat could be the meat of the future especially in health-conscious Western countries as it has no cholesterol and hardly any fat, since the fat is concentrated in the camel's hump, which can weigh up to 36 kg and can be easily discarded. If a camel's fat was distributed over it's body like humans, it would insulate the body and make it harder to cool down. A mature camel (6-7 years old) weighs between 250 kg to 680 kg, and stands from 1.8 m to 2m tall at the shoulders. A camel's thick wool can make it appear larger, especially during the winter. Their head and body length is 3m, not including a rope like tail which is over 50cm long.

Camels are mobile browsers and have a deeply split upper lip. This is ideally suited to stripping leaves from even the most prickly trees and shrubs. With their long neck, they

can reach 3.5m high and can feed on tough thorny plants that even sheep and goats would pass over. They can do this because of stiff hairs on their nose that permit them to push their way into thorny plants and thick skin inside their mouth that thorns cannot pierce. They also like extremely salty plants that grow at salt lakes and other locations. Inside the camel's mouth are 34 strong sharp teeth, that can be used as weapons, as well as for feeding. Although camels will normally select the freshest vegetation available, when food is scarce, they are omnivore.

Camels are called ruminant feeders, because they do not chew their food before swallowing it. Instead, later after feeding, they regurgitate some of it (the cud) and finish chewing it. Then, three chambered stomach can complete the digestion. When a camel cannot find food, the hump will shrink, droop to one side, or even slide off the camel's back to one side. However, the hump will rapidly return to size in a few weeks once the camel finds food. In the wintertime, camels can gather enough moisture from the plants they eat to go as much as 50 days without water. However, in the summertime, they may only go 5 days without water (Denise Chan and Neil Carey, 2007).

Camels minimize water loss through their waste. The camel's kidneys can concentrate their urine to the consistency of syrup with twice the salt content of sea water, and their fecal pellets are so dry immediately after voiding. Camels do not sweat, instead they allow their body temperature to rise and fall from 36.5-42°C, that is 5.5°C. In humans, an increase of only 1°C is a sign of illness; and, a core temperature rise of 3°C will result in vital organ damage and eventually death. Camels can drink brackish or salty water tha t would be unfit for most other animals. The camel keeps as cool as it can by resting when the weather is extremely hot, and feeding at night or in the morning. It will lay down in a shady place, if it can find one, or face in the direction of the sun to minimize how much of its body is exposed to the rays. During the heat of the day, a group of camels may press against each other, because their body temperatures are lower than the air temperature. In captivity, a camel will usually work only six months of the year.

They will become ill or die, if too much work is demanded from them (Denise Chan and Neil Carey, 2007).

Sexual activity occurs in both males (bulls) and females (cows) on a seasonal basis. Increased daylight is believed to trigger the breeding urge. While sexual activity in cows can commence at 2-3 years, a calf is not normally born until the cow is 5 years old. Ovulation is induced by mating. The cycle averages about 27 days. Depending on the season, food availability, stress, etc., pregnancy length varies from 374 days (12.5 months) to 419 days (14 months). Each pregnancy will usually result in a single calf being born, although twins have been known to occur. At birth, a calf's eyes are open, and it already has a thick woolly coat. A baby camel will weigh 30-40 kg and will not have a hump at birth. They will not start developing a hump until they begin eating solid food. After only a few hours, a calf can run and will call to its mother with a soft "baa," a little like a lamb. The mother and the camel will live together for several years, if not forcibly kept apart. In captivity, the calf is weaned at one year of age, and weighs about 150-180 kg (Denise Chan and Neil Carey, 2007).

An average milking camel will produce 20-25L of milk a day. A camel with well-formed udders may produce 30-40L a day. Camels can give milk even under harsh conditions; and, unlike dairy cows, do not need much or any supplements to do so. A camel can produce 20L of milk a day without drinking any water for up to 10 days. Camel milk is chemically similar to cattle milk, but has a higher proportion of vitamin C. It also has more fat, protein, and minerals than either cow's or goat's milk. The period of production (lactation) is longer than for cattle and the daily production lower. While dairy herds are kept in parts of the Middle East, camel milk is said to be an acquired taste. The average cow gives birth every 2-3 years producing 8 calves by the age of 20 (Denise Chan and Neil Carey, 2007).

2.4. Dromedary Camel Population and Husbandry Practices

Old World camels (OWCs) comprise the dromedary camel (one-humped, *Camelus dromedarius*), historically inhabiting the Middle East and the Horn of Africa. The OWCs have an estimated world population of 18 million across the arid and semi-arid environments of African and Asian countries. In Africa, the dromedary population of about 15 million accounts for about 74% of the world camel population, and of these, 60% are found in East African countries (Somalia, 6.2 million; Sudan, 2.8 million; Ethiopia, 1.7 million, Kenya, 0.9 million). The United Arab Emirates has approximately 360,000 dromedaries (Rhodes *et al.,* 2015). They are extremely important for the livelihood of pastoral communities and cultural life. The countries which have the most camels in descending order are Somalia, Sudan, Ethiopia, India, Pakistan, and Mauritania (Table 1) (Farah *et al.,* 2007).

Table 1: Estimated camel populations of Africa and the world

Country	**Camel population (in 1000)**	**Country**	**Camel population (in 1000)**
Africa:			
Algeria	240	Morocco	36
Chad	725	Niger	415
Djibouti	70	Nigeria	18
Egypt	120	Senegal	4
Ethiopia	1070	Somalia	6200
Kenya	830	Sudan	3200
Libya	72	Tunisia	231
Mali	467	West Sahara	105
Mauritania	1230		
Other regions:			
China	326	Mongolia	360
India	1030	Pakistan	800
Iraq	76	Saudi Arabia	400

In general, breeds of camels are not differentiated and classified as breeds of other domestic species. In most camel rearing societies, breed classifications are based on names of the ethnic group, clan as well as on the geographical localities where these camels are raised, rather than upon phenotypic characteristics. In Kenya for example there are three main types of camels, classified as Somali breed, Rendille/Gabbra breed and Turkana breed. Similarly in Ethiopia, camel breed classification is based on Ethnic groups of the regions in which the camels are reared like Borena camel, Kereyu camel, Somali camel, and Afar camels. But information regarding detailed characterization of camel breeds, their production potential, husbandry and management practices are not well documented and lacking.

The threat posed by increasing and prolonged periods of drought, particularly in arid and semi arid areas of Africa, has forced pastoralists to undergo adaptive strategies such as herd diversification, where emphasis on camel husbandry is becoming a priority. Increasing numbers of pastoralists in Ethiopia are herding camels in response to less predictable weather patterns, because camels are more drought hardy than any other species of animals. In this regard, pastoralists have long been renowned for their diversified herd management practices that camels constitute a major livelihood means. Camels can continue to provide milk well into a drought period with the result that pastoral children from camel-herding families are less susceptible to malnutrition. The trade of live camels to North Africa and the Middle East is also vibrant and prices are higher than at any time in the last 10 years, a great financial benefit for families with surplus camels to sell. According to recent studies made by Tufts University in Ethiopia the trade value of camels in 2010 was close to 61 million (Aklilu and Catley, 2011).

Camels are the back bone of the pastoral economy and subsistence for vast majority of pastoralists. These animals ensure the adverse climatic conditions in the arid areas and are the most efficient animals in converting fodder into energy for work and products like milk and meat. The outstanding performance of camels in drought prone areas made pastoralists to trust camels where camel production is the desire of every pastoralist

although the growing price of camel is unaffordable to every pastoralist. At present it takes far longer time for a medium class pastoralist to save enough to buy and own a camel. Despite the far reaching role of camels in supporting the life of pastoralist, little attention has been given to it in the past. At present, however, the focus by government on camel development is encouraging (OPADC, 2012).

2.5. Constraints and Prospects to Camel Production in Ethiopia

Camels were probably domesticated about 400 years ago. The camels were domesticated in southern Arabia Peninsula probably the area of Yemen and Oman. It has subsequently been distributed to the rest of the world (Wilson, 1998). Of the estimated 17 million camels of the world, 15 million are one-humped, and the vast majority of these (12 million) are found in Africa, especially in the five neighboring East African countries of Somalia, Sudan, Djibouti, Ethiopia and Kenya. The rest are mainly found in Asia (CSA, 2011).

In Ethiopia, an estimated 1.7 million camels are present which are mainly distributed in arid and semi arid areas of the country (CSA, 2011). The arid and semi- arid areas of the country that constitute more than 60% of the total area and home of 7.8 million pastoral and agro-pastoral communities are suitable for camel production. The eastern and southern parts of the country, namely Somali, Afar and Borena are the major areas where camel husbandry is widely practiced. In these areas, the livelihood of the pastoral communities is certainly ensured by dromedaries (Wossene, 1991).

Camel has won a very big interest of the pastoralists as a means of transport and a reliable means of wealth accumulation and resilience mechanism against drought. The livestock population trend was decreasing for the last 10-15 years and the composition was in favor of small stock and camel. Camels contribute to the wellbeing of many people across the world. It is a hardy animal with a unique physiological, behavioral and anatomical constitution that enabled it to survive in incredibly harsh climates. Camels

can tolerate severe dehydration with no hypertonicity of the blood plasma while it is capable of safe and rapid rehydration with neither hemolysis nor hypervolemia. It also acclimatize with a wide range of environmental temperature as low as 36^0C as high as 41.5^0C. These natural adaptive capacities have made camel the preferred species of livestock in the pastoral communities for centuries and has earned the name 'an animal of the future' (Megersa, 2010).

In Ethiopia, camels represent a subset of major livestock resources. Camel production is practiced by pastoral communities under diverse constraints in dry and marginal areas. The dominance of other ruminant species over camels perhaps might have masked the potential contributions of these animals to the national and household economy. Infectious and parasitic diseases appear to be the major constraints that are hampering the potential performances of the animals. Scarce research information on disease reveals that camels may be either carriers of, susceptible to or suffering from a vast array of infectious and parasitic diseases. Camel calf morbidity and mortality have been reported to be hindrances to production enhancement and population growth, with a mortality reported to be as high as 50% from pastoral areas. As a result, the camels have been neglected, or at least their importance underestimated by the society as such (Megersa, 2010).

Given the anticipated impact of global climate change, camels will become increasingly important in the dry lands and the development of the camel sector will catalyze economic growth and contribute significantly to achieving food security by creating employment and reducing poverty at the national and household levels. For camel production to contribute its full potential to national economic growth, there is a need to progress towards intensification, diversification and commercialization of the production systems. However, a framework to guide the operationalization of these policies and strategies and direct prospective investors in the camel sub-sector as well as enable the sustainable utilization of modern value-added technologies are lacking (OPADC, 2012).

Camels have been marginalized as a species due to factors that include the underestimation of the population size, the under-rating of their economic significance, and the fact that they are disproportionately owned by marginalized pastoralists. These perceptions have to change for valid reasons. This change could be started by re-assessing the true camel population in the country (through a reliable census) and by conducting an evaluation of the existing and potential economic contribution (such as milk, meat, hides, and transport) of the species at the household and the national level. The marginalization of camels in the country is reflected in the dearth of camel specialists in the fields of veterinary medicine, production, feed, and other camel husbandry disciplines. This apparent gap has led to camel mortalities from both known and unidentified diseases, and the under-utilization of camel-based resources (meat, milk, and hides, for example). The potential for commercial camel production has been constrained by the evident lack of specially formulated processed feed for camels (Aklilu and Catley, 2011).

Although the livestock export business has shown very positive growth in recent years, there are questions over whether this scale of growth can be sustained. At present, the main pastoralist areas that supply livestock and meat for formal exports are subject to an uncertain policy environment and possible restrictions on access to the grazing and water resources that are needed to raise cattle, sheep, and goats in dryland environments. Comparable resource issues exist in mid- and high altitude areas, with the problem of limited grazing land being widely reported, over many years (Aklilu and Catley, 2011).

The adverse effects of climate change have continued to affect most livestock species. But, the camel is naturally made with tolerant biophysical, anatomical and behavioral traits that it will continue to produce milk for pastoral communities suffering from climatic hardships. In today's challenging world where pastoral production systems have to be resilient for shocks from various disasters, camel have been introduced as a resilience mechanism against drought. It is highly wanted for herd diversification by increasing number of pastoralists. Considering the importance to pastoral livelihoods;

noting the increasing trend of acquiring camels by pastoral communities; and realizing the income generation potential of camels and the role in the pastoral household economy and nation at large. Even though different efforts were made to increase the benefit from camel production, more needs to be done to realize its potential fully and to make it a source of economic benefit to pastoral communities. The camel is a more reliable milk provider than other classes of livestock in arid areas during both dry seasons and drought years. There is also an increasing demand for camel milk and meat in local towns with increasing demand at Kenya side Moyale. A traditional camel milk market chain has already been established along Yabello-Moyale Kenya milk shade (Megersa, 2010).

Despite all its ecological and economic importance and significant role in the life of pastoral community, until recently the animals were neglected by researchers and development planners in Ethiopia. Research agendas, promotion programs, regular vaccination and animal health service deliveries are almost always excluding camels. The depth of information on camels and camel production has not been adequate to solve its multifaceted problems. A particular problem has been that the traditional knowledge harvested over centuries have not been appreciated, and the local competence of pastoral people have not been assessed and compared to the more modern scientific approach (Megersa, 2010). Small amount of work has been done on the market and marketing aspects of the camels. Increasing adaptation and promotion for development of the camels has also been reported by Oromiya Pastoralist Areas Development Commission (OPADC, 2012). Owing to the increasing human population and declining per capita production of food in Africa, there is an urgent need to develop marginal resources, such as arid land, and optimise their utilisation through appropriate livestock production systems of which camel production is the most suitable without doubt (Farah *et al.*, 2007).

3. MATERIALS AND METHODS

3.1. Study Area

This study was conducted from February, 2014 to July, 2016 to investigate the epidemiology of tuberculosis in camels slaughtered at Akaki municipality abattoir, Central Ethiopia. The abattoir is the branch abattoir of Addis Ababa abattoirs enterprise where camels are slaughtered in addition to cattle and small ruminants. The abattoir is located at Akaki town which is one of the sub-cities of Addis Ababa, the capital city. Akaki is located in the southern part of Addis Ababa at a latitude and longitude range of 8^0 58'N, 38^0 47'E. It has a humid subtropical climate that is mild with dry winters, hot summers and moderate seasonality. It has mean annual temperature of 15.9^{o}c, total average precipitation 1089mm and annual sunshine 2439hours. It lies in the central highlands of Ethiopia. In the camel slaughter house of the abattoir, as pre-slaughtering procedure, camels were visually inspected for the presence of any abnormality which may need emergency slaughtering. Otherwise, camels meant for slaughtering were bitten over the base of their neck using woodstick as stunning procedure which was un-humane way of stunning procedure. Then fallen down camels were positioned in sitting position for slaughtering and further processing of the carcass. In the camel slaughter house, there is un-interrupted and powerful water supply with good hygienic practice and the slaughtering of camels was during the day time (in the afternoon) that can facilitate meat inspection and post mortem examination procedures. The meat inspection procedure was done by meat inspectors but the inspection was superficial on carcasses and major viscera like liver. The camels slaughtered at the abattoir were mainly to meet consumer demand of Somali residents at Addis Ababa Bole Bulbula area where the camel meat sold at camel butcheries in that locality. During the study, camels slaughtered at the Akaki abattoir were originating mainly from the catchment (source) areas namely Borena and Fentale (Metehara) pastoral livestock production systems of Ethiopia (figure 4).

The main catchment areas possess large number of camels, in Fentale pastoral area, there are 61,425 camels and in Borena pastoral area posses an estimated population of 174,185 camels (CSA, 2008; OPADC, 2012).

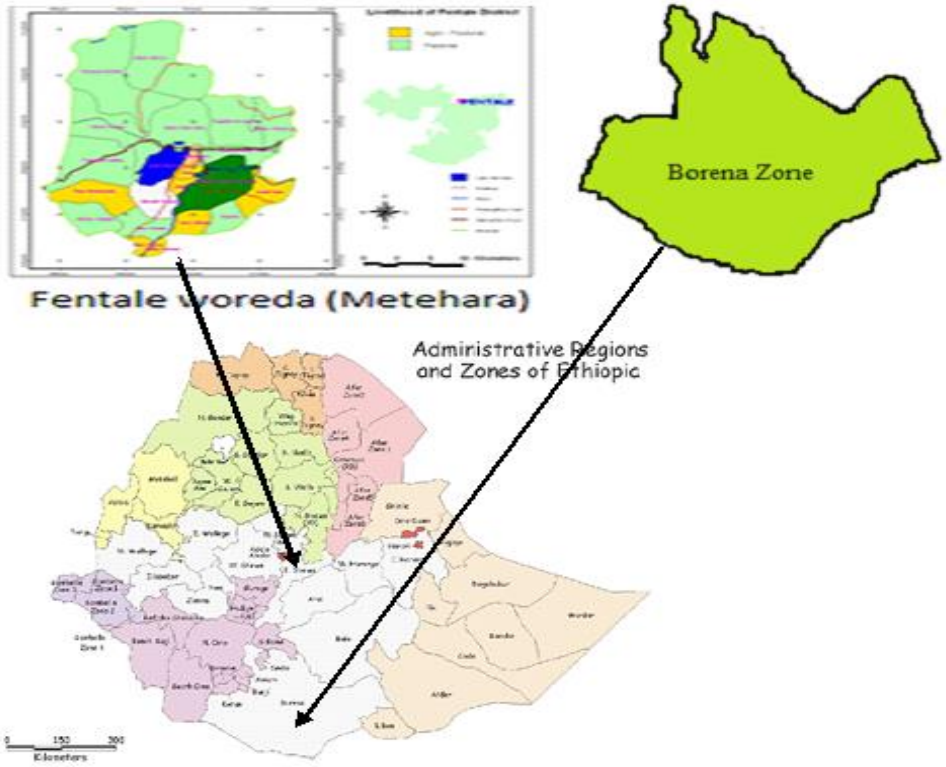


Figure 4: Map of Ethiopia with zones, showing the origin of camels (Borena and Fentale/Methara) slaughtered at the Akaki abattoir

Fentale District (Metehara)

Fentale is one of the districts in the Oromia Regional state of Ethiopia, part of the Misraq Shewa Zone located in the Great Rift Valley, about 250 kms East of Addis Ababa. Fentale is bordered on the southeast by the Arsi Zone, on the southwest by

Boset, on the northwest by the Amhara Region, and on the northeast by the Afar Region. The administrative center of Fentale is Metehara. The prevailing climate in Metehara is arid (NMSA, 2003). Most parts of this woreda range from 900 to 1000 meters above sea level; Mount Fentale (2400 meters) is the highest point. Rivers include the Awash and the Germama; Lake Basaka is an important body of water in this woreda. In 11 of the 18 kebeles of Fentale, the predominant agricultural practice is pastoralism. Camels, goats and cattle are the most common livestock; migration to the border areas of Boset woreda for grazing during normal years is common, but in years of low rainfall, herdsmen will migrate as far as Negele Arsi (OPADC, 2012).

Borena Zone

Borena is located in the Oromia National Regional State, about 600 kms South of Addis Ababa. The Borana plateau of 95.000 km^2 gently slopes from high mountain massifs in the north (1650 m.a.s.l) to the south bordering Kenya (1000 m.a.s.l) with a slight variation due to central mountain ranges, and scattered volcanic cones and craters. The climate is generally semi-arid with annual average rainfalls ranging from 300 mm in the south to >700 mm in the north (NMSA, 2003). Animal husbandry is characterized by extensive pastoral production system and seasonal mobility. Cattle are the dominant animal species followed by goats, camels and sheep. As aridity increases, the principal stock shifts gradually from cattle combined with small stock to camels, with a relative degree of the social and cultural values accounting for differences. Camel herd movement may move the whole herd to water points and to relatively better areas where green fodder is available, or by herd splitting where lactating animals are kept around homesteads and moving the rest to distant located forage areas. Seasonal herd mobility was observed particularly during the dry season for foraging and watering purposes, and to some extent during wet season to avoid disease occurrences. Health care is mainly practiced by herders and traditional healers (Megersa, 2010).

3.2. Study Population

The study animals were camels of Borena and Metehara origin which were slaughtered at Akaki Abattoir, Central Ethiopia. The camels slaughtered at the abattoir during the investigation were from the main catchment sites of Borena and Metehara pastoral livestock production systems of Oromiya regional state. On arrival, the camels will stay for few days in the holding pen until they are slaughtered. On average, about 5 to 8 camels were slaughtered on dialy bases during the study period depending on the consumer demand of Somali residents at Addis Ababa Bole Bulbula. For comparative tuberculin testing, apparently healthy camels which were staying for at least three days in the holding pen of the abattoir were considered. For pathological investigation of gross visible tubercle lesions during post mortem examination, camels slaughtered each day in the abattoir were considered. A total of 2070 camels were examined during the study period.

Camels were carefully identified: age, sex, origin and body condition score (BCS) were recorded. Body condition score was determined by hump structure of the camels according to the previously established guideline indicated in the website: (http://www.camelsaust.com.au/livebodycond.htm): and the scores ranges from 1 to 5 based on the amount of fat in the hump and then categorized into three groups: poor, moderate and good. Age category was determined by using the dental eruption and wears as described by Schwartz and Dioli (1992); accordingly, camels were categorized as ≤7 years of age and >7 years of age. The host risk factors such as parameters of age, sex, body condition and origin origin of camels were properly recorded to assess for the presence of possible association of these risk factors with the prevalence of tuberculosis in camels. For comparative tuberculin skin testing, apparently healthy camels, which were stayed in the holding pen for at least three days before slaughtering, were considered to facilitate the reading of tuberculin skin reaction after 72 hours of injection.

3.3. Study Design and Sampling

This study was cross sectional study design with abattoir based epidemiological investigation of tuberculosis in apparently healthy camels slaughtered at Akaki municipality abattoir. Apparently health camels were sampled based on systematic sampling and on the number of days before slaughtering for comparative tuberculin testing. For pathological investigation, camels which were slaughtered on the daily bases were followed during post mortem examination to detect for the presence of any visible tubercle lesion from the lymph nodes of the head, lung tissue, and lung associated lymph nodes and also for pathology scoring of leions. For evaluating the performance of the single intradermal comparative cervical tuberculin test (SICCT) to diagnose tuberculosis in camels against gross pathology as gold standard diagnostic test; tuberculin tested camels were followed during post mortem examination, with an average of six lymph node tissues per camel, were sampled whether with gross lesion or without visible lesion for mycobacteriological culture isolation and molecular characterization of isolates.

3.4. Sample Size Determination

To estimate the prevalence of tuberculosis in camels using the SICCT and gross pathological lesions, the formula for random sampling of Thrusfied (2007) were employed. For this, 95% confidence level, 5% desired absolute precision, and expected prevalence of 10% (Mamo *et al.,* 2011) was considered.

$$n = \frac{1.96^2 * P_{exp}\,(1-P_{exp})}{d^2}$$

Where: n = required sample size,

P_{exp} = expected prevalence of 10%

d^2 = desired absolute precision of 5%,

1.96 = Z value of the 95% confidence level

Thus, the sample size was calculated as 198. However, to increase the precision of sample estimates as it is abattoir based epidemiological study, sample size was increased about two fold for tuberculin testing and more than fourfold for post mortem pathological examination. Thus, 387 camels were tested for the SICCT to estimate tuberculin reactor prevalence and assess associated risk factors. In total, 2070 camels were investigated during the study period (Figure 5).

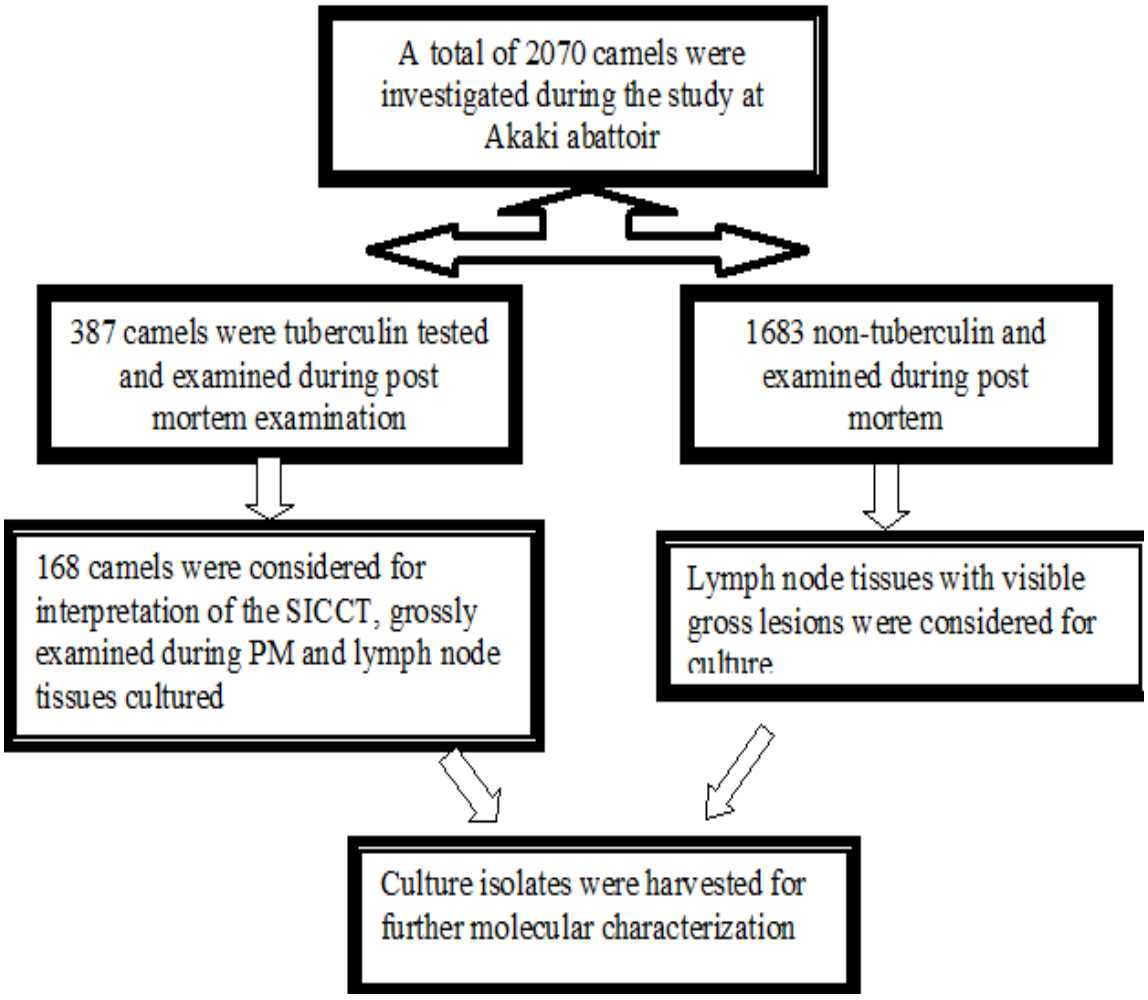


Figure 5: Diagram showing the number of camels examined during the study period.

3.5. Sample Collection

Pathological Examination and Tissues sampled

Any suspected gross tuberculous lesions from the lung, lung associated lymph nodes and lymph nodes of the head were sampled for pathology score and mycobacteriological culture isolation. These sampled lymph nodes (LNs) were retropharyngeal, Parotid, Sub-mandibular, trachea-brocheal, and Cranial and Caudal mediastinal LNs. Samples were aseptically collected into sterile universal bottles containing 5 ml of 0.9% saline solution for mycobacteriological culture isolation. The samples were kept in an icebox with solid packs, transported to Aklilu Lemma Institute of Pathobiology (ALIPB) and College of Veterinary Medicine (CVM) and stored at +4°C and sometimes at -20°C until mycobacteriological culturing was carried out in the TB laboratory.

3.6. Methodology

3.6.1. Comparative Tuberculin Test

Tuberculin skin testing is the OIE standard test for the detection of bovine tuberculosis. It involves intra dermal injection of bovine and avian tuberculin-purified protein derivative (PPD) and the subsequent detection of swelling (delayed hypersensitivity) at the site of injection 72 hours later (OIE, 2009). Single intra-dermal comparative cervical tuberculin (SICCT) test was conducted using bovine and avian tuberculin purified protein derivatives (PPD) provided by Veterinary Laboratories Agency, Addlestone, Surrey, UK. An aliquot of tuberculin was injected intra-dermally with appropriate dose (0.1ml of 2500IU/ml of bovine PPD and 0.1ml of 3000IU/ml of Avian PPD) on the right side of the neck of the camels at about 10 to 15 cms distance. Skin thickness before and after injection was recorded for individual camel using graduated caliper.

Initially, following the standard procedure, the skin at the middle of the right side of the neck of camels were cleaned, shaved and the thickness were measured and the site marked prior to injection, the dose of tuberculin was then injected intra-dermally. The injection site was examined for swelling and thickness after 72 hours. The difference between the skin reactivity to bovine PPD and avian PPD was recorded for each injection sites after 3 days. The reaction was considered positive when bovine PPD was ≥4.0mm greater than that of avian PPD; inconclusive if it is only from 1 to 4 mm greater, and negative if the reaction was equal to or smaller than that obtained at the avian site of injection (Wernery *et al.*, 2007). According to OIE (2009), for the SICCT, change in the skin thickness that is change in PPD-Bovine (B2-B1= ΔB) minus PPD-Avian (A2-A1= ΔA) was recorded. Thus, the difference ΔB - ΔA ≥4mm is considered positive reactors, between 2mm and 4mm is considered positive suspect, and ≤ 2mm thickness is considered as negative. To increase the performance of the SICCT to detect tuberculosis in camels, a cutoff value of ΔB-ΔA ≥2mm as recommended for Ethiopian cattle by (Ameni *et al.*, 2008) adopting for camels and the OIE standard ≥4mm were used to classify as positive reactors and below which considered as negative reactors. In addition the cutoff value ≥3mm recommended in this study for the SICCT to detect tuberculosis in camels against gross pathology was also used for this classification.

3.6.2. Post mortem examination and pathology scoring

Postmortem examination was performed following the procedure as previously described (Corner, 1994; Mamo *et al.*, 2011). Tissues from the lung, lymph nodes such as sub-mandibular lymph node, retropharyngeal lymph node, trachea-bronchial lymph node, cranial and caudal mediastinal lymph node and occationally, mesenteric lymph nodes were examined in detail during post-mortem examination in the abattoir under a bright-light source. The lobes of the left and right lungs were inspected and palpated externally. Then, each lobe is sectioned into about 2cm thick slices to facilitate the

detection of lesions with sterile surgical blades. Similarly, lymph nodes were sliced into thin sections (about 2mm thick) and inspected for the presence of visible lesions.

Palpation and incision of the lung, palpation and incision of the parotid, retropharyngeal, sub-mandibular, trachobronchial, cranial and caudal mediastinal lymph nodes were examined. Whenever gross lesions suggestive of TB were detected in any of the tissues, the tissues were classified as having lesions. However, for the diagnostic test evaluation, tuberculin tested camels' lymph node tissues were sampled with lesion and without evident lesion. Lymph nodes were sliced in to thin sections of 2mm and lung tissues were cut in to slices of 2cm using separate sterile surgical blades and tissue samples were collected in the universal bottles containing normal saline (0.9%) for mycobacteriological culture isolation. The cut surface was examined under a bright light source for the presence of abscess and tubercle lesions (Bogale *et al*., 2004; Mamo *et al.*, 2011).

Pathology scoring for the severity of gross lesions was undertaken on tissues with abscesses and tubercle lesions to determine the severity of the lesions based on semi quantitative procedure developed by (Vordermeier *et al.*, 2002; Ameni *et al*., 2006) and adopted for camel tuberculosis lesion scoring. Briefly, lesions in the lung scored separately as follows: 0, no visible lesions; 1, no gross lesions but lesions apparent on slicing of the lung lobe; 2, fewer than five gross lesions; 3, more than five gross lesions; 4, gross coalescing lesions. The scores of the individual lobes were added up to calculate the lung score. Similarly, the severity of gross lesions in individual lymph nodes was scored as follows: 0, no visible lesions; 1, a small lesion at one focus (just starting); 2, small lesions at more than one focus; 3, extensive necrosis. Individual lymph node scores were added up to calculate the lymph node score. Finally, both lymph node and lung pathology scores were added up to determine the total pathology score per camel.

Camels with macroscopic lesions varying from firm or hard white, grey, or yellow nodule with a yellow, caseous, necrotic centre that was dry and solid to thin walled

suppurative abscesses were classified as post mortem positive (Warnery and Kinne, 2012). Tuberculous lesions from slaughtered camels were aseptically collected into sterile universal bottles containing 5 ml of 0.9% saline solution for mycobacteriological isolation. The samples were kept in an icebox with solid packs, transported to Aklilu Lemma Institute of Pathobiology (ALIPB) and College of Veterinary Medicine (CVM) and stored at +4°C and at -20°C until mycobacteriological culturing was carried out in the TB laboratory.

3.6.3. *Mycobacteriology*

3.6.3.1. Mycobacteriological Culture Isolation

Isolation of Mycombacteria was performed in accordance with OIE (2009) protocols. Briefly, tissue specimens for culture were collected in sterile universal bottles in 5 ml of 0.9% saline solution and then transported to the laboratory in a cold chain using ice box packed with ice packs to keep the low temperature. Tissue samples were sliced, macerated in sterile mortar and pistol using surgical blades and forceps to get fine pieces and then homogenized for 10 minutes in 5 ml of normal saline. Homogenate of about 7 ml was transferred to centrifuge tube and decontaminated with equal volume (7ml) of 4% NaOH ; centrifuged at 3000rpm for 15 minutes, the supernatant was discarded and the sediment was neutralized by adding 1% (0.1%) HCL, employing phenol red as a pH indicator; Neutralization was achieved when the suspension changed from purple to yellow.

After neutralization, 0.1 ml of the suspension from each sample was dispensed, spread on culture tubes with slants of Lowenstein-Jensen (LJ) medium. Duplicates of LJ medium was used, one enriched with sodium pyruvate and the other enriched with glycerol. Each sample suspension was inoculated on to the set of Lowenstein-Jensen (LJ-pyruvate) and glycerol (standard LJ). Cultures were incubated aerobically at 37^{o}C

for up to 12 weeks with weekly observation for growth of colonies. When visible colonies are observed, Ziehl-Neelsen staining was performed to confirm the presence of acid fast bacilli.

Characteristics growth patterns and colonial morphology can provide a presumptive diagnosis of *M. bovis*; however every isolate needs to be confirmed. It is necessary to distinguish *M. bovis* from the other members of the 'tuberculosis complex' specifically *M. tuberculosis*-the primary cause of tuberculosis in humans (Quinn *et al.*, 2011; WHO, 2009). Positive cultures confirmed by Ziehl-Neelsen staining were preserved with freezing media while at the same time heat killed in water bath at 80^{o}C for 45minutes. The frozen and heat killed isolates were stored at (-20^{o}C) for further mycobacteriology and molecular typing analysis (Mamo *et al.*, 2011).

3.6.3.2. Microscopic examination (Zeihl Neelsen Acid-fast staining)

Microscopic direct smear examination of culture isolates from camel tissue samples from pure colony growth on Lowenstein Jenson (LJ) media were made with the classic Zeihl-Neelsen staining technique for presumptive diagnosis of acid fastness, following procedure described by (OIE, 2009; Quinn *et. al*., 2011). The heat fixed smear was stained with carbon fuchsine, heated gently and allowed to stand for 10 minutes. The stain is then poured off and the smears washed with tap water and decolorized with 25% sulpheric acid, then 96% ethyl alcohol (acid alchole) for 1 minute each with the slides being washed under tap water between each step. The smears were then counter stained with methylene blue for 3 minutes, dried and examined for the presence of acid fast bacilli (AFB) under light microscope employing a 100X oil immersion objective (Quinn *et. al*., 2011).

3.6.4. Molecular Typing of culture Isolates

3.6.4.1. Multiplex Polymerase Chain Reaction (PCR) Assay

The multiplex (PCR) differentiate *M. tuberculosis* complex from *M. avium* complex, *M. intracellularae* and other mycobacterial species. The PCR targets the sequence of the genus Mycobacterium within the 16S rRNA gene (G1, G2), sequences within the hypervariable region of 16S rRNA that is known to be specific to *M. intracellularae* (MYCINT-F) and *M. avium* (MYCAV-R), and the MTB70gene specific for *M. tuberculosis* complex (TB-1A, TB-1B). Mycobacterial genus typing was conducted as described previously by Wilton and Cousins (1992).

Heat killed acid fast bacilli (AFB) positive samples were used as a source of DNA template. DNA amplifications was done in thermocycler with 20 µl reaction volumes consisting: 5 µl of genomic DNA as a template, 8 ml HotstarTaqMasterMix (MgCL2, dNTP, Taq polymerase and PCR buffer) (Qiagen, United Kingdom) for each sample, 0.3 µl internal primer per sample, 0.3 µl forward and reverse primer per each sample and 5.2 µl per sample of Qiagen water. The primers used for amplification were MYCGEN-F, 5'AGA GTT TGA TCC TGG CTC AG 3' (35ng/µl); MYCGEN-R, 5'TGC ACA CAG GCC ACA AGG GA 3' (35ng/µl); MYCAV-R, 5' ACC AGA AGA CAT GCG TCT TG 3' (35ng/µl); MYCINT-F, 5'CCT TTA GGC GCA TGA TGT CTT TA 3' (75ng/µl); TB1-F, 5' GAA CAA TCC GGA GTT GAC AA 3' (20ng/µl); TB-1-R, 5' AGC ACG CTG TCA ATC ATG TA 3' (20ng/µl). *M. tuberculosis* strains (H37Rv) and *M. avium* were used as positive control while Qiagen water was used as negative control.

The reaction mixture was then heated in Programme Thermal Controller (VWR, UK) cycle using the following amplification program: 95^{o}C for 10 minutes for enzyme activation; 95 ^{o}C for 1 minute for denaturation; 61^{o}C for 0.5 minute for annealing; 72^{o}C for 2 minutes for extension, involving 35 cycles all in all; and final extension at 72 ^{o}C

for 10 minutes. The products were electropherosed in 1% agarose gel in 10x TAE running buffer. Ethidium bromide at appropriate ratio (eg. 1:10), 100bp DNA ladder, and orange-6x loading dye were used in gel electrophoresis, the resulting band produced were analyzed accordingly. All members of the genus *Mycobacterium* produce a band of 1030bp, *M. avium* or *M. avium subspecies paratuberculosis* produces a band of 180bp, *M. intracellularae* a band of 850bp while members of *M. tuberculosis* complex produce a band with 372bp (Mamo *et al.,* 2011).

3.6.4.2. Region of Difference-4 (RD-4) Deletion Typing

PCR analysis on the basis of RD regions has been found to be an important differentiating tool between members of the *M. tuberculosis* complex. RD4 is 12.7kb genetic segment that is deleted from *M. bovis* BCG strain, but present in *M. microti, M. africanum*, and *M. tuberculosis* (Gordon *et al.,* 1999).

The RD4 deletion typing was applied to isolates that showed band for *M. tuberculosis complex* by Multiplex PCR. For this deletion typing, the procedure described by Cadmus *et al.,* (2006) was followed. Accordingly, each sample was tested in a separate PCR tube. Primers directed against the RD4 were used to generate a deletion profile that would allow species identification of the isolate. Primers that were used to check for the presence of RD4 locus includes RD4intF ACA CGC TGG CGA AGT ATA GC, RD4flankF CTC GTC GAA GGC CAC TAA AG and RD4flankR AAG GCG AAC AGA TTC AGC AT to check for the presence of RD4 locus. The Hot StarTaq Master Mix system from Qiagen was used for PCR with primers described previously. *M. tuberculosis* strain (H37Rv) and *M. bovis* strain (2122/97) was used as positive controls while Qiagen water as negative control. The reaction mixture was 10 µl of HotStarTaq Master Mix, 0.3 µl×3 of each primer (Flank Rear, Front and Intermediate), 2.1µl DNA template and 7 µl distilled water to a final volume of 20 µl.

The reaction mixture was heated in Programme Thermal Controller (VWR, UK) using an initial hot start of 95^{o}C for 15 minutes, followed by 35 cycles of 95^{o}C for 1 minute, 55^{o}C for 1 minute, and 72^{o}C for 1 minute; a final extension step of 72^{o}C for 10 minutes to complete the cycle. The PCR products were electrophoresed in 1% agarose gel in 16 TAE running buffer, Ethidium bromide at a ratio of 1: 10, 100bp DNA ladder and orange 66 loading dye were used in electrophoresis. The gel was visualized in Multi-imageTM light cabinet using Alpha innotech version 1.2.0.1(Alpha Innotech Corporation). The presence of RD4 (*M. tuberculosis*, *M. africanum*) gives a product size of 335bp (RD4int+RD4FlankR) and its absence (*M. bovis*) gives a product size of 446bp (RD4FlankR+RD4FlankF).

3.6.4.3. Spoligotyping (Spacer Oligonucleotide Typing)

Spoligotyping was performed as previously described by Kamerbeek *et al.*, (1997) and according to the spoligotype kit supplier's instruction (Ocimum Biosolutions Company, Iisselstein, the Netherlands). The direct repeat (DR) region was amplified by PCR using oligonuleotide primers derived from the direct repeat (DR) sequence. A total reaction volume of 25µl of the following reaction mixture was used for the PCR: 12.5µl of HotStarTaq Master Mix (Qiagen: this solution provides a final concentration of 1.5mM $MgCl_2$ and 200µM of each deoxynucleotides triphosphates), 2µl of each primer (20pmol each), 5µl suspension of heat-killed cells (approximately 10 to 50ng) and 3.5µl distilled water.

The mixture was heated for 15 minutes at 96^{o}C and then subjected to 30 cycles of 1 minute at 96^{o}C, 1 minute at 55^{o}C, and 30 seconds at 72^{o}C. The amplified product was hybridized to a set of 43 immobilized oligonucleotides, each corresponding to one of the unique spacer DNA sequences within the DR locus. After hybridization, the membrane was washed twice for 10 minutes in 2x SSPE (1x SSPE is o.18 M NaCl, 10 mM NaH2PO4, and 1 nM EDTA at pH 7.7), 0.5% sodium dodecyl sulfate at 60^{o}C and then

incubated in 1:4000 diluted streptavidin-peroxidase (Boehringer) for 45 to 60 minutes at 42^{o}C. The membrane was washed twice for 10 minutes in 2x SSPE-0.5% sodium dodecyl sulfate at 42^{o}C and rinsed with 2x SSPE for 5 minutes at room temperature. Hybridizing DNA was detected by the enhanced chemiluminiscence method (Amersham) and by exposure to X-ray film according to the description by the manufacturer. The results of spoligotyping were converted into octal and binary formats. These binary and octal formats of the strains were entered into query box so that the name of the strains were retrieved from the database (http://www.pasteur-guadeloupe.fr:8081/SIT-VITDemo/(SITVIT1Database, www.*Mbovis*.org) if the spoligotype pattern of the strain in question fits the pattern that has already been registered in the SPolDB4 database (Brudey *et al.,* 2006).

3.7. Data Analysis

Data from tuberculin skin testing, gross pathology, mycobacteriology and molecular analysis were entered into Microsoft excel sheet, coded and analyzed using statistical packages: STATA (Intercooled stata verion 13) and SPSS (version 20). Statistical tests such as descriptive statistics were used to determine prevalence based on SICCT skin reactivity and gross pathological tubercle lesions and appreciate the mean severity of pathological lesions. Pearson chi-square test was used to assess for the presence of association of risk factors of age, sex, origin, and body condition of camels with prevalence of tuberculosis in camels. The multivariable logistic regression analysis with Odds ratio (with its 95% confidence interval) was used to assess the strength of association of these risk factors with the prevalence. The Reciever Operating Characteristics (ROC) analysis was used to assess the area under the curve (AUC) for the sensitivity and specificity evaluation of the SICCT at different cutoff value to diagnose tuberculosis in camels using gross pathology for tubercle lesion as gold standard. For statistical significance, 95% confidence level and P-value of 0.05 were considered. To assess test agreement between the SICCT and gross pathology, Kappa statistics was used.

Molecular data for characterization of strains were entered in the data base www.*Mbovis*.org, and http://www.pasteur-guadeloupe.fr:8081/SIT-VITDemo/(SITVIT1Database, retrieved on February, 2017). Online tool, Run TB-lineage http://tbinsight.cs.rpi.edu/run_tb_lineage.html was also used for grouping lineages using Conformal Bayesian Network (CBN) analysis and sub-lineage using knowledge based Bayesian network (KBBN). The binary and octal formats of the strains were entered into query box, the name of the strains were retrieved from the database if the spoligotype pattern of the strain in question fits the pattern that has already been registered in the SPolDB4 database (Brudey *et al.*, 2006) If the pattern of the strain in question has not been registered in SPolDB prior to this study, the strain was considered as an orphan.

4. RESULTS

4.1. Prevalence of Tuberculosis in Camels based on the SICCT Test

The single intra-dermal comparative cervical tuberculin (SICCT) test of the 387 camels indicated that overall positive tuberculin reactor prevalence of 9.82% (38/387) with (95% CI: 6.84% - 12.8%) and 17.05% (66/387) with (95% CI: 13.3% - 20.82%) at cutoff values ≥4mm and ≥2mm, respectively. There was no statistically significant association (P>0.05) of risk factors of age, sex, body condition, and origin of camels at a cutoff value ≥4mm although higher prevalence of 10.37% was recorded in females than males (5.0%) (table 2).

Table 2: Association of risk factors with positive tuberculin reactivity at a cutoff value ≥4mm

Variables	No. Examined (%)	No. Positive (%)	χ^2 result	P-value
Age				
≤ 7 years	93 (23.5%)	9 (9.68)	0.0028	0.958
>7 years	294(76.5%)	29 (9.86)		
Sex				
Male	40 (10.3%)	2 (5.00)	1.17	0.279
Female	347(89.7%)	36 (10.37)		
Body Cond.				
Poor	209 (54%)	21 (10.05)		0.927
Moderate	70 (18.1%)	6 (8.57)	0.1518	
Good	108 (27.9%)	11(10.19)		
Origin				
Borena	323 (83.5%)	31 (9.6)	0.1083	0.742
Metehara	64 (16.5%)	7 (10.94)		

Statistically significant association was observed for the origin of camels (χ^2=13.461, P=0.00) and body condition (χ^2=6.54, P=0.038) with the prevalence at a cutoff value ≥2mm (table 3).

Table 3: Association of risk factors with positive tuberculin reactivity at a cutoff value ≥2mm

Variables	No. Examined (%)	No. Positive (%)	χ^2 result	P-value
Age				
≤ 7 years	93 (23.5%)	18 (19.35)	0.458	0.499
>7 years	294 (76.5%)	48 (16.33)		
Sex				
Male	40 (10.3%)	9 (22.5)	0.935	0.334
Female	347 (89.7%)	57 (16.43)		
Body Cond.				
Poor	209 (54%)	29 (13.88)	**6.54***	**0.038***
Moderate	70 (18.1%)	19 (27.14)		
Good	108 (27.9%)	18 (16.67)		
Origin				
Borena	323 (83.5%)	45 (13.93)	**13.46***	**0.000***
Metehara	64 (16.5%)	21 (32.81)		

Note: * Chi-square and P-value with statistically significant association.

The multivariable logistic regression analysis at a cutoff value ≥4mm, being female (AOR= 2.226, 95% CI: 0.5099-9.719) was found to be associated risk factor for positive tuberculin reactivity. Similarly, the multivariable analysis at a cut off value ≥2mm, being moderate body conditioned (AOR= 1.583, 95% CI: 0.7399-3.385) was identified as risk factor for higher tuberculin reactivity (Tables 4 and 5).

Table 4: Multivariable logistic regression of risk factors at a cut off value ≥4mm

Variables	No. Examined (%)	No. Positive (%)	COR (95% CI)	AOR (95% CI)
Age				
≤7 years	93 (23.5%)	9 (9.68)	1	1
>7 years	294(76.5%)	29 (9.86)	1.0213 (0.4649-2.244)	0.9998 (0.4503-2.220)
Sex				
Male	40 (10.3%)	2 (5.00)	1	1
Female	347(89.7%)	36 (10.37)	**2.1994** (0.5092-9.500)	**2.226** (0.5099-9.719)
Body Cond.				
Poor	209 (54%)	21 (10.05)	0.985 (0.456-2.126)	0.958 (0.4401-2.088)
Moderate	70 (18.1%)	6 (8.57)	0.8267 (0.2912-2.347)	0.8332 (0.2877-2.413)
Good	108 (27.9%)	11(10.19)	1	1
Origin				
Borena	323 (83.5%)	31 (9.6)	0.8645 (0.3629-2.059)	0.8049 (0.3339-1.940)
Metehara	64 (16.5%)	7 (10.94)	1	1

**Note:* *COR= Crude Odds Ratio, AOR= Adjusted Odds Ratio*

Table 5: Multivariabe logistic regression of risk factors of tuberculosis at cutoff ≥2mm

Variables	No. Examined (%)	No. Positive (%)	COR (95% CI)	AOR (95% CI)
Age				
≤ 7 years	93 (23.5%)	18 (19.35)	1	1
>7 years	294(76.5%)	48 (16.33)	0.813 (0.446-1.482)	0.836 (0.446-1.565)
Sex				
Male	40 (10.3%)	9 (22.5)	1	1
Female	347(89.7%)	57 (16.43)	0.677 (0.306-1.49)	0.867 (0.377-2.000)
Body Cond.				
Poor	209 (54%)	29 (13.88)	0.806 (0.4247-1.528)	0.781(0.405-1.505)
Moderate	70 (18.1%)	19 (27.14)	**1.863 (0.897-3.867)**	**1.583 (0.739-3.385)**
Good	108 (27.9%)	18 (16.67)	1	1
Origin				
Borena	323 (83.5%)	45 (13.93)	0.331(0.180-0.609)	0.356 (0.191-0.664)
Metehara	64 (16.5%)	21 (32.81)	1	1

Using the cutoff point ≥3mm for the SICCT to detect tuberculosis in camels which is recommended in this study showed overall positive tuberculin reactor rate of 5.2% (95%CI: 4.07% - 6.33%). Statistically significant association (χ^2 = 5.208, p<0.05) was observed between the origin of camels and reactor prevalence at ≥3mm cutoff value (table 6). However, the multivariable logistic regression analysis revealed no strong association of host risk factors with positive tuberculin reactivity at this cutoff point.

Table 6: Association of risk factors with positive tuberculin reactivity at a cutoff value ≥3mm

Variables	No. Examined (%)	No. Positive (%)	χ^2 result	P-value
Age				
≤ 7 years	93 (24%)	4 (4.3)	0.188	0.45
>7 years	294 (76%)	16 (5.4)		
Sex				
Male	40 (10.3%)	4 (10)	2.125	0.14
Female	347 (89.7%)	16 (4.6)		
Body Cond.				
Poor	209 (54%)	6 (2.9)	5.965	0.051
Moderate	70 (18.1%)	7 (10)		
Good	108 (27.9%)	7 (6.5)		
Origin				
Borena	323 (83.5%)	13 (4.02)	**5.208**	**0.032**
Metehara	64 (16.5%)	7 (10.9)		

4.2. Prevalence of Tuberculosis in Camels based on Gross Pathological Lesions

Of the total 2070 camels investigated during post mortem examination for any suspected gross tuberculosis lesions, 156 camels were detected with suspected gross tuberculosis lesion with lesion prevalence of 7.54% (95% CI:6.45%-8.7%). During the study, majority of the camels were with poor body condition, of Borena origin, older aged, and unproductive female camels.

Among investigated camels, 81.74% (1692/2070) were old age (>7 years of age) and 18.3% (378/2070) were adult camels (≤7 years of age); 83.7% were female camels and 16.33% were males. Higher lesion prevalence of 7.74% (131/1692) was detected in older camels (>7 years) than adult camels (≤7years) with lesion prevalence of 6.61%. Statistically significant association (P=0.000, χ^2=26.2) was observed between body condition and prevalence of tuberculosis in camels with lesion prevalence of 12.1%, 6.7%, and 5.2% in poor, moderate, and good body conditions, respectively. However, there was no statistically significant association (P>0.05) was observed between other risk factors of age, sex, and origin of camels with lesion prevalence (Table 7).

Table 7: The Chi-square test for the association of risk factors with lesion prevalence

Variables	No. Examined (%)	No. Positive (%)	χ^2	P-value
Age				
≤7 years	378 (18.3)	25 (6.61)	0.5647	0.452
>7 years	1692 (81.74)	131 (7.74)		
Sex				
Male	338 (16.33)	20 (5.9)	1.52	0.218
Female	1732 (83.7)	136 (7.9)		
Body Cond.				
Poor	594 (28.7)	72 (12.1)	**26.2**	**0.000***
Moderate	510 (24.64)	34 (6.7)		
Good	966 (46.7)	50 (5.2)		
Origin				
Borena	1739 (84)	131 (7.53)	0.002	0.99
Metehara	331 (16)	25 (7.6)		

The multivariable logistic regression analysis revealed that being poor body conditioned was found to be associated risk factor for the detection of gross tubercle lesions and significantly associated (P=0.000) with lesion prevalence of tuberculosis in camels [Adjusted OR= 2.55 (95% CI: 1.743-3.734)] (Table 8).

Table 8: Multivariable logistic regression for association of risk factors with lesion prevalence

Variables	No. Examined (%)	No. Positive (%)	COR (95% CI)	AOR (95% CI)
Age				
≤7 years	378 (18.3)	25 (6.61)	1	1
>7 years	1692 (81.74)	131 (7.74)	1.185 (0.761-1.846)	1.261 (0.8023-1.982)
Sex				
Male	338 (16.33)	20 (5.9)	1	1
Female	1732 (83.7)	136 (7.9)	1.355 (0.835-2.1996)	1.189 (0.726-1.946)
Body Cond.				
Poor	594 (28.7)	72 (12.1)	**2.527 (1.734-3.682)**	**2.55 (1.743-3.734)**
Moderate	510 (24.64)	34 (6.7)	1.309 (0.835-2.0512)	1.312 (0.835-2.061)
Good	966 (46.7)	50 (5.18)	1	1
Origin				
Borena	1739 (84)	131 (7.53)	0.9972 (0.6391-1.556)	1.071 (0.6814-1.683)
Metehara	331 (16)	25 (7.6)	1	1

The frequency of severity of lesion (pathology score of the lymph nodes) showed, 73.7% of the lesioned camels were with pathology score of 1, 16.03% with score of 2, and 10.9% with severe lesion (score of 3). Frequency of lesion distribution showed, 84% of the lesions detected were in camels >7 years, whereas 16% in camels of ≤7 years of age. Moreover, higher frequency of lesion with 87.2%, 46.2%, and 84% were recorded in female, poor conditioned, and Borena origin camels, respectively. Pathology score for severity of gross lesions interms of age, sex, body condition and origin of camels were also indicated in the table below (Table 9).

Table 9: Pathology score for severity of typical tubercle lesions in terms of host risk factors

Variables	No. Lesioned (%)	Pathology Score (%)		
		1	2	3
Age				
≤7 years	25 (16)	20 (17.4)	4 (16)	1 (5.9)
>7 years	131 (84)	95 (82.6)	21 (84)	16 (94.1)
Sex				
Male	20 (12.8)	19 (16.5)	1 (4)	1 (5.9)
Female	136 (87.2)	96 (83.5)	24 (96)	16 (94.1)
Body Cond.				
Poor	72 (46.2)	53 (46.1)	11(44)	8 (47.1)
Moderate	34 (21.8)	24 (20.9)	5 (20)	6 (35.3)
Good	50 (32.1)	38 (33.0)	9 (36)	3 (17.6)
Origin				
Borena	131 (84)	101 (87.8)	19 (76)	12 (70.6)
Metehara	25 (16)	14 (12.2)	6 (24)	5 (29.4)
Total	**156 (7.54)**	**115 (73.7)**	**25 (16.03)**	**17 (10.9)**

From the 156 camels detected with gross visible lesions, the distribution, frequency, and severity of the lesions were assessed. Thus, the distribution and frequency of the lesions were high in sub-mandulbular lymph node (SMLN) (54.8%), followed by retro-pharyngeal lymph nodes (RPLN) (16.94%), trachea-broncheal lymph nodes (TrBLN) (10.73%), parotid lymph node (PLN) (7.34%), lung (6.2%), and the least frequency being detected in caudal mediastinal lymph node (CaMLN) (2.82%) and cranial mediastinal lymph node (CrMLN) (1.13%) (Figure 6).

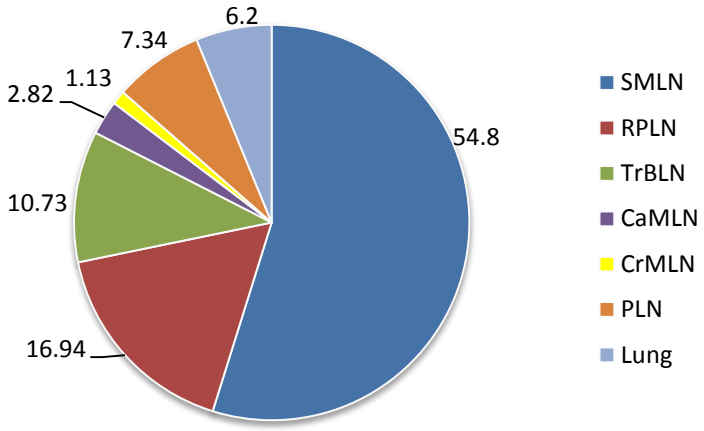


Figure 6: Frequency and lesion distribution in lung and lymph node tissues

Pathology score for severity of lesions of lung tissue, lung associated lymph nodes and lymph nodes of the head showed, severe lesions was detected in sub-mandibular lymph node more frequently than the other lymph nodes with the mean severity of 2.47±0.24, followed by retro-pharyngeal (0.35±0.19) and caudal mediastinal lymph nodes (0.35±0.19) (table 10).

Table 10: Pathology score for severity of tubercle lesions from different tissues of camels

No.	Tissues examined	Mean ± SE of Pathology Score		
		1	**2**	**3**
1	CrMLN	0.01±0.009	0.08±0.08	-
2	CaMLN	0.01±0.009	0.00±0.00	0.35±0.19
3	SMLN	**0.57±0.05**	**1.12±0.19**	**2.47±0.24**
4	RPLN	**0.17±0.035**	**0.40±0.14**	**0.35±0.19**
5	TrBLN	**0.07±0.024**	**0.40±0.14**	**0.35±0.19**
6	PLN	0.11±0.03	-	-
7	Lung	0.07±0.024	0.24±.13	-

Of the total 156 camels detected with gross typical tuberculosis lesions, 92.9% (145/156) exhibited at least one lesion in their tissues examined while 7.1% of the camels (11/156) showed more than one lesion in their tissues.

4.3. Diagnostic Test Performance of SICCT against Gross Pathology

Of the 168 camels considered for assessing the performance of the SICCT at different cutoff points to detect tuberculosis in camels against gross pathology as a reference test, positive reactor rate of 61.3% (60.9%-61.8%), 60.1% (59.6%-60.6%), and 22.6% (22.2%-23.02%), 11.9% (11.6%-12.23%) for the cutoff points ≥1.5mm, ≥2mm, ≥2.5mm and ≥3mm, and ≥3.5mm, respectively. The detection of gross tubercle was with lesion detection rate of 16.7% (16.3%-17.1%) and culture positivity of 21.4% (20.9%-21.8%). The frequency of positive reactors, lesion detection rate, and culture isolation rate in terms of host risk factors were summarized in the table below (Table 11).

Table 11: Frequency of positive reactors, gross lesion and Mycobacteriological culture

Variable	No. (%) Examin	Number (%) positive reactors of the SICCT at					No. (%) PM	Culture No. (%)
		≥1.5mm	≥2mm	≥2.5mm	≥3mm	≥3.5mm		
Age								
≤7 years	42 (25)	30 (71.4%)	30 (71.4%)	9 (21.4%)	9 (21.4%)	6 (14.3%)	6 (14.3%)	10 (23.8%)
>7 years	126 (75)	73 (57.9%)	71 (56.3%)	29 (23.0%)	29 (23.0%)	14 (11.1%)	22 (17.5%)	26 (20.6%)
Sex								
Male	34 (20)	21 (61.8%)	20 (58.8%)	7 (20.6%)	7 (20.6%)	4 (11.8%)	2 (5.9)	8 (23.5%)
Female	134 (80)	82 (61.2%)	81 (60.4%)	31 (23.1%)	31 (23.1%)	16 (11.9%)	26 (19.4)	28 (20.9%)
BCS								
Poor	70 (42)	39 (55.7%)	38 (54.3%)	11 (15.7%)	11 (15.7%)	8 (11.4%)	17 (24.3)	14 (20%)
Moder.	50 (30)	37 (74%)	36 (72%)	18 (36%)	18 (36%)	8 (16%)	5 (10)	8 (16%)
Good	48 (28)	27 (56.3%)	27 (56.3%)	9 (18.8%)	9 (18.8%)	4 (8.3%)	6 (12.5)	14 (29.2%)
Origin								
Borena	121 (72)	77 (63.6%)	75 (61.9%)	29 (23.9%)	29 (23.9%)	14 (11.6%)	21 (17.4)	25 (20.7%)
Metehara	47 (28)	26 (55.3%)	26 (55.3%)	9 (19.1%)	9 (19.1%)	6 (12.8%)	7 (14.9)	11 (23.4%)
Total	**n = 168**	**103 (61.3%)**	**101 (60.1%)**	**38 (22.6%)**	**38 (22.6%)**	**20 (11.9%)**	**28 (16.7%)**	**36 (21.4%)**

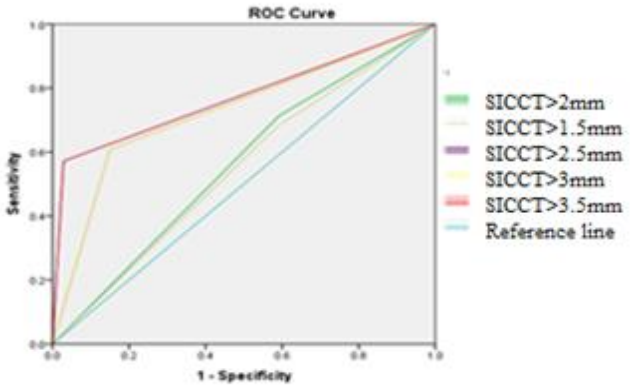
The interpretation of the SICCT at different cutoff points to detect tuberculosis in camels as compared to gross pathology for tubercle lesion as a reference to define disease status revealed that the interpretation of SICCT at cutoff value ≥3mm was found to be better informative in discriminating diseased cases than the other cutoff value with sensitivity of 60.7% and specificity of 85% at this cutoff value (Table 12).

Table 12: Diagnostic test performance of SICCT as compared to gross pathology as reference

SICCT	Gross Pathology to define true disease status				
	Se (%)	Sp (%)	χ^2	P-value	Kappa
≥ 2mm	71.4	41	1.45	0.16	0.06
≥ 1.5mm	68	41	0.84	0.24	0.05
≥ 2.5mm	**60.7**	**85**	**27.9**	**0.000**	0.4
≥ 3mm	**60.7**	**85**	**27.9**	**0.000**	0.4
≥ 3.5mm	57.1	97	65.6	0.000	0.613

Without considering gross pathology as gold standard test, kappa statistics revealed that agreement between the SICCT and gross pathology was found to be significant with kappa value 0.4 [p=0.000, χ^2=27.9, OR=8.89 (95% CI: 3.6 - 21.3)] at cutoff points ≥3mm with moderate agreement for the SICCT and gross pathology.

Regarding the validity of the SICCT at different cutoff points according to gross pathology, the receiver operating characteristics (ROC) analysis revealed area under the curve for the SICCT cutoff points ≥2.5mm and ≥3mm was [0.729 (0.615 - 0.842)] and [0.771 (0.654 - 0.889)] for ≥3.5mm cutoff point and this was statistically significant (P= 0.000) for sensitivity and specificity evaluation (Figure 7).



SICCT Cutoff points	AUC (95% CI)	Asym. Significance
≥2mm	.561(.447-.674)	.311
≥1.5mm	.546(.431-.661)	.439
≥2.5mm	**.729(.615-.842)**	.000
≥3mm	**.729(.615-.842)**	.000
≥3.5mm	**.771 (.654-.889)**	.000

Figure 7: The receiver operating characteristics analysis for the performance of the SICCT at different cutoff points

4.4. Cultural Isolation and Molecular Characterization of Isolates from Camels

Mycobacteriological culture

Isolates obtained from any of the lymph node tissues of the 36 culture positive camels were considered as separate and were identified as acid fast bacilli (AFB) positive isolates for Mycobacteria with isolation rate of 21.4% (36/168). Hence, a total of 80 acid fast bacilli (AFB) positive isolates were harvested from the different lymph node tissues; where 51.3% of the isolates were harvested from pyruvate enriched LJ-media while 48.8% (39/80) on glycerol enriched LJ-media (table 13).

Table 13: Culture isolates of mycobacterium from tissues of dromedary camels

No.	Tissues examined	No of Isolates		No. of Isolates (%)
		Pyruvate	Glycerol	
1	CrMLN	6	6	**12 (15)**
2	CaMLN	10	10	**20 (25)**
3	SMLN	9	4	**12 (15)**
4	RPLN	4	4	**8 (10)**
5	TrBLN	9	7	**16 (20)**
6	PLN	5	6	**11 (13.8)**
	Total	**41 (51.3%)**	**39 (48.8%)**	**80 (7.84%)**

The isolation of Mycobacterium from different lymph node tissues showed the highest isolation rate from the caudal mediastinal LN (25%) followed by trachea-broncheal LN (20%), sub-mandibular LN (15%), cranial mediastinal LN (15%), and the least in retro-pharyngeal LN (10%) (Figure 8).

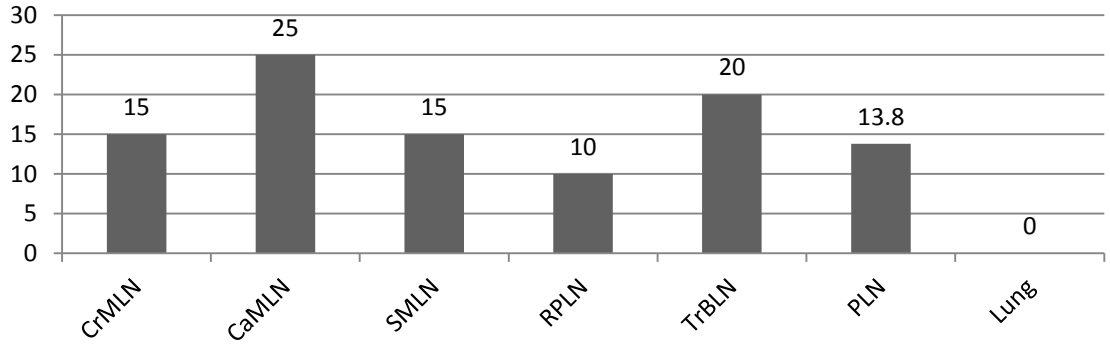


Figure 8: Isolation frequency of *Mycobacteria* from different lymph node tissues of camels

Molecular Typing of Culture Isolates

Molecular characterization or genotyping of the 80 acid fast positive isolates based on multiplex PCR analysis revealed PCR products with 48.8% of the isolates (39/80) bands/signals for Mycobacterium and identified as *Mycobacterium tuberculosis* complex and the remaining isolates were probably Mycobacteria other than *Mycobacterium tuberculosis* complex or non-tuberculous Mycobaceteria (NTM) (Annex 1).

Species differentiation of the isolates that showed bands for *M. tuberculosis complex* using PCR based deletion typing for region of difference (RD4) revealed 23.1% (9/39) of the isolates identified as *M. bovis* while 76.9% (30/39) isolates were as *M. tuberculosis* (Annex 2). PCR based spoligotyping for strain differentiation of the *Mycobacterium tuberculosis complex* species depicted as *M. bovis* and *M. tuberculosis* by RD4 typing revealed signals for different spoligotype patterns.

The 39 isolates from different lymph node tissues identified as *M. tuberculosis* complex were spoligotyped and four isolates showed no spacers an hence considered as negative. Unfortunately, all the isolates revealed spoligotype patterns with spacer 9 which should be normally absent from *M. bovis* but uniquely present in *M. tuberculosis* strains as it can be appreciated in the autorad film (Figure 9).

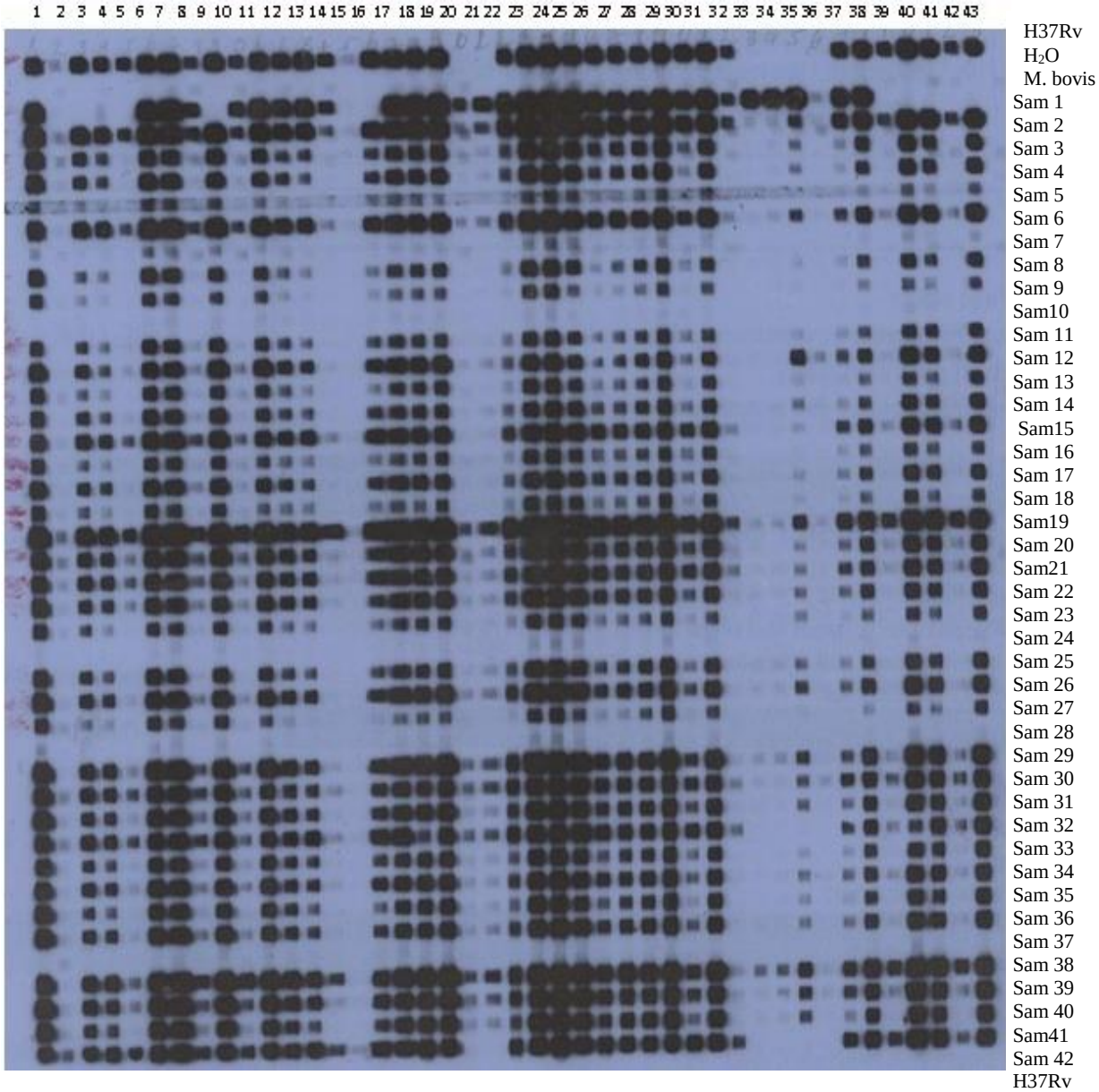
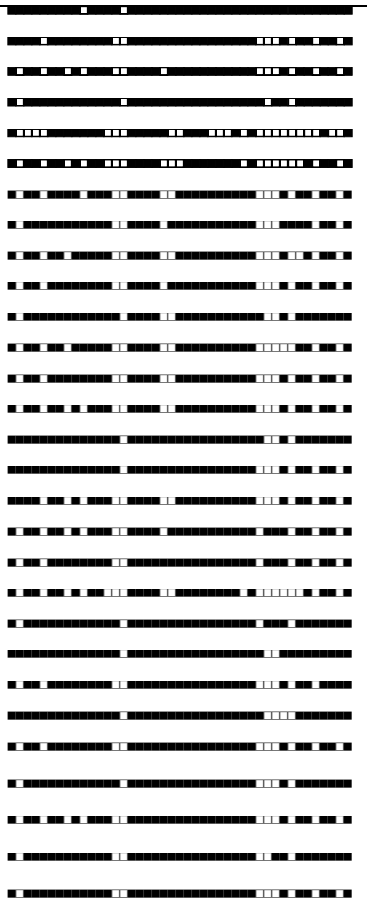


Figure 9: The spoligotype autorad film of the isolates of *M. tuberculosis* complex identified as *M.tuberculosis* strains based on the presence of spacer 9.

Of the 39 isolates spoligotyped, majority of the strains 84.6% (33/39) were identified as orphans with no shared SIT number in the Spoligotype data base and not registered prior to this work. While two strains identified as 1088, and 118 with shared SIT number in the data base. The lineage of the strains based on Conformal Bayesian Network (CBN) analysis revealed the dominant *M. tuberculosis* strains identified were Euro-American lineage 84.6% (33/39) while 12.8% (6/39) of strains with Indo-Oceanic (ancestral) lineage, and one strain as *M. africanum* lineage (2.6%) (Table 14).

Table 14: The major lineage, clades and of the spoligotype patterns of the strains of *M. tuberculosis* identified from lymph node tissue culture isolates of camels

SIT number	Isolates with similar pattern	Major Lineage By CBN	SIT/VIT Clade by KBBN	Octal number	Binary format
Orphan	2	IO	Manu1	777767777777771	
Orphan	2	EA	Manu2	757747777742661	
Orphan	1	EA	Manu2	755347577742661	
Orphan	1	EA	Manu2	577767777766771	
Orphan	1	EA	T	417707634240041	
Orphan	1	EA	T	755307437640261	
Orphan	1	EA	H37Rv	557347477742661	
Orphan	1	IO	Manu2	577747577743661	
Orphan	1	EA	H37Rv	555747477742261	
Orphan	1	EA	Manu2	557747577742661	
Orphan	1	EA	H37Rv	577767477762771	
Orphan	1	EA	T	555747477740661	
Orphan	1	EA	H37Rv	557747477742661	
Orphan	2	EA	H37Rv	555347477742661	
Orphan	2	EA	Manu2	777767777762771	
Orphan	1	EA	Manu2	777767777742661	
Orphan	1	EA	H37Rv	755347477742661	
Orphan	1	*M. afri.*	Manu-ancestor	555347577756661	
Orphan	1	IO	Manu1	557747777756661	
Orphan	1	EA	T	555307477640261	
Orphan	2	IO	Manu1	577767777756771	
1088	1	EA	Manu2	777767777763771	
Orphan	1	EA	Manu2	557747777742671	
118	1	EA	T	777767777760771	
Orphan	2	EA	Manu2	557747777742661	
Orphan	1	EA	Manu2	577767777742771	
Orphan	1	EA	Manu2	555347777742661	
Orphan	1	EA	Manu2	577747777746771	
Orphan	1	EA	Manu2	577747777742661	

Note: *CBN= Conformal Bayesian Network, KBBN= Knowledge Based Bayesian Network, IO= Indo-Oceanic, EA=Euro-American*

The 39 isolates of *M. tuberculosis* were grouped into 29 spoligotype molecular patterns, of which 64.1% (25 isolates) with single (specific) spolipattern. Again the 39 *M. tuberculosis* strains were clustered into five families (sub-lineages) as *Manu-1* (12.8%), *Manu-2* (44%), *T-family* (12.8%), *H37Rv* (17.9%), and *Manu-ancestor* (2.6%).

From host perspectives, of the 36 culture positive camels, 69.4% (25/36) camels were identified with different spoligotype patterns. The distribution of strains from host perspectives showed 76%, 88%, 56%, and 72% of the isolates spoligotyped were identified from camels >7 years, female, poor condition, and borena origin camels, respectively (table 15). Strains of *M. tuberculosis* identified with the SIT-1088 was from camels >7 years age, female and of borena origin while the other strain of *M. tuberculosis* with SIT-118 was identified from poor conditioned female camel of Metehara (Fentale) origin.

Table 15: Frequency of strains identified from different lymph node tissues interms of host variables

Variables	No. (%) strains identified
Age	
≤7 years	6 (24%)
>7 years	19 (76%)
Sex	
Male	3 (12%)
Female	22 (88%)
BCS	
Poor	14 (56%)
Moderate	-
Good	11 (44%)
Origin	
Borena	18 (72%)
Metehara	7 (28%)

5. DISCUSSION

5.1. Prevalence of Tuberculosis in Camels based on the SICCT Test

The current study based on comparative intradermal tuberculin test revealed overall individual prevalence of 9.82% at a cutoff value ≥4mm, 5.2% at cutoff value ≥3mm and 17.05% at a cut off value ≥2mm. The present finding at a cutoff value ≥4mm was slightly lower but comparable to the previously reported prevalence of Mamo *et al.*, (2011) with abattoir based prevalence of 10.4% for camels of Ethiopia but it was higher than prevalence report of Mamo *et al.*, (2009) for camels at Dire Dawa, and Gumi *et al.*, (2012) for camels in Southern Ethiopia. Similarly, it was significantly higher than previously reported prevalence of some other authors at different sites in Ethiopia (Kassaye *et al.*, 2013; Beye *et al.*, 2014) for camels at Akaki and Eastern part of Ethiopia. It is comparably higher than the incidence reported for camels in Egypt by Manal *et al.*, (2008). At the same cut off value ≥4mm and ≥3mm, the present finding was significantly lower than the previous prevalence report of Abubakar *et al.*, (2014) for camels slaughtered at Kano abattoir, Nigeria. These variations might be probably due to variations in the composition of study population, study site, management and environmental factors, study design, methodology, and sample size factors.

Regarding age as a risk factor, in the present study at a cutoff value ≥4mm, prevalence of 9.68% and 9.86% was recorded in camels ≤7 years and camels >7 years, respectively. Moreover, at a cutoff value ≥2mm, prevalence of 19.35% for camels ≤7 years and 16.33% for camels >7 years was recorded and prevalence of 4.3% and 5.4% at a cutoff value of ≥3mm for camels ≤7 years and >7 years old, respectively. It was found that there was no statistically significant association (P>0.05) between tuberculosis prevalence and age categories. This finding was in agreement with that of Kasaye *et al.*, (2013) for camels at Akaki abattoir, Central Ethiopia. However, some previous studies in Ethiopia (Ameni and Aklilu, 2007; Kebede *et al.*, 2008; Gumi *et al.*, 2011; Mamo *et al.*, 2013), from Ecuador (Proano-Perez *et al.*, 2009) and study from India (Thakur *et al.*,

2010) reported significant association of age with positive tuberculin reactivity in cattle. The significance of age as a risk factor was not appreciated in this study and this might be due to the number of camels investigated in the age categories where large number of older camels (76.5%) was slaughtered during the study period than adult age categories (23.5%).

About 89.7% of the camels investigated were females. The prevalence at the two cutoff values were not significantly (P>0.05) associated with sex. The current finding was similar with the reports of Nuru *et al.,* (2015) for cattle at Amhara region. However, an Ethiopian study (Zeru *et al.*, 2014) reported significant association of sex with positive skin reactivity. In the current study, there was large number of unproductive females and older age camels which were slaughtered during the investigation. Moreover, only very few number of male camels were slaughtered that might attribute to the variation in prevalence recorded between males and females at a cutoff value ≥2mm although this was not statistically significant (P>0.05). The study of Abubakar *et al.,* (2014) at Kano abattoir, Nigeria, with higher prevalence in male camels (23.9%) than female (21.8%) camels using lateral flow assay and his finding was not statistically significant.

As to the body condition of camels investigated, at a cutoff value ≥2mm, higher prevalence of 27.14% was observed in camels with moderate body condition than poor (13.88%) and good (16.67%) body conditions and associated with positive tuberculin reactivity and it was statistically significant (χ^2=6.54, p= 0.038). This finding was similar with the reports for cattle by different authors (Nuru *et al.,* 2015; kebede *et al.,* 2008; Zeru *et al.,* 2014). In the current study, about 53% of the camels were with poor body condition, 20% with moderate, and the rest 27% with good body conditions. Animals with poor body condition assumed to have association with high skin reactivity however, poor body conditioned animals have relatively weak immunological responses to TB and subsequently susceptible to infection (Griffin *et al.,* 1993; O'Reilly and Daborn, 1995). Camels with poor body conditions might have in apparent and chronic infectious diseases like Tuberculosis and good body conditioned camels might have

strong immune response against Tuberculosis infection. Moreover, the proportions or the number of camels in different categories was not comparable and this might have attributed to slight variation in the prevalence with body conditions.

Majority of the camels investigated during the study were Borena camels (83.5%) and some Metehara origin camels (16.5%). Prevalence of 9.6% and 10.94% was recorded for camels of Borena and Metehara origin, respectively at a cut off value ≥4mm and it was not statistically significant (P>0.05). However, prevalence of 13.93% for Borena and 32.81% for Metehara origin camels at a cutoff value ≥2mm was observed and this was statistically significant (χ^2= 13.46, P= 0.000). Moreover, prevalence of 10.9% for Metehara and 4.02% for borena at ≥3mm which was significant (χ^2=5.02, p<0.05). Some other recent studies reported strong association between breed type and tuberculin positive reactivity for cattle in different sites at a cutoff value ≥4mm (Admasu *et al.*, 2014; Romha *et al.*, 2014; Zeru *et al.*, 2014; Nuru *et al.*, 2015). This prevalence report was significantly higher for Metehara camels than Borena camels. This might be probably for the reasons of the disproportionate number of camels investigated during the study where the majority of the camels slaughtered at Akaki abattoir were Borena origin camels. The husbandry and other mamangement factors might have also contributed for the variation. Moreover, management and husbandry practices where migration, mixed livestock practices might also contribute to interspecies transmission of TB and other contagious diseases and possibly the pastoral livestock keeping in Borena and Metehara area might have some contribution to this variation in prevalence.

5.2. Prevalence of Tuberculosis in camels based on Gross Pathological Lesions

Information regarding the occurrence and epidemiology of Tuberculosis in camels is very scarce in Ethiopia and many other camel rearing countries in the world. In this abattoir based epidemiological investigation, detection of gross pathology typical for tubercle lesion with the overall lesion prevalence of 7.54% was recorded. This finding is

slightly lower than from the reports of Mamo *et al*., (2011) for camels in Eastern Ethiopia and Elmossalami *et al.,* (1971) for camels at Kazakistan; but it was slightly higher than the finding of Mamo *et al.,* (2009) for camels at Eastern and Southern Ethiopia; Again, the current finding was higher than from previous reports of Mason (1917) for camels at Cairo abattoir and the work of Zubair *et al.,* (2004) for camels in Pakistan. The current finding was lower than from that of Zerom *et al.,* (2013) for camels in Eastern Ethiopia. However, the present finding was higher than previous reports of Kassaye *et al.,* (2013) for camels slaughtered at Akaki abattoir and Manal *et al.,* (2008) for camels which were kept in close contact with cattle in Egypt, and also from that of Elmosalami *et al.,* (1971) in camels at Kazakistan. This variation might be due to difference in geographic location, husbandry practice, study design, predisposition of host species might all be factors for lesion prevalence variations.

During the study, 81.74% of the camels were old aged (>7 years) and 18.3% were adult (≤7 years) camels. Lesion detection rate was higher in older camels with lesion prevalence of 7.74% than adult camels (6.61%). In the current study, the association of age as a risk factor with lesion prevalence of tuberculosis in camels was not statistically significant (P> 0.05) and this finding was in agreement with previous works by different authors (Kassaye *et al.,* 2013; Mamo *et al.,* 2011) for camels. Mamo *et al.,* (2011) reported in his study that the frequency of lesion detection was higher in older than younger camels. Other researchers have also reported in cattle that older animals are affected by TB (Kazwella *et al.,* 2001; Cleaveland *et al.,* 2007; Inangolit *et al.,* 2008; Munyeme *et al.,* 2008a) which could be due to the fact that older animals have weaker immune system and might have higher rate of exposure to infection in the course of the longer life span. This fact can be substantiated by the chronic nature of the disease tuberculosis in which case the animals might have acquired the infection at younger age and developed the disease at an older age (Radostits *et al.,* 2007).

Regarding sex, about 83.7%, and 16.3% of the study camels were unproductive female and male camels, with lesion prevalence of 7.9% and 5.9% were detected in female and male camels, respectively. The present finding was consistent with previous studies of some authors (Kassaye *et al.,* 2013; Mamo *et al.,* 2011) for camels. Similarly as Mamo *et al.,* (2011) reported that lesion was more frequently observed in female camels as compared to male camels. This could be due to the fact that female camels were brought for slaughter at their older age after completion of their productive and reproductive age as it is substantiated by some authors (Inangolet *et al.,* 2008; Munyeme *et al.,* 2008b).

Poor body conditioned camels revealed significantly higher lesion prevalence (12.1%) than the lesion prevalence of 6.7% and 5.18% in moderate and good body conditioned camels, respectively. The high prevalence reported in poor conditioned camels in this study was in agreement with previous reports of (Kassaye *et al.,* 2013; Mamo *et al.,* 2009). Poor conditioned animals are nutritionally and immunologically compromised to defend and combat infectious agents. It was also consistent with previous reports which indicated that the animals with poor body condition have relatively low resistance to infectious agents (Radostitis *et al.,* 2007).

There was no difference in the lesion prevalence recorded for Borena and Metahra origin camels. This finding is consistent and comparable with previous works of (Kassaye *et al.,* 2013; Mamo *et al.,* 2009). However, Kassaye *et al.,* (2013) reported higher lesion prevalence in Metehara origin camels than Borena camels. Majority of the camels investigated during this study were Borena origin camels (84%) with very few of Metehara origin camels (16%). This uncomparable number of camels of Borena and Metaehara origin slaughtered during the investigation might have influenced the appreciation of origin of the camels as a risk factor of tuberculosis where the lesion prevalence was almost similar. Moreover, environmental factors, husbandry and management practices where these camels brought from, might have influenced the status and frequency of the disease.

The distribution, frequency, and severity of gross tuberculosis lesions were found to be high in sub-mandibular lymph node (54.8%) followed by retropharyngeal lymph node (16.95%) and trachea-broncheal lymph node (10.7%). This might indicate the predominant portal route of entry of mycobacterial agents in camels might be by inhalation. Although not investigated in detail during this study, the presence of TB lesions in mesenteric lymph nodes also indicates the existence of infection through ingestion (Radostits *et al.,* 2007). Similar studies of some other researchers revealed slight variation in the frequency, distribution and severity of gross visible lesions detected in different tissues. Previous reports of (Tsegaye *et al.,* 2010) showed lesions were predominantly detected more frequently in mediastinal lymph node followed by retropharyngeal lymph node; Kassaye *et al.,* (2013) also indicated that more frequent lesion being detected in the right cardiac lung and cranial mediastinal lymph node; whereas, Mamo *et al.,* (2011) reported that severe and more frequent tuberculous lesions in mesenteric lymph node and mediastinal lymph node. Reports from developed countries indicated that tuberculosis lesions are found most frequently in the lymphatic tissues of the thoracic cavity. In intensive husbandry system, 90% of the lesions occur in the respiratory tract (Corner *et al.,* 1994; Neil *et al.,* 2001). The report by Ameni *et al.,* (2007) showed that 94.5% of the lesions were detected in mesenteric lymph nodes in animals that kept on pasture. Mason (1912) also mentioned that all cases of Tuberculosis in OWCs had lesions at the lungs and associated thoracic lymph nodes, where typical caseo-necrotic lesions can be particularly extensive. Ethiopian studies have described 54% of infected OWCs with lesions in the lung and associated lymph nodes, and 38% with mesenteric lymph node lesions, followed by retropharyngeal lymph node involvement (Mamo *et al.,* 2009).

Of the total 156 camels with tuberculosis lesion, 7.1% (11 camels) were with more than one lesion in different lymph nodes and the remaining 92.9% (145 camels) with at least one lesion in their lymph node tissues. The severity of tuberculosis lesions showed 9.04% of the tissue lesions were with severe pathology (score of 3) and 78.5% of tissues with mild pathology of score 1. Comparable finding was previously reported by Kinne

et al. (2006). Mamo *et al.,* (2011) indicated that the detection of severe lesions was more frequent in mesenteric lymph node followed by mediastinal lymph node in camels. The current finding revealed that tuberculosis lesions were extensive in slaughtered camels of Borena and Metehara origin at Akaki abattoir, although in general, camels seem to be resistant against a number of diseases affecting other animal species. Tuberculosis is an existing phenomenon in camels and the findings in this study had revealed that tuberculosis in camel could occur and affect health and production of camel in pastoralist areas with potential public health concern, since pastoralists share the same environment (confinement) with their animals and the consumption of raw or unpasteurized milk and milk products in pastoralist communities is common practice.

5.3. Diagnostic test performance of the SICCT test against gross pathology

The performance of the single intra-dermal comparative cervical tuberculin test (SICCT) to detect tuberculosis in camels at different cutoff points as compared to gross pathology for tubercle lesion as reference test to define disease status were assessed and the first of its kind for the interpretation and validation of the SICCT to detect tuberculosis in dromedary camels of Ethiopia. Post mortem examination for the detection of gross tubercle lesion in the 168 camels showed 28 camels with gross lesion detection rate of 16.7% (16.3% - 17.1%) and 36 camels with mycobacteriological culture positivity of 21.4% (20.9% - 21.8%). In the present study, the performance of single intradermal comparative cervical tuberculin (SICCT) at different cutoff points against gross pathology as a reference to detect tuberculosis in camels was found to be more informative with superior performance at cutoff point ≥3mm. Sensitivity of 60.7% and specificity of 85% for the cutoff value ≥3mm were recorded.

In this study better performance of SICCT for positive test interpretation in camels in Ethiopia can be obtained if the cutoff value is lowered from the OIE standard cutoff value of ≥4mm to ≥3mm as compared to gross pathology. Similar findings was reported

by Awah-Nduke *et al.,* (2014) for cattle in Cameroon where the author indicated better performance of the SICCT can be obtained at the ≥3mm and ≥3.5mm as compared to macroscopic lesion detection. In previous studies, lowering the cutoff value from the OIE standard has been recommended by different researchers for the African cattle for the better performance of SICCT for sensitivity and specificity evaluation (Ameni *et al.,* 2008; Muller *et al.,* 2009; Awah-Ndukum *et al.,* 2014) which substantiate the present finding. Better performance of SICCT in Ethiopian cattle if the cutoff value for positive test interpretation was lowered from ≥4mm OIE standard to >2mm for bovine tuberculosis in cattle (Ameni *et al*., 2008). A lowered cutoff point will increase the test sensitivity, albeit at the cost of a decreased specificity. The OIE- recommended cutoff value was established mainly in developed countries for *Bos taurus* cattle and different cutoff values are applied according to a particular country's disease status and objective of its disease control programme. The fact that the performance of tuberculin skin test (TST) is affected by environmental and host factors and the nature of the tuberculin used (De la Rua-Domenech *et al.,* 2006; Ameni *et al.,* 2008; Francis, 1973; Marcotty, 2009; Humblet *et al.,* 2010).

The receiver operator characteristics (ROC) analysis, sensitivity and specificity evaluations of the SICCT at different cutoff points revealed severe interpretation in this study was observed at ≥3mm cutoff point as compared to detection of gross lesion. The area under the curve of the ROC analysis was significantly higher ($P<0.05$) for the SICCT cutoff points ≥2.5mm, ≥3mm, and ≥3.5mm with detection of gross lesion as a reference. Thus, the sensitivity and specificity evaluation of the SICCT at cutoff point ≥3mm was found to be better informative in discriminating diseased case than the other cutoff points according to detection of gross lesion. Moderate sensitivity of 60.7% and specificity of 85% at the cutoff value ≥3mm was recorded when the SICCT compared with detection of gross tubercle lesion. However, the sensitivities obtained in this study particularly at the cutoff point ≥3mm was found to be lower than the value reported by Ameni *et al.,* (2008) who reported 68.8% at ≥2mm cutoff point for cattle in Ethiopia, Awah-Ndukum *et al.,* (2014) who reported 67.8% at ≥3.5mm against detection of gross

lesion for cattle in Cameroon and much lower than that of Delafosse (Delafosse *et al.*, 2002) who reported 94% at ≥4mm in Chad. In this study, although best individual sensitivity of 71.4% observed at ≥2mm cutoff point of the SICCT with detection of gross tubercle lesion as reference test recorded was lower than the sensitivity of 80% stated by OIE (OIE, 2009) at the recommended >4mm cutoff point (De la Rua-Domenich *et al.,* 2006), but it was with very low specificity.

Various factors can influence the sensitivity of tuberculin skin test and the hypersensitivity reactions can fluctuate considerably depending on the animal. Delayed hypersensitivity reactions provoked by tuberculin injection can become established 3 to 6 weeks after exposure of the host to bacilli agents while recently infected animals may not react sufficiently to tuberculin injection (Francis, 1971). Anergy has been reported to cause false negative reactions during tuberculin skin test but the reasons are still poorly understood (Inangolet *et al.,* 2008). However, recently infected animal, animal under stress due to malnutrition, gastrointestinal parasitoses, other concurrent infections and animal with generalized TB would be anergic and fail to react to tuberculin skin test (Ameni and Medhin, 2000; Inangolet *et al.,* 2008). Thus, animals presenting the SICCT skin thickness of ≤4mm should not be excluded that they are not affected by Tuberculosis particularly bovine TB, especially animals in highly endemic areas and animals sensitized to environmental mycobacteria as reported in Cameroon (Awah-Ndukum *et al.,* 2012). These animals could actually be infected but low reacting or not reacting at all because their immune system may not be sufficiently stimulated for a positive response to occur at the ≥4mm OIE recommended cut-off point (Ameni and Medhin, 2000; Inangolet *et al.,* 2008). Also, conditions such as stress may compromise their immune function (Thoen *et al.,* 2009) and animals may be sensitized to environmental mycobacteria (Palmer *et al.,* 2006). Furthermore, in late stages or towards the end of the course of the disease, the capacities of the infected hosts may become saturated and the expected hypersensitivity reactions may not be observed (Lepper *et al.,* 1997). Also, about 1% to 5% some animals may be totally anergic during their entire lifespan (De la Rua-Domenech *et al.,* 2006; Pollock and Neil, 2002). These phenomena

are responsible for the fluctuating sensitivities of tuberculin skin tests according to environments and amongst animal populations. This study revealed that severe interpretation of tuberculin skin tests, at cut off value less than the OIE recommended cut off value of >4 mm, is essential for optimal diagnosis of tuberculosis in pastoralist camels of Ethiopia. Therefore, the interpretations of the SICCT, to diagnose tuberculosis in camels, could be done at ≥3mm cutoff point, given the epidemiological factors including host factors, managemental and environmental context of the region or country.

5.4. Cultural Isolation and Molecular Characterization of Isolates

In the present study, mycobacteriological culture isolation from different lymph node tissues of Borena and Metehara origin camels slaughtered at Akaki abattoir, Central Ethiopia, revealed 36 camels with mycobacteriological culture positivity of 21.4% (36/168). The culture positivity was lower than what have been reported previously in camel by Mamo *et al.,* (2011) and Zeron et al., (2013). The lower culture positivity might be related with the non-optimal condition of the culture for the possibility of the occurrence of non-tuberculous Mycobacteria (NTM) which was assumed to be the major isolates causing pathology in camels as indicated by (Mamo *et al.*, 2011; Zerom *et al.*, 2013). Although the frequency, distribution and severity of gross visible tubercle lesions were predominantly detected in sub-mandibular lymph node and retropharyngeal lymph node, high frequency of culture positivity was recovered from caudal mediastinal lymph node, trachea-broncheal lymph node and the least isolates being recovered from retro-pharyngeal lymph node. Regarding culture positivity of each organs/tissues, the highest culture positivity recorded in the caudal mediastinal lymph node and trachea-broncheal lymph node suggesting the respiratory route could be the main route of infection in camels. Similarly, other authors have reported high culture positivity in lung tissue and thoracic lymph nodes in cattle (Corner, 1994; Ameni *et al*., 2010; O'Reilly and Dabron, 1995). Contrary to this, Mamo *et al.,* (2011) reported high culture positivity in the

retropharyngeal lymph node and mesentric lymph node. Moreover, Kassaye *et al.*, (2013) reported high culture positivity from right cardiac lung lobe (66.67%), cranial mediastenal (20%) and retropharangeal lymph nodes (16.67%). The current finding revealed that Mycobacteria can be isolated from tissues with visible tubercle lesions and also from tissues without evident macroscopic lesions in camels.

It was suggested that majority of TB-like pathology in Ethiopian pastoral camels may be caused by non-MTBC mycobacteria (Mamo *et al.*, 2011; Zerom *et al.*, 2013), and this aligns with Elmossalami *et al.*, (1971) who isolated *M. bovis* and *M. tuberculosis* and non-tuberculous mycobacteria (NTB) such as *M. kansasii* and *M. smegmatis* from tuberculous like lesions in camels at Cairo abattoir, causing similar caseous nodules like those caused by *M. bovis* and *M. tuberculosis* (Elmossalami *et al.*, 1971; Rhodes *et al.*, 2015). In Ethiopia, NTM have been isolated from camel (Mamo *et al.*, 2011; Zerom *et al.*, 2013) and from cattle (Berg *et al.*, 2009) with tuberculosis lesions in different regions of the country which indicates their wider geographic distribution and role as a cause of tuberculosis lesions in livestock of the country.

Genotyping of Mycobacteria from camel lymph node tissue culture isolates based on multiplex PCR analysis revealed 48.8% of the isolates (39/80) were with bands/signals for *Mycobacterium tuberculosis complex* and the remaining isolates were probably Mycobacetria other than tuberculous Mycobacteria. Species differentiation of the 39 acid fast positive isolates using PCR based deletion typing for region of difference-4 (RD4) revealed signals for *M. bovis* with 23.1% (9/39) and *M. tuberculosis* with 76.9% (30/39). The spoligopatterns of the isolates referring to online tools such as the SIT/VIT data base and TB-insight with run TB lineage and Spotclust (SpolBD3) of the *M. tuberculosis* strains revealed only two strains with shared SIT/VIT to the data base while the remaining strains were orphans with no shared SIT/VIT number prior to this report.

Of the 39 *M. tuberculosis* strains identified, only two strains have spoligopatterns registered in the SIT/VIT data base while the remaining 37 strains do not have patterns that match with the patterns registered in the SIT/VIT data base. The strains of *M. tuberculosis* identified were clustered into 29 different spoligopatterns of which about 58.9% (23/39) of the isolates were clustered as a single (unique) strain with specific spoligopattern while the remaining were clustered into six different patterns. It was observed that different lymph node tissue culture isolates of individual camels identified as strains with different spoligopatterns, family, and different lineage that might suggest the probability of the occurrence of diversity of strains and probably mixed infection could occur in camel tuberculosis with different strains of *M. tuberculosis* complex.

The identification of *M. tuberculosis* strains from camels has also been reported by different authors: Gumi *et al.,* (2012) from camels in southern Ethiopia, Zerom *et al.,* (2013) from camels in Eastern Ethiopia, and Elmosalami *et al.,* (1971) from Egypian camels. Moreover, Mamo *et al.,* (2012) identified *M. tuberculosis* from goats at Afar pastoralist area, Mulugeta *et al.,* (2013) from pigs in central Ethiopia and Ameni *et al.,* (2011) identified *M. tuberculosis* from extensively managed cattle in central Ethiopia indicating the possibility of transmission from farmers to their pastured cattle. Majority of the strains 84.6% (33/39) were identified as orphans with no shared SIT in the Spoligotype data base while two strains identified as SIT-1088 and SIT-118 in the data base. *M. tuberculosis* strain with SIT-1088 identified in the present study was also identified in pigs was identified in Ethiopia by Mulugeta *et al.,* (2013). Gumi *et al.,* (2012) reported strain of *M. tuberculosis* with SIT-149 from camels in Southern Ethiopia. Conformal Bayesian Network (CBN) analysis revealed the dominant strains were Euro-American lineage (84.6%), Indo-Oceanic (12.8%) lineage, and one strain as *M. africanum* (2.6%). It was indicated that Euro-American lineage was the most common lineage of *M. tuberculosis* in Ethiopia as indicated (Zufan *et al.,* 2017).

However, Mamo *et al.,* (2011) identified *M. bovis* strains from camels of Ethiopia and similar strains has also been identified by Berg *et al.,* (2009) and Biffa *et al.,* (2010) from cattle in southern pastoralist area of Ethiopia and Oloya *et al.,* (2007) in pastoralist area of Uganda indicating the possibility of interspecies transmission of strains among pastoralist livestock. The isolation of the human adapted *M. tuberculosis* from the camels of pastoralist area could probably be related with the habit of sharing the same dwelling with their pastoralist attendant and camels might have contracted the infection. Camels might be maintaining the infection and can be the source of infection for the pastoralists and warrants further investigation on the transmission dynamics of *M. tuberculosis* complex strains circulating in pastoralist areas and the potential role of camels in the epidemiology of tuberculosis in general.

6. CONCLUSION AND RECOMMENDATIONS

In this cross sectional epidemiological investigation of tuberculosis in camels slaughtered at Akaki abattoir, central Ethiopia, the prevalence of tuberculosis based on single intra-dermal comparative cervical tuberculin testing and gross pathological tubercle lesions was comparable to some other previous studies in different sites by different authors for cattle and small ruminants and very few reports for camels in Ethiopia and many other countries in the world. Statistically significant association was observed between body condition of camels and tuberculosis prevalence where high prevalence recorded in poor body conditioned camels. Extensive gross evident lesion detection was more frequently from sub-mandibular lymph node, retropharyngeal lymph node and trachea-broncheal lymph node; whereas, culture positivity was detected more in caudal mediastinal lymph node and trachea-broncheal lymph node than the other examined lymph nodes. This can assist to elucidate that the main route of entry for Mycobacterial infection in camels is via inhalation. Moreover, the extensive lesion detection and cultural isolation of Mycobacteria suggested that tuberculosis evidently common and prevalent in camels. The overall cultural isolation of mycobacterium from camel lymph node tissues was found to be relatively low from previous reports. Although majority of the culture isolates were identified as *Mycobacterium tuberculosis* complex, the involvement of non-tuberculous Mycobacteria other than *M. tuberculosis* complex was probable. Thus, the involvement of non-tuberculous mycobacteria causing tubercle like lesions should not be excluded and optimized culture conditions should be considered for their investigation in camel tuberculosis.

Although the single intra-dermal comparative cervical tuberculin testing is an OIE recommended test for the diagnosis of tuberculosis in live animals and for the international trade of live animals particularly for camels with recommended cutoff point ≥4mm, the current finding revealed that different cutoff points can be established to the existing local conditions of environmental factors, other host related factors and

the prevalence of the disease in a given area. It is also concluded that the SICCT if lowered from the OIE recommended cutoff value of ≥4mm to ≥3mm would be better informative to discriminate diseased cases for sensitivity and specificity evaluations to detect tuberculosis in pastoralist camels in Ethiopia. The current finding recommended that for the better performance of SICCT in Ethiopia and probably many other camel rearing countries of the world, if the cutoff value for positive test interpretation is lowered from the OIE recommended cutoff value would be of high practical relevance. Moreover, the performance of the SICCT as compared to the detection of gross tubercle lesion was found to be with moderate sensitivity and specificity at the cutoff values ≥3mm and the area under the ROC curve was highly significant (P=0.000) at this cutoff point. In general, the performance of diagnostic tests is often setting dependent. Thus, evaluations of diagnostic tests for a given region are a prerequisite for the implementation of local disease surveillance schemes and control measures.

Cultural isolation and molecular characterization of Mycobacterium from tissues of camels revealed the occurrence of circulating strains of *M. tuberculosis* in camels of Borena and Metehara origin where majority of the strains identified were orphans indicating the probability of recent infection that might be acquired from their pastoralist attendants with TB infection. The Spoligopattern of strains of *M. tuberculosis* identified in this study might also be circulating in pastoralists and camels in different pastoralist areas of Ethiopia and the need for further investigation on the patterns of *M. tuberculosis* complex strains circulating in the pastoralist areas of the country. Moreover, the possibility of the potential role of camels in the epidemiology of *M. tuberculosis* infection in humans in pastoralist area and the potential transmission dynamics, including the need to assess the economic and public health significance in pastoral communities in Ethiopia should be substantiated by further molecular epidemiological investigation of tuberculosis in dromedary camels and pastoralists.

RECOMMENDATIONS

- ✓ Despite a number of drawbacks, the SICCT is still the most frequently used test for ante-mortem diagnosis of bovint TB in Africa and to date, no satisfactory serological tests exist, which would allow the detection of *M. bovis* infection. Thus, the SICCT with recommended cutoff point of ≥3mm should be used as screening test to detect tuberculosis infection in dromedary camels in Ethiopia.
- ✓ Tuberculin skin reaction and the detection of extensive tissue lesions indicative of tuberculosis are not conclusive enough in diagnosing the disease. These should be substantiated by more confirmatory tests like mycobacteriological culture isolation, histopathology, immuno-histochemistry, serological and molecular techniques under appropriate circumstance.
- ✓ The isolation of Mycobacterium from tissues with evident lesions should not exclude the possibility of harvesting the organism from tissues without evident lesions. Therefore, in assessing the performance of diagnostic tests, it should be realized and emphasized that tissue samples should be collected from lesioned and also from animals without evident macroscopic lesions.
- ✓ Identification of strains of *M. tuberculosis* from camels in our investigations can help to elucidate the potential role of camels in the epidemiology of tuberculosis in humans and the possibility of identifying additional strains of *M. tuberculosis* complex from different pastoralist camels in Ethiopia and indicate the need to assess the transmission dynamics of tuberculosis between pastoralists and their camels.
- ✓ Detailed and more discriminatory molecular tools should be used for analysis of isolates from camels and possibly pastoralists, would be important for tracing back the source of infection, assess transmission dynamics of strains of *Mycobacterium tuberculosis* complex circulating in pastoralist area would be helpful for further surveillance and control options.

- ✓ Detailed epidemiological investigations should be conducted in camel rearing pastoral communities of Ethiopia with particular emphasis to the epidemiology, molecular epidemiology of strains of *Mycobacterium tuberculosis* complex circulating in camels and zoonotic significance of camel tuberculosis where the habit of consuming raw camel milk is very common in pastoralist communities.
- ✓ Detailed post mortem examinations during abattoir meat inspection should be conducted to safe guard the public against the threat posed by a number of zoonotic infectious diseases that could possibly by-pass during meat inspection.
- ✓ Camels are the back bone of the majority of pastoral societies of Ethiopia and many other countries where camels are raised. Therefore, the socio-economic importance, the health and production potential of camels should be well assessed.
- ✓ Awareness creation as to the public health significance of the disease particularly in camel rearing areas, where patoralists share the same dwelling and consume raw animal products, should be addressed as there may be possibility of transmission of the disease from camels to human being and vise versa.

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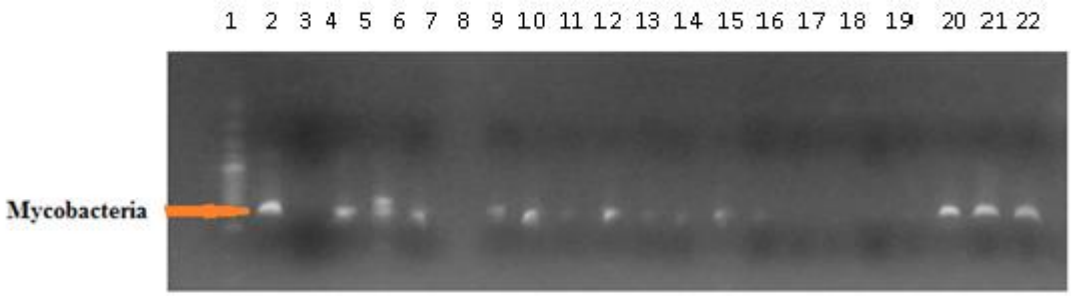
8. APPENDICES

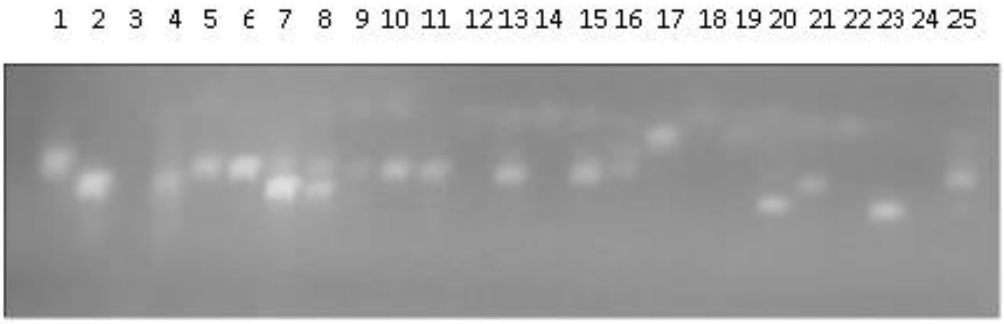
Annex 1: Multiplex PCR

Gel electrophoresis separation of the PCR products of the multiplex PCR analysis of tissue culture isolates of camels slaughtered at Akaki abattoir. Note: lane 1: 100bp DNA ladder, lane 2: M. tuberculosis complex (positive control), lane 3: Qiagen H2O (negative Control), lane 4 to 22: sample isolates. Lane 9, 11 and 14, and lane 20-22 (positive samples with signals for M. tuberculosis complex) and lane 7, 15-19 (negative samples, no signals).



Annex 2: Region of difference-4 (RD4) typing of tissue isolates of camels

Gel electrophoresis separation of PCR products for the Region of Difference (RD4 typing) of Mycobacterium tuberculosis complex isolates to species level. N.B: Lane 1: M. tuberculosis H37Rv (positive control), lane 2: M. bovis (positive control), lane 3: Qiagen H_2O (negative control), lane 4 to 25: (sample isolates). Lane 5, 6, 10, 11, 13,15, 21 and 25 with signal bands for M. tuberculosis H37Rv; lane 4, 7, 8, 20, and 23 with signals for M. bovis.

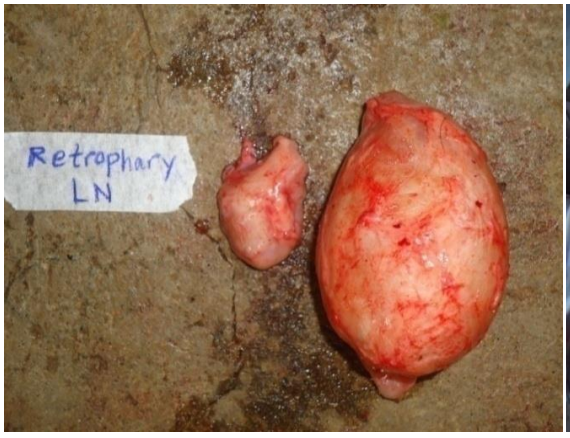


Isolates identified with signals as *M. bovis* were from caudal mediastinal lymph node (CaMLN) where lane 5, 8, and 9 correspond to samples 120, 127, 175, respectively. Whereas, sample isolates confirmed as *M. tuberculosis* with lanes 6, 7, 11, and 12 comprised mainly of cranial and caudal mediastinal lymph nodes including trachea-broncheal, retropharyngeal and one isolate from sub-mandibular and parotid lymph nodes.

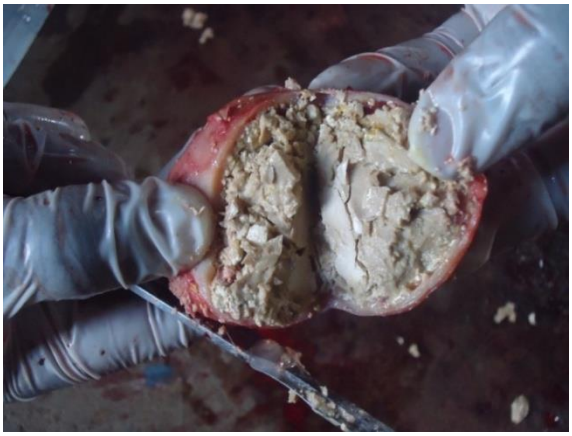
Annex 3: Individual camel lymph node tissue isolates identified as strains with different spoligopattern, and different lineage of *M. tuberculosis* strains which could suggest the possibility of mixed infection

No.	Camel ID	Spoligopattern	SIT	Family	Lineage
1.	P147CRMLN	777767777777771	orphan	Family33	Indo-Oceanic
	P147RPLN	777767777762771	orphan	Family33	Euro-American
	P147CAMLN	577767777756771	orphan	Family33	Indo-Oceanic
	P147CRMLN	555347777742661	orphan	Family33	Euro-American
2.	P155PLN	777767777742661	orphan	Family33	Euro-American
	P155CRMLN	577767777756771	orphan	Family33	Indo-Oceanic
	P155CAMLN	555347477742661	orphan	Family33	Euro-American
	P155SMLN	557347477742661	orphan	Family33	Euro-American

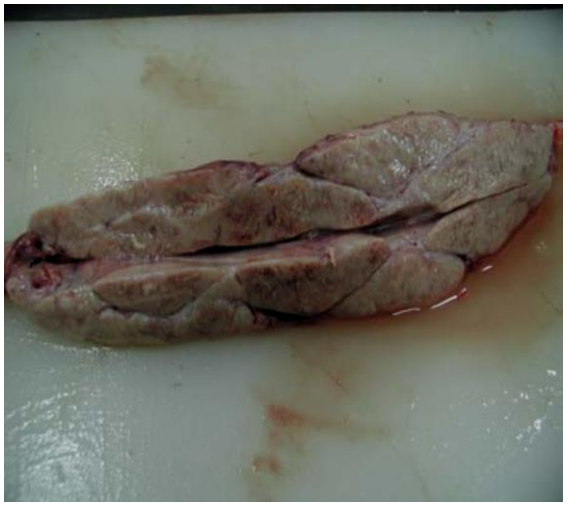
Annex 4: Pictures Taken during the study at Akaki abattoir



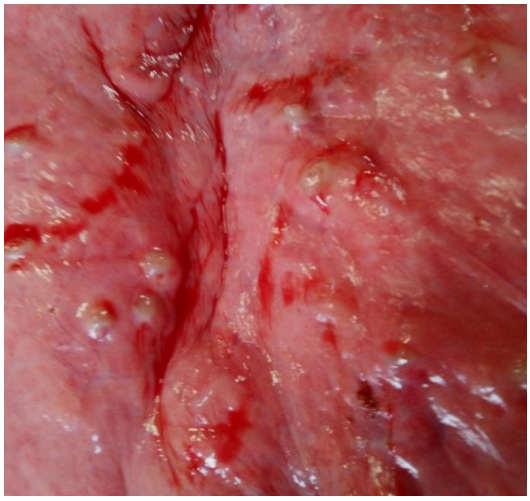
Enlarged retro-pharyngeal lymph node



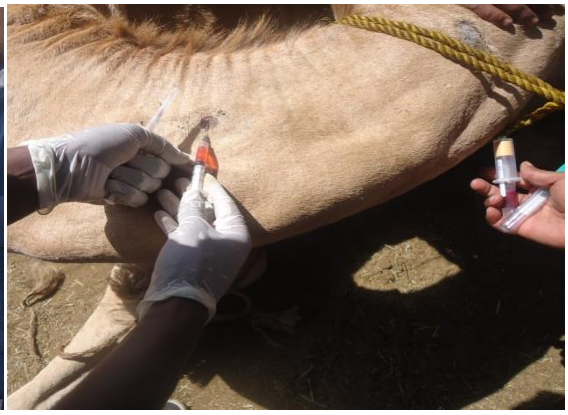
Greyish mass of calcified in lymph node



Swollen and firm sub-mandibular lymph in node with greyish discoloration



A circumscribed caseous nodular masses tuberculosis suspected camel lung

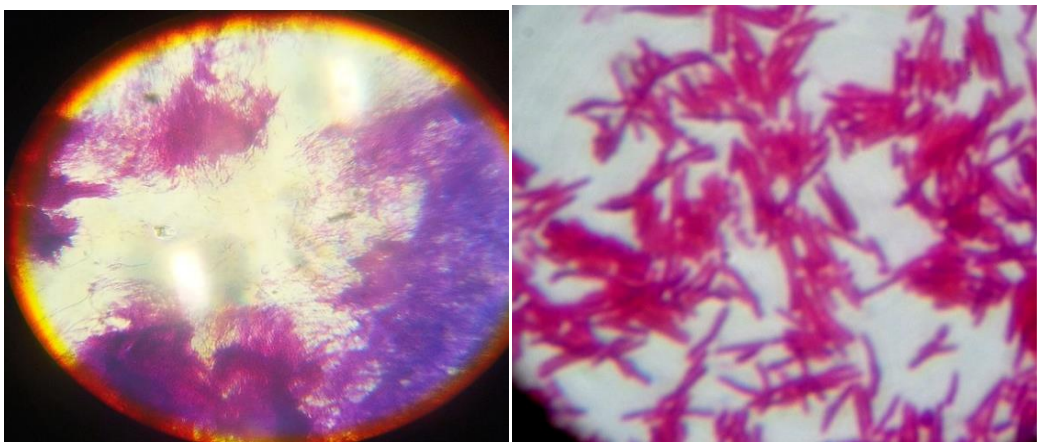


While restraining, measuring skin fold and injecting tuberculin

Camels in the holding pen of camel slaughter house at Akaki abattoir, some with branding and poor body condition

Annex 5: Colonial growth of Mycobacterium on Lowenstein Jenson media

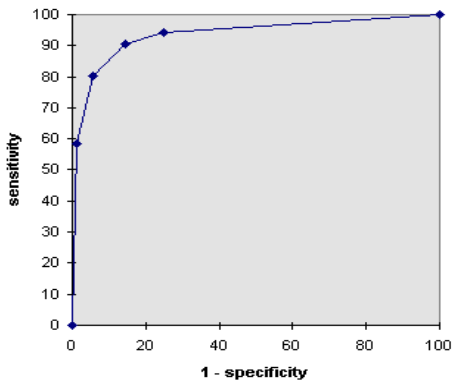
Growth of Mycobacteria on Lowestein Jenson media



Long, rod and pinkish clumped of acid-fast bacilli under microscope

Annex 6: Interpretation of Area under the curve of ROC analysis

Receiver-operating characteristic (ROC) curves are an excellent way to compare diagnostic tests. They were initially developed in World War II, by the British. They were building the "Chain Home" series of radar detectors to identify incoming German planes. But the radar detectors would also detect flocks of birds and other "false positive" signals. So, when we use the term Receiver Operating Characteristic (ROC) curve, the receiver we were originally talking about was radar receiver in World War II.



That's an ROC curve! It simply graphs sensitivity on the y-axis against 1 - specificity on the x-axis. (Note: we say "1-specificity" but in reality it is also "100% - specificity". As noted earlier, a specificity of 0.3 equals a specificity of 30%). The area under the ROC curve (AUROCC) is a reflection of how good the test is at distinguishing (or "discriminating") between patients with and without a disease.

The best possible test (100% sensitive and 100% specific) would have an area under the curve of 1.0 since the entire graph would fall beneath the curve. While it is possible that the "perfectly bad test" would have an area of 0.0, in reality we would just redefine what is positive and negative and it would become a perfect test. Thus, the AUC-ROC generally has a range of 0.5 to 1.0, with the following general interpretation:

AUC	Interpretation
1.0	Perfect test
0.9 to 0.99	Excellent test
0.8 to 0.89	Good test
0.7 to 0.79	Fair test
0.51 to 0.69	Poor test
0.5	Worthless test

If you have two tests, you can compare the AUROCC of the tests to determine, which is better overall at distinguishing patients with and without disease.

Annex 7: preparation of Lowenstein-jensen media

Ingredients

1. Mineral salt solution	
Potassium dehydrogen phosphate	**2.4gm**
Magnesium sulphate	**0.24gm**
Magnesium citrate	**0.6gm**
Asparagine	**3.6gm**
Glycerol	**3.6g4ml**
Pyruvate	**12gm**
Distilled water	**600ml**
2. Malachite green solution (2%)=5.1ml	
Malachite green dye	**2gm**
Sterile distilled water	**100ml**
3. Homogenized whole egg.	
Fresh hen's eggs not more than 7 days.	

Preparation

- The first 5 reagents were dissolved in 550ml distilled water and 12ml glycerin and then autoclaved at 85^0c for 25 min.
- Cool the solution to 30 ^{0}c.
- Add 16gm of ferric ammonium citrate (in a sterile solution of 50ml) autoclaved separately.
- Add 625ml of whole egg.
- Filter egg homogenate through sterile surgical gauze to remove clumps then add to warm medium with thorough mixing.
- Fill 10ml of medium in to glass tubes and allow solidifying at an angle such that the surface of the medium extended three-fourth ups the tube.
- The tubes were incubated at 85^0c for 30 min then stored upright at 4^0c for 1 month (max)

Annex 8: Ziehlneelsen staining method

1. Carbolfuchsin	
Basic fuchsin	0.3g
Ethanol (95%)	10ml
Phenol	5g
Distilled water	95ml
2. Acid alcohol	
C_2h_5oh (95%)	97ml
Concentrated HCL	3ml
3. Counterstain	
Methylene blue	1g
Distilled water	100ml
4. Alkali tap water	

- Make a smear of on slide and fix it over the flame methanol fixation.
- Flood the smear with carbolfuchsin and heat from the below till steam comes out.
- Allow the hot carbolfuchsin to act for 3 to 5 min. Do not boil the stain or allow it to dry on the slide.
- Wash the slide with tap water.
- Decolorize with acid alcohol for about 15-20 seconds until the bacterial smear appears faint pink or colour less. Wash it with tap water.
- Counter stain with methylene blue for about 30 seconds.
- Wash with tap water, blot dry the slide.
- Examine the slide under microscope with oil immersion objective and make a drawing of a field under microscope
- Acid fast bacteria will take pink / red colour while non acid fast stain blue

Annex 9: Procedure of culturing inoculums

- The inoculum is distributed evenly over the surface of the slants.
- The tubes are allowed to remain in a slanted position at 37°C for approximately 1 week with screw caps loose.
- The tubes are returned to a vertical position when the free moisture has evaporated from the slants.
- The lids are tightened and the tubes are placed in baskets in an incubator at 37°C.
- The slants are incubated for at least 6 months and observed weekly for the presence of any growth from the sixth week onwards.
- Cultures will be considered positive based on the colony characteristics and presence of acid- fast bacilli in the smear.

Annex 10: General facts relevant with camel's water condition

Camels are capable of losing safely 30% of their body's weight in water, which would kill any other animal. They can do this because:

~ Fluid levels in their circulatory system are maintained by taking fluid from the body's tissues.

~ The hydrophilicity (attracts water) of a camel's hemoglobins means its red blood Cells. Resist dehydration.

~ Red blood cells (erythrocytes) are normally circular, except in the family *Camelidae*, where they are oval. These small oval erythrocytes can circulate more easily at increased blood viscosities.

~ Camel blood contains 94% water, just like humans. But during dehydration, their blood can lose up to 40% of its water safely. According to doctors, human blood must stay very close to 94% water. If it loses 5% of its water, you go blind; at 10%, you can't hear and go insane; 12%, and your blood is as thick as molasses. Unable to circulate blood, you're dead.

In effect, these biomechanisms for coping with dehydration, allow camels to use water in their body tissue as storage capacity, because the water can be used without any damage to the camel's health.