POTENCY OF CONVENTIONAL COMPARATIVE INTRADERMAL BOVINE TUBERCULOSIS TEST AND ITS EFFICIENCY OVER INTERFERON GAMMA TEST IN MIXED HERD GOATS

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN VETERINARY PUBLIC HEALTH AND FOOD SAFETY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2015

ABSTRACT

Diagnosis of bovine tuberculosis (BTB) in asymptomatic goats is a big challenge towards control of the disease in mixed herds. This is because not all of the available diagnostic methods in cattle are easily applicable and capable of detecting the disease in goats. In this study, two BTB diagnostic tests commonly used in cattle were employed to diagnose BTB in goats. The study established the potency and effiency of conventional comparative intraderma test (CIT) over interferon (IFN)-γ test. Goats (n= 71) and 10 cattle used in this study, were from mixed herds randomly selected from urban and periurban of Morogoro municipality. Blood sample for IFN-γ test was collected from each animal, which was immediately followed by intradermal injection of Bovine Purified Protein Derivative (PPD-Bovine) and Avian Purified Protein Derivative (PPD-Avian) 12 cm apart after proper shaving of middle third of the neck on right side. The blood samples were collected from jugular vein and investigated at the Sokoine University of Agriculture (SUA) laboratory. After 72 hours, CIT results were obtained by re-measure of the same skin fold. Results showed that CIT isolated 5 reactors while IFN-γ isolated 9 reactors from the sample size. Comparative intradermal test results proved to have no significant better potency and efficiency over the IFN-γ test ($P<0.05$; 95% confidence interval). Results from both tests were confirmed with pathological lesions observed in two sacrified goats. It is concluded that CIT has no peculiar potency and efficiency in diagnosis of BTB in asymptomatic goats. Therefore, IFN-γ test could be a sound option in diagnosis of BTB in carrier asymptomatic goats in a mixed herds and thus aid in a long run, the control of the disease.

DECLARATION

I, Henson Kainga, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and have neither been nor concurrently submitted for a higher degree award in any other institution.

....

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The above declaration is confirmed

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Prof. Joshua J. Malago **Date**

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ACKNOWLEDGEMENTS

This study was fully supported by the Malawi Government through Southren Africa Development Community –Transboundary Animal Diseases (SADC-TADs) Project, which is highly appreciated.

My sincere thanks go to Prof. J. J. Malago, my supervisor and Head of Veterinary Pathology Department for his invaluable time, expertise, scientific ideas and constructive critics all the way from research proposal development to the end of the study.

My gratitudes go to Prof. I. Kimera, cordinator of my studies and Head of Veterinary Medicine and Public Health Department. More thanks also goes to Prof. R. R. Kazwala, Dean of Faculty of Veterinary Medicine, Prof. L.S.B. Mellau and Prof. D.M. Kambarage for sharing with me their technical, experience and expertise in the field of bovine tuberculosis.

I owe a debt of gratitude to farmers within Morogoro municipality for allowing me to work on their animals. More thanks to Masaai Bomas located at Melela, Mlandizi and LITA Morogoro campus, for their wonderful cooperation. I extend my gratitudes to Dr. E. Mwakijungu, Dr. R. Samson, Dr. G. C. Kamwendo, Mr. Z. Mabagala, Mr. J. Mlay and Mr. J. Malakalinga for their technical support through out the study period.

Last but not least, I thank God for His wonderful mercies during this study. May all Glory and Honor be unto Him. Thanks to Dr. B. Chimera and Dr. P. Chikungwa, the Directorate of Veterinary Service in Malawi, for the Scholarship award.

DEDICATION

This work is dedicated to my beloved wife Carolyn Chisomo, my dad Wilson Belon Kainga and my late mother, Margret Hellen Ofisi Kainga.

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LIST OF ABBREVIATIONS AND SYMBOLS

"	Seconds
%	Percentage
˚ C	Degrees Celcius
<	Less than
≥	Greater than or equal to
AIDS	Acquired Immune Deficiency Syndrome
APC	Antigen Presenting Cells
BTB	Bovine Tuberculosis
CCTT	Comparative Cervical Tuberculin Test
CFTT	Caudal Fold Tuberculin Test
CFU	Colon Forming Unit
CIT	Comperative Intradermal Test
Cm	Centimeter
CMI	Cell Mediated Immune
E.g.	For example
HIV	Human Immuno-deficiency Virus
Hrs	Hours
I.e	it is
IFN-γ	Interferon gamma
IL-2	Interleukin 2
ml	Milliliter
mm	Millimeter
M. avium	*Mycobacterium avium*
M. bovis	*Mycobacterium bovis*

NGO	Non Governmental Organisation
^{o}	Degrees
OIE	Office International des Epizooties
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PPD-Avian	Avian Purified Protein Derivative
PPD-Bovine	Bovine Purified Protein Derivative
PZA	Pyrazinamide
SADC	Southren Africa Development Community
SUA	Sokoine University of Agriculture
TADs	Transboundary Animal Diseases
Th	Helper T cell

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Bovine Tuberculosis

Bovine tuberculosis (BTB) is a disease caused by the bacterium Mycobacterium bovis *(M. bovis)*. It can infect most mammalian species although bovids (cattle and buffalo) and especially cattle are the main hosts. *Mycobacterium bovis* is a slow growing, facultative intracellular, aerobic and Gram-positive bacterium with a dysgonic colony shape when cultured on Löwenstein-Jensen medium (Müller, 2010). Because of being slow to develop clinical signs or lesions, tuberculosis is an important disease in many countries including Malawi and Tanzania (Müller, 2010).

Bovine tuberculosis is one of the infectious diseases in cattle and goats. Infection with the *Mycobacteria bovis* in goats can lead to localized lesions that may persist for a number of months causing poor body condition and loss of production (Ozturk *et al*., 2010). In most areas of Malawi and Tanzania farmers manage cattle and goats together. It is uncommon to find most communal grazing grounds populated by cattle and goats forming what are called mixed herds. Mixed herds are becoming points of interest when focusing on diseases with reasonably long incubation period like BTB.

The infection causes a disease that develops slowly in goats but may be the cause for disease establishment and spread in a mixed herd such that, goats can play a role of perpetual reservoir of the disease within and between herds (RSA Manual, 2013). Napp *et al*. (2013) provided evidence of goats being domestic reservoirs for BTB in a mixed herd. Since goat rearing is a main source of meat and milk for poor people where by goat is popularly known as “poor man's cow” (Pandey *et al*., 2013), control of tuberculosis in

goats is not only pertinent to rural livelihood but also to the control of BTB among animals and humans.

1.2 Mycobacteria Infection and Immune Interaction Process

Following infection, mycobacteria can spread throughout the body where the host immune system macrophages attempt to kill them by phago-lysosome fusion and acidification (Müller, 2010). Phagocytosis of particulate antigens serves as an initial activating stimulus. However, macrophage activity is further enhanced by cytokines secreted by activated helper T (Th) cells, by mediators of the inflammatory response, and by components of bacterial cell walls. One of the most potent activators of macrophages is interferon gamma (IFN-γ) secreted by activated Th cells. The most important antigen presenting cells (APCs) in tuberculosis infection are probably dendritic cells, which also play a major role in modulating the host immune response (Cosivi *et al.,* 1998). The secretion of cytokines and mycobacterial antigen presentation on the surface of dendritic cells help to trigger an adaptive cell mediated immune (CMI) response (Müller, 2010; Ozturk *et al*., 2010; Marassi *et al*., 2013). This CMI response is commonly known as the Th immune response and characterized by secretion of high levels of IFN-γ and interleukin 2 (IL-2) by Th1 (Müller, 2010). The production of cytokines by Th1 can activate macrophages in order to become highly microbiocidial, but when the macrophages are not activated in proportion to the rapidly multiplying bacteria, the disease can be overwhelming for the immune system (Lambert *et al*., 2006; EFSA Scientific Opinion, 2012).

Bovine tuberculosis has proved to be a universal disease to other species like, human, pig and goat and these species are most susceptible (Schwan, 2009; Rahman *et al*., 2013). Since goats are considered to be disease reservoirs, Wood and Jones (2001) suggested the

use of Interferon gamma (IFN-γ) test as a primary diagnostic test when implementing test and slaughter policy in goats.

This is a welcomed idea if there is enough information on efficiency of the test when conducted in goats, bearing in mind that goats show certain degree of resistance to BTB (EFSA Scientific Opinion, 2012; Rahman *et al*., 2013).

1.3 Immune-based *Mycobacteria S*pecies Diagnostic Methods

Currently, there are no conventional diagnostic methods for the disease in goat species. The diagnosis methods of the disease is well developed in cattle. However, the ability to identify infected animal is an essential first step in the campaign to control the disease in goats. There are two immune based diagnostic tests namely Comparative Intradermal Test (CIT), and IFN-γ test that are used in cattle and both have potential to do well in goats (Marassi *et al*., 2013). The CIT with bovine and avian tuberculin is used mainly to differentiate between animals infected with *M. bovis* and those sensitised to tuberculin due to exposure to other mycobacteria or related genera. The decision to use single or comparative test generally depends on the prevalence of tuberculosis infection and on the level of environmental exposure to the other sensitising organisms **(**El-Mahrouk and El-Balawy, 2010). If a single skin test is performed, the reactions in sheep and goat, infected with *M. avium*, can be interpreted as false positive or para-allergic. In the CIT, reactions to avian tuberculin are usually stronger than the reactions to bovine tuberculin. False-negative results, particularly in the case of anergic goats, allow infected animals to remain within the herd and to compromise the success of the disease control program. The IFN-γ assay is relatively fast and easy test to perform and has the sensitivity to identify animals in early stages of the disease. The sensitivity of CIT is known to be 65.6% in cattle. However, this performance depends on prevalence of tuberculosis infection and on the

level of environmental exposure to the other sensitising organisms (El-Mahrouk and El-Balawy, 2010).

There are two approaches of conducting CIT in goats, namely Caudal fold tuberculin (CFTT) test which is less prefered and Comparative cervical tuberculin (CCTT) test. Comparative cervical tuberculin test is a confirmatory skin test to determine if a responder's positive CFTT is most likely due to *M. bovis* or *M. avium*. The procedure involves shaving of two sites in the middle third of the neck on one side 12 cm apart, one above the other. At each site, a fold of skin is measured using a caliper and the measurement recorded in millimeters. Bovine Purified Protein Derivative (PPD-Bovine) 0.1 ml and Avian Purified Protein Derivative (PPD-Avian) 0.2 ml is injected intradermally. The upper site is used for the PPD-Avian and the lower site for the PPD-Bovine. The skin thickness is measured at 72 hours post injection and the differences in skin thickness is recorded. The differences in pre and post test skin thickness determine the test results. Results are interpreted according to the recommendations of the Office International des Epizooties (OIE), (Council Directive 64/ 432/EEC): at ≥ 4 mm cutoff for cattle and also at ≥ 2 mm cutoff for goats . Thus, at cutoff ≥ 4 mm, if the increase in skin thickness at the injection site for PPD-Bovine is greater than the increase in skin thickness at the injection site for PPD-Avian and PPD-Bovine minus PPD-Avian is less than 2 mm, between 2 and 4 mm, or 4 mm and above, the animal is classified as negative, doubtful, or positive reactor based on CIT, respectively. At cutoff ≥ 2 mm, if the difference between PPD-Bovine and PPD-Avian is greater or equal to 2 mm, the animal is considered as positive, while if the difference is less than 2 mm, the animal is considered negative. When the change in skin thickness is greater at PPD-Avian injection site, the animal is considered positive for *Mycobacteria* species other than *M. tuberculosis* complex. A flock

(herd) is considered as positive if it has at least one tuberculin reactor animal (Whipple *et al*., 2001; López-Sánchez *et al*., 2006).

In this study, CIT principles were borrowed from cattle and employed in goats to evaluate its potency. Its perfomance efficiency when employed in BTB asymptomatic goats. Thereafter, its potency and perfomance efficiency was compared to that of modern known IFN-γ test which is getting established in early diagnosis of BTB in cattle. It is now speculated that the IFN-γ test detects animals that escape conventional CIT testing, probably because it can detect animals earlier after infection than CIT testing (Wood and Jones, 2001; Whipple *et al*., 2001; Lambert *et al*., 2006). However, direct comparisons of the two tests in the same study have not been made in goats. This will increase the overall diagnostic sensitivity to detect infected animals and will thus have a major impact on disease control. The knowledge developed has immediate application in most developing countries including Malawi and Tanzania where Non Governmental Organisations (NGOs) are distributing unscreened BTB goats to people living with HIV/AIDS as well as other vulnerable households. The efficient diagnostic test is recommended to be used for screening the goats before distribution.

1.4 Problem Statement and Justification

Mycobacterium bovis is a zoonotic organism and should be treated as a risk/hazard group III organism with appropriate precautions to prevent human infection occurring (OIE, 2009). The major challenge with the disease in all species is CIT lacks early diagnostic ability, the problem is more worse in goat flocks because of increased rate of unreliable test kits/tools. This is becoming a BTB screen and cull challenge realising that most community goat flocks are managed together with cattle in communal grazing grounds where they form mixed herds. It is interesting to note that the mixed herds are comprised

of BTB susceptible species (cattle and goats) but of different degree of resistance to the disease causing agent. Existence of mixed herd denotes continuous harboring and build-up of BTB infection. However, the agent can be transmitted through consumption of contaminated milk, meat and close contact with infected cattle. The bacteria is naturally resistant to pyrazinamide (PZA), a first line anti-tuberculosis drug in humans; making Goverments and Organisations to spend much money on medication (WHO, 2014). It has a very devastating effect on people when one considers the epidemic of HIV/AIDS in Malawi and Tanzania (Cadmus *et al*., 2004). Bovine tuberculosis is primarily of economic importance as it has a direct effect on milk, meat and animal reproduction (WHO, 2014). Moreover, national and international trade and other economic sectors are indirectly affected by the disease (Silaigwana *et al.,* 2012). To tackle this, diagnosis of the disease at its early stage is pertinent to curb *Mycobacterium bovis* spread to other animals. However, there is no test approved for goats despite the fact that goats rearing industry has been in place from time immemorial. Worse more, goats are often reared mixed with cattle that are usually diagnosed with BTB, creating a potential source of transmission for the disease. The knowledge gained is of immediate importance to different stakeholders. Farms that have both goats and cattle should implement the knowledge gained to minimise BTB cases and promote BTB free farms. For policy makers and extension workers, the knowledge should be used in minimising the BTB risk factors especially where there is practice of communal grazing of goats and cattle. The knowledge should give guidance to NGOs that are distributing goats to vulnerable households. Some of the beneficiaries are people living with HIV/AIDS. Therefore, the study is an eye opener to all stakeholders involved in promoting better human health as well as enhancing social-economic of poor communities through keeping goats.

1.5 Objectives

1.5.1 General objective

The study had a general objective stated below;

To explore the potency and efficiency of conventional comperative intradermal test over interferon-gamma BTB test in goats.

1.5.2 Specific objectives

The study had four specific objectives as stated below;

i. To determine the performance of conventional comparative intradermal BTB test in mixed herd goat flocks.
ii. To assess the performance of interferon-gamma BTB test in mixed herd goat flocks.
iii. To evaluate the performance of conventional comparative intradermal BTB test over Interferon-gamma BTB test in mixed herd goat flocks.
iv. To determine and compare location-dependent BTB prevalence in mixed herd goat flocks within Morogoro Municipality i.e. Milela, Mlandizi, Bigwa, Magadu, Folkland, Mazimbu and Kihonda.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Definition and Aetiology of Bovine Tuberculosis

Bovine tuberculosis (BTB) is a chronic bacterial disease of animals and humans caused by *Mycobacterium bovis*. In a large number of countries BTB is a major infectious disease among cattle, other domesticated animals, and certain wildlife populations. Transmission to humans constitutes a public health problem (OIE, 2009; Schwan, 2009).

2.2 Sub-Saharan Bovine Tuberculosis Documentaries for Goats

In Ethiopia and Nigeria, there has been BTB diagnosis exercise in both goats and cattle using CIT (Schwan, 2009; Tschopp *et al*., 2011). In most sub-Saharan Africa, BTB control measures are not applied (OIE, 2007 in Boukary *et al.,* 2012). In addition, BTB in goats has hardly been studied in the sub-Saharan context where epidemiologic aspects of the disease remain largely unknown (Cosivi *et al*., 1998). In Malawi and Tanzania, there are less deliberate efforts to diagnose BTB in goats due to high slaughter rate of goats and labour demands of CIT such that control of BTB is limited to meat inspections in abattoirs (Müller, 2010). Furthermore, there are very complex interactions between the rural pastoral livestock systems and the semi-intensive system practised in and around urban settings, that make the diagnosis to be tedious and labour demanding (Cosivi *et al*., 1998).

In developing countries particularly in low income group, BTB is still prevalent and is responsible for significant economical loss in animal production through reduced milk yields and low reproductive performance. In these areas, consumption of unpasteurized milk is a regular practice due to lack of pasteurization of milk and tradition and culture of consuming raw milk and other dairy preparations (Pandey *et al*., 2013; Müller, 2010).

Katale *et al*. (2012) worried of high incidences of vending of unprocesed milk of both goats and cattle along the streets of big cities of Tanzania which is a conduct happening contrary to the strict BTB control measures directed by the country. Reports developed by researchers (Kazwala *et al*., 2001*a*, 2001*b* and 2006; Katale *et al*., 2012) elaborated on the public health dangers that arise from vending of raw food products obtained from suspected BTB infected livestocks. In Tanzania, there is increased in goat meat and milk vending locally without cooling facilities and packed in hygienically poor bottles and pails (Katale *et al*., 2012). Office International des Epizooties (2009) recognised that in cattle, clinical evidence of tuberculosis is usually lacking until very extensive lesions have developed.

2.3 Bovine Tuberculosis Diagnostic Methods in Malawi and Tanzania

Current diagnostic methods of BTB include *in vivo* tests like CIT, *in vitro* cellular test based on quantification of IFN-γ, post mortem diagnosis of macroscopic lesions of tuberculosis, microscopic examination of tuberculosis lesion, culture, biochemical characterization and molecular methods (Surujballi *et al*., 2009). In Malawi and Tanzania, BTB diagosis is mostly done in slaughterhouses, mainly based on the screening of typical tuberculous macroscopic lesions of the organs rather than on Ziehl-Neelsen stained smears, culture, biochemical and molecular methods (Katale *et al*., 2012). Polymerase chain reaction (PCR) methods and bacterial culture are specific tests that can only be performed post mortemly, and may present variable results. Under these conditions, they are more useful as confirmatory tools than primary diagnostic tests for the epidemiologic control of outbreaks (Kazwala *et al*., 2001b).

The antemortem test currently used for diagnosis of BTB in both countries is the tuberculin skin test (Kazwala *et al*., 2001*a*, 2001*b* and 2006; Katale *et al*., 2012). In its

various formats i.e. the single intradermal test and the comparative cervical test, both used in ruminants, the test has served as an international standard for BTB diagnosis for a long time (Wood and Jones, 2001; Surujballi *et al*., 2009). Tuberculin, a concentrated sterile culture filtrate of tubercle bacilli grown on glycerinated beef broth and, more recently, on synthetic media, provides a means of detecting the disease (Napp *et al.,* 2013). However, notwithstanding its long history and international acceptance, the skin test has problems. The other problem associated with CIT is lack of PPD specificity; tuberculin Purified Protein Derivatives (PPD) prepared from *M. bovis* displays cross-reactivity with other *Mycobacterium* species, which adversely affects the specificity and sensitivity of skin tests bearing in mind that, infections in cattle may not be detected for the first 3 weeks (Surujballi *et al*., 2009; EFSA Scientific Opinion, 2012). The last problem of CIT is the mode of the administration of the test and the subjective nature of the result's assessment that necessitate considerable technical expertise and experience. Furthermore, the need to score the results after 3 days can pose safety hazards and be impractical in the case of wild animals (Cosivi *et al.,* 1998; McGeary, 2008; Müller, 2010).

2.4 Similarities and Differences of Comparative Intradermal Test and Interferon Gamma Test in Cattle

The ability to identify infected animals is an essential first step in the campaign to control BTB. The efficiency of the test greatly increases the ability of preventing the disease spread within the herd and contributes to appropriate safeguards (Cosivi *et al.,* 1998). Both BTB tests, skin test (*in vivo*) and the IFN-γ assay (*in vitro*) measure cellular response to infection. Therefore, depending on the particular stage of the disease, particularly during anergy, each of these tests may be negative since this response may no longer be detectable (Wood and Jones, 2001; Whipple *et al*., 2001; Lambert *et al*., 2006). The sensitivity and specificity of IFN-γ assay have been shown to be higher than conventional

CIT in several studies (López-Sánchez *et al*., 2006; Surujballi *et al*., 2009; Lambert *et al*., 2014). While BTB could be diagnosed by CIT in 3-6 weeks post infection, IFN-γ assay detects the infection as early as 14 days post infection (El-Mahrouk and El-Balawy, 2010). The IFN-γ test provides results within 24 hours after collection of blood and also removes the operators' errors. On the contrary, CIT requires at least 72 hours to have the results. Further, CIT accomodates Lambert *et al*. (2006) operators's error more than IFN-γ test. The IFN-γ assay is easy to perform as it does not require farm visits to read the test results (Whipple *et al*., 2001). High cost of kits and incubation of heparinised blood with antigen within a few hours of collection are the disadvantages of IFN-γ assay. The current study focused on the two tests in goats managed in mixed herds of goats and cattle. Similar tests were conducted in cattle for reference of the herd`s BTB status (Ozturk *et al*., 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 The Study Area

The study was conducted in different urban and periurban areas of Morogoro municipality in Morogoro region. Morogoro region lies between latitude 5^{o} 58" and 10^{o} 0" to the south of the equator and between longitude 35^{o} 25" and 35^{o} 30" to the east. As recommended, samples collected from animals for IFN-γ assay were processed within 16 hours from the time of collection. This made Morogoro a suitable location for the study since it is near Sokoine University of Agriculture (SUA) laboratories where analysis was done.

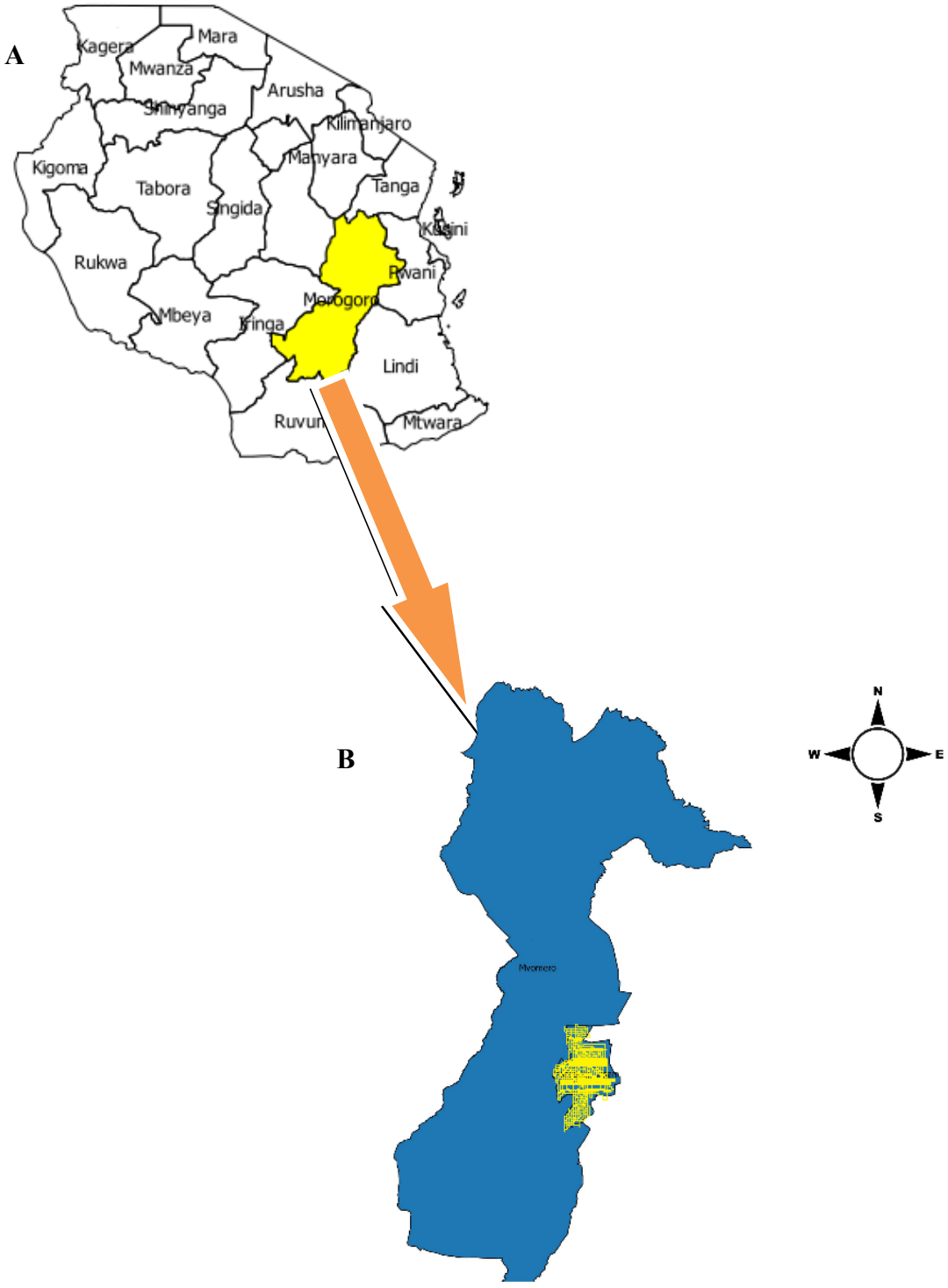

Figure 1: Tanzania map showing Morogoro region

Legend: A = Tanzania map showing Morogoro region in yellow;
B = Morogoro urban (yellow) and Mvomero district (blue) withdrawn from Morogoro region's map

Source: Drawn with QGIS software 2.6.1 using the Tanzania Shape file 2012

3.2 Research Design

The study employed cross-sectional research design where data was collected from goats and cattle by conducting CIT. Prior to that, blood samples were collected from jugular vein, carried in cooler boxes and transported to Tuberculosis laboratory (Faculty of Veterinary Medicine, SUA, Morogoro) for IFN-γ BTB test.

3.2 Sample Size and Sampling Methods

Data in this study was collected from 71 goats and 10 cattle that were reached at by applying formula for unknown population $n = Z^2SD^2/e^2$, (Kothari, 2004). Multistage sampling methods were used in sampling the area to be surveyed. A purposive sampling in selecting mixed herds production of goats and cattle was employed (Appendix I). Randomly, 2 asymptomatic positive goats cases were sacrifised to confirm the results of CIT and IFN-γ test by recording pathological lesions observed. Tissues of interest were lungs and pulmonary lymph nodes (cranial, tracheobronchial and mediastinal).

3.3 Methodology for Data Collection

The CIT was performed on the mid-neck of right side (Appendix II). As for the methodology for IFN-γ test, the assay is based on the release of IFN-γ from sensitised lymphocytes during a 16–24-hour incubation period with specific tuberculin antigens (PPD) (Appendix III). The procedure started with blood collection from the animals followed by CIT (Plate 1 and 2). The collected blood was processed in laboratory (Plate 3).

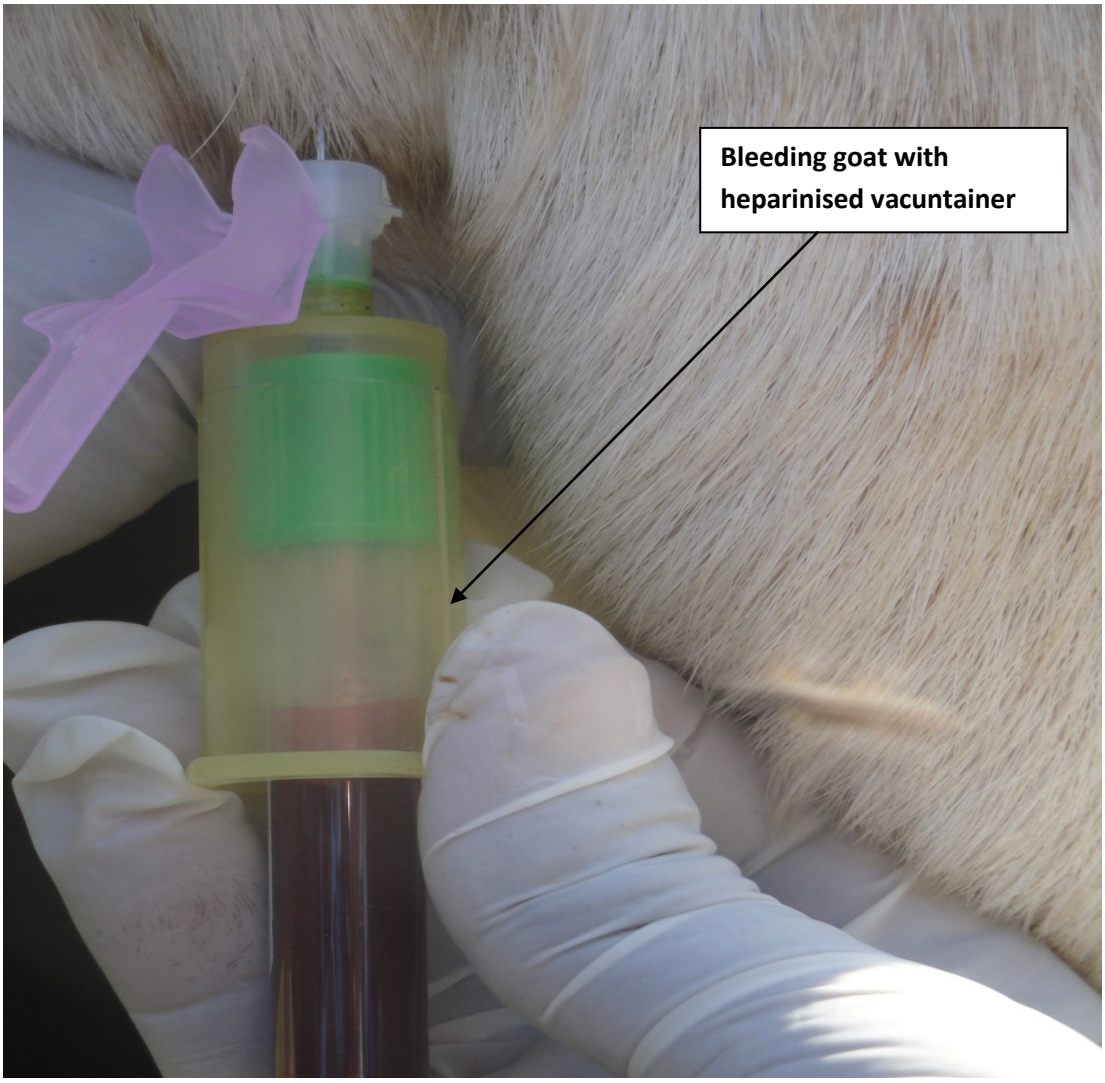

Plate 1: Blood collection from jugular vein using heparinized vacuntainer

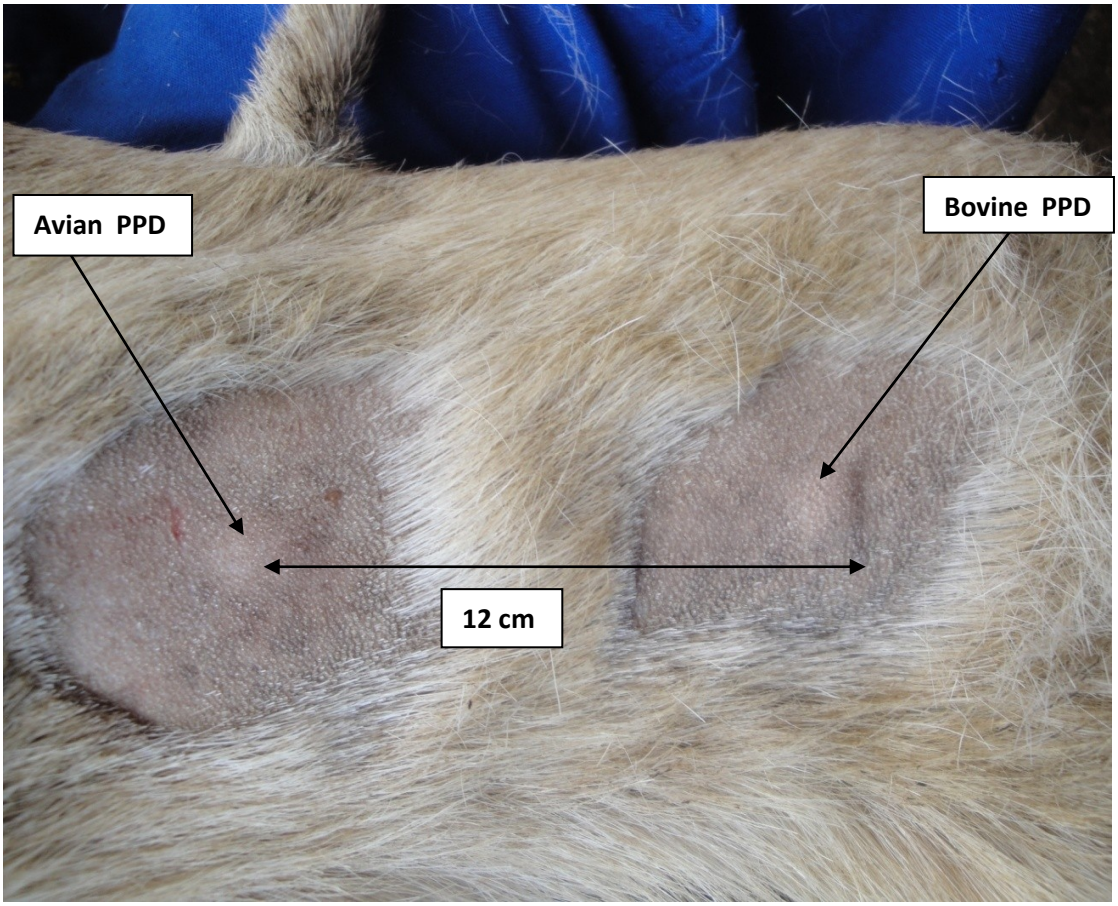

Plate 2: Comparative intradermal test on two skin site in a goat

Middle third part of the neck of a goat. Anterior shaved and swollen part of Avian Purified Protein Derivative (Avian PPD) and posterior shaved and swollen part of Bovine Purified Protein Derivative (Bovine PPD). The PPDs were deposited at 12 cm apart.

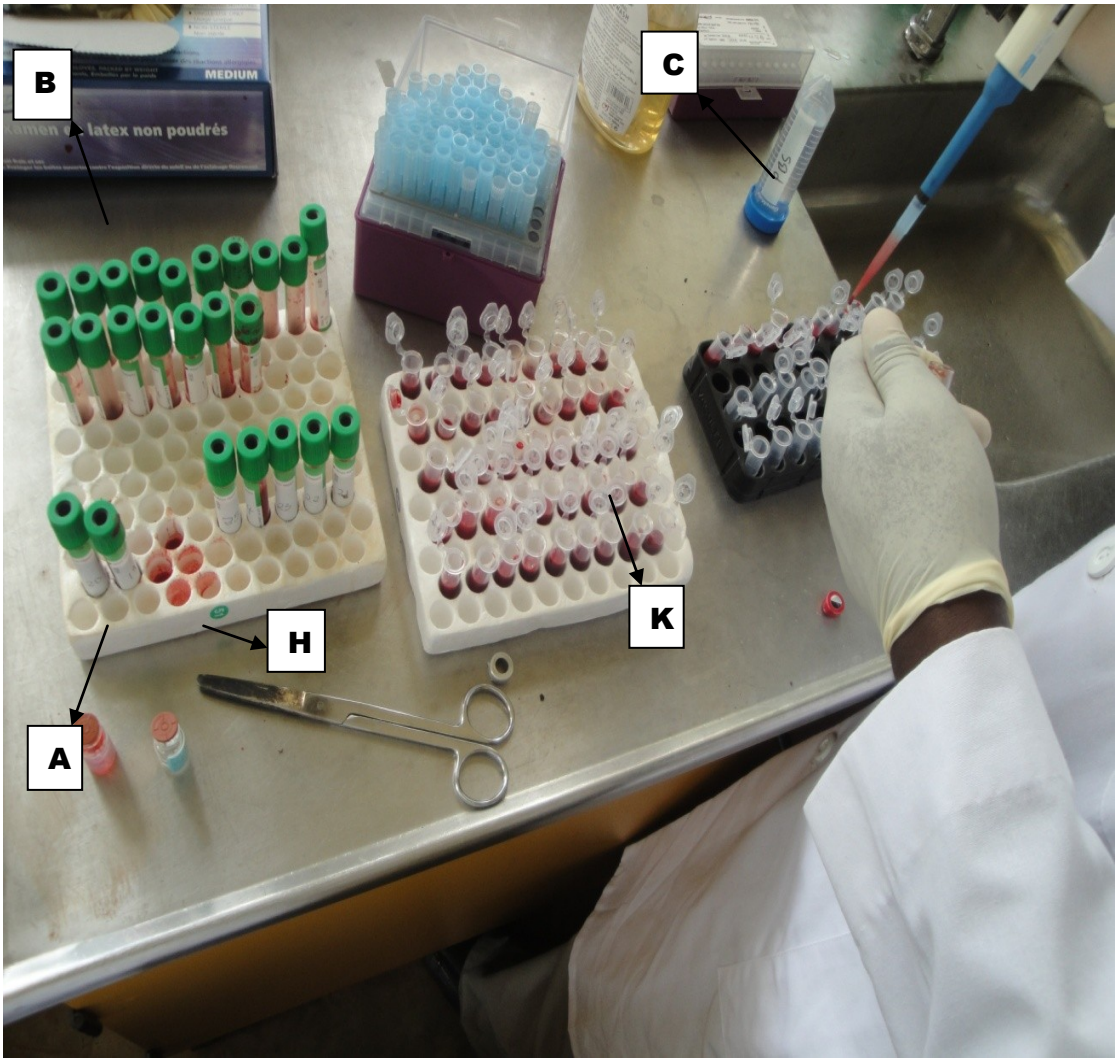

Plate 3: **Interferon gamma assay in the laboratory**

Whole blood in heparin vacuntainers (B) being transfered to three tubes each sample for specific sensitisation (K); sensitizing antigen Avian Purified Protein Derivative (Avian PPD) (A), Bovine Purified Protein Derivative (Bovine PPD) (H) and Phosphate Buffer Saline (PBS) (C) ready for incubation at 37 ˚ C for 24 hours.

3.4 Data Analysis

The collected data was compiled, entered using Microsoft excel data base and analyzed using the statistical software EPI INFO version 7 (Coulombier *et al*., 2001). The measures of levels and variability as regard to performance and efficiency of each BTB test was determined. Results on mean difference of two tests were considered significance if $P<0.05$ at 95% confidence interval.

CHAPTER FOUR

4.0 RESULTS

4.1 Performance of Conventional Comperative Intradermal Test in Goats

On the basis of CIT, the animal prevalence of BTB was 7% (5/71) at a cutoff ≥ 2 mm. At ≥ 2 mm cut-off point, there were two classified as doubtful after having 1.8 mm. They necessitated redo of the test. Finally, the two were grouped in non reactors (Table 1).

Table 1: Number and percentage of bovine tuberculosis reactors and non reactors

Item	Number	Percentage
BTB reactors	5	7
BTB non reactors	66	93
Total	**71**	**100**

* BTB is bovine tuberculosis

4.2 Performance of Interferon Gamma Test in Goats

On the basis of IFN-γ test, the animal prevalence of BTB was 13% (9/71). The test detected 9 positives including the 5 and 2 CIT reactor and doubtful goats respectively (Table 2). Comprehensive summary of results are on appendix 4.

Table 2: Number and percentage of bovine tuberculosis reactors and non reactors

Item	Number	Percentage
BTB reactors	9	13
BTB non reactors	62	87
Total	**71**	**100**

* BTB is bovine tuberculosis

4.3 Performance of Comparative Intradermal Test over Interferon Gamma test in Goats

There was no significant difference in efficiency of screening the possible BTB infected asymptomatic goats between the CIT and IFN-γ tests. However, IFN-γ had better performance over CIT. Basing on mean difference of performance of the two tests, there is no significant difference (Table 3).

Table 3: Mean difference of performance for comparative intradermal test and interferon gamma test

	P value	t Critical value
a. P(T<=t) one-tail	0.011146793	1.745883669
b. P(T<=t) two-tail	0.022293586	2.119905285

*P<0.05 at 95% confidence interval

4.4 Bovine Tuberculosis case Distribution Between Locations

Distribution of cases screened seems to be higher (11/14 i.e. 71%) in periurban mixed herds than among urban mixed herds (3/14 i.e. 29 %) (Plate 4).

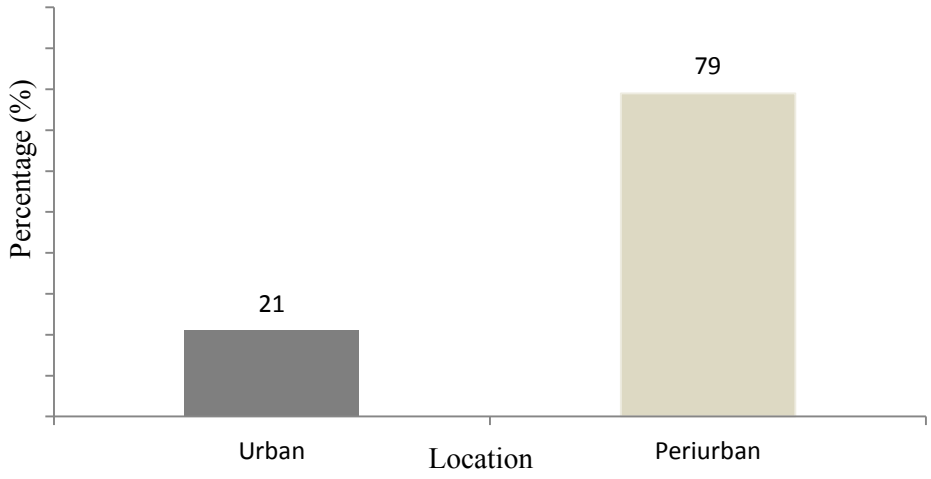

Figure 2: Distribution of bovine tuberculosis cases in goats in urban and periurban Morogoro Municipality

4.5 Pathological Lesions Recorded During Postmortem

Two goats among the asymptomatic reactors list were targeted at random for pathological lesions examination. Fortunately, one goat was doubtful from CIT test results and the other was reactor to both CIT and IFN-γ tests. However, they were both from periurban farmers. At first, organs of interest were lungs and pulmonary lymph nodes (cranial, tracheobronchial and mediastinal) later we extended observation to other organs. The reactor goat, had a yellowish caseous material in cranial, tracheobronchial and mediastinal lymph nodes and lungs while doubtful goat, had a number of lesions in mesentery and mesenteric lymph nodes, which upon incision with surgical blade greenish discharge was observed. No significant lesions were recorded from its lungs and pulmonary lymph nodes.

4.6 Goats with Significant Results to Comparative Intradermal and Interferon Gamma Tests

Upon conducting the two tests in same goats, 9 were reactors and 62 were non reactors. The IFN-γ tests picked 5 reactors, 2 doubtful and 2 of the non reactors from CIT test results as reactors. Comprehensive summary of results are on appendix 4, however, (Table 4) describes test perfomance of the reactors and doubtful goats.

Table 4: Goats with significant results to comparative intradermal and interferon gamma tests

S/N	ID	CIT RESULTS	CIT INFERENCE	BOVGAM RESULTS		BOVGAM INFERENCE
		PPD (B-A)		**PPD B-PPD A**	**PPD B-PBS**	
1	4763	2.7	**Reactor**	0.111	0.118	**Reactor**
2	4817	1.8	**Doubtful**	0.1295	0.13	**Reactor**
3	3	2.1	**Reactor**	0.1305	0.1005	**Reactor**
4	40/iii	2.2	**Reactor**	0.1375	0.147	**Reactor**
5	2	1.8	**Doubtful**	0.147	0.16	**Reactor**
6	7	1.7	**Non Reactor**	0.1	0.1002	**Reactor**
7	9	-1.7	**Non Reactor**	0.1775	0.1775	**Reactor**
8	20	2	**Reactor**	0.109	0.104	**Reactor**
9	8b	2	**Reactor**	0.118	0.135	**Reactor**

*CIT is Comparative Intradermal Test, Avian PPD is Avian Purified Protein Derivative, Bovine PPD is Bovine Purified Protein Derivative, PBS is Phosphate Buffer Saline

NOTE:

a. Cut off for CIT was 2 mm; positive ≥ 2 mm and negative ≤ 2 mm.

b. Cut off for Bovgam was 0.100 PPD OD value for both differences {PPD Bovine less PPD Avian as well as PPD Bovine less OD value of PBS}.

4.7 Test Findings from the 10 Cattle

Both CIT and IFN-γ tests, detected no animal with BTB at cutoff ≥ 4 mm (Table 5). This necessitated redo of the test but the areas (Milela Mlandizi and LITA Morogoro campus) has been under BTB research in cattle for the past 4 consecutive years where screen and cull strategy is employed. For detailed findings (Appendix 5).

Table 5: Number and percentage of bovine tuberculosis reactors and non reactors

Item	Number	Percentage
BTB reactors	0	0
BTB non reactors	10	100
Total	**10**	**100**

* BTB is bovine tuberculosis

CHAPTER FIVE

5.0 DISCUSSION

Part of the problem of BTB control has been the difficulty in the disease diagnosis. This is attributed to a number of factors including the fact that majority of animals infected with BTB do not show clinical signs in low grade infections (OIE, 2009). Bovine tuberculosis is very difficult to diagnose in goats on clinical examination alone, as the signs of the disease are not very specific and animals may be latently infected without exhibiting any obvious clinical signs. Bovine tuberculosis should be considered in cases of chronic loss of condition and appetite, reduced milk yield and debilitating disease, with or without respiratory signs (AHVLA, 2014).

The performance of CIT in mixed herd goats has shown to be capable of 7%, at cut off ≥ 2 mm. On the other hand, the performance of IFN-γ in mixed herd goats is 13 percent, indicating that IFN-γ in goats has potential of performing comparatively better. Detection of IFN-γ in blood is fast because the levels of IFN-γ keep on increasing more at every encounter of immune response to BTB infection (Ozturk *et al.,* 2010). Additionally, application of IFN-γ test makes the exercise simple and easy because it reduces the number of times to handle animals after blood collection. In addition, serum can be stored for later analysis without need for immediate processing. This allows for batch processing, application of confirmatory tests, option for allotment to multiple laboratories, and archiving for future tests (Palmer and Waters, 2006).

Relatively low potency of CIT was also experienced by Kassa *et al*. (2012) and recommended multiple tools to be employed when screening BTB in goats. A number of

studies have demonstrated that, by applying both skin test and IFN-*γ* test in parallel, between 12-38 percent additional diseased animals can be detected that had escaped diagnosis by skin testing. This increases the overall detection rate in these studies from 65-80 percent when using skin testing alone to 88-95 percent when both tests were applied in parallel (Vordermeier *et al*., 2008; Abdellrazeq *et al*., 2014). In this particular study, detection rate has improved by 6% through IFN-*γ* test that detected 4 additional diseased animals.

In the current study, there is no significant difference between the means of the two tests. Thus CIT has no better potency and efficiency over IFN-γ test. However, performance of IFN-γ test in this study has been better than that of CIT. This is so because, the IFN-γ test targets a biochemical component available in blood from the onset of infection as a product of immune response to disease agent. In contrast, CIT works as an hypersensitivity body reaction which can be immediate or delayed, mild or excessive, and is bound to be missed at times. The IFN-γ is widely used to detect early infection in cattle or to confirm the results of the CIT, and is considered to have adequate sensitivity for diagnostic purposes (Lambert *et al.*, 2006; Vordermeier *et al*., 2008; Abdellrazeq *et al*., 2014). Wood and Jones (2001) in a comprehensive review reported that IFN-γ test could identify BTB positive cattle at an earlier time point compared to CIT. In some cases positively reacting animals were identified at 14 days post infection, in a similar performance evidence as in goats (Whipple *et al.*, 2001; Lambert *et al.*, 2006; Schiller *et al*., 2011). Injection of stimulating antigens when conducting CIT needs extra care due to smaller thickness of goat skin than that of cattle. This situation needs to hold patience of time and speed especially when dealing with large flock. Otherwise, many injection shots will be done either outside the skin or subcutaneous which can accounts for wastage of time and resources as well as inaccurate results.

It is interesting to note that BTB was detected in both locations of mixed herds i.e; periurban and urban investigated in this study, albeit there was a subtle difference in the prevalence between locations. It is noted to be high in periurban than urban having prevalence of 29% vis a vis 71%. This could be an indication that BTB can infect any goat regardless of location as long as it is exposed to BTB in one way or another.

Other authors reported that age and breed are some of the risk factors associated with BTB infection in goats and cattle. This was taken care of in this study by having local breeds that were above 2 years old and inclusion of Government farm (LITA farm). This farm has large numbers of Norwegian type dairy breeds mixed with Friesian-Holstein crosses and Aryshire cross dairy cattle. The study included different breeds of different ages with minimun of 2 years such that aspects of age and breed specific was under control. It has been observed that the two factors have no impact on BTB screening in goats of this study (Cadmus *et al*., 2004; Silaigwana *et al*., 2012). Nevertheless, prevalence could be low in urban bacause of high slaughter rate and low mixer of flocks at communal grazing yards (understocking of grazing grounds). The latter accounts for low dose uptake which should be equivalent to 10^4-10^5 CFU, through respiratory routes for effective infection (Palmer and Waters, 2006).

Post moterm lesions observed in two sacrificed goats included partially disseminated BTB lesions which involved lung, intestine and lymph nodes of thoracic and abdominal cavities. Upon incision of the lung, lesions showed a yellowish caseous material indicating a characteristic of tuberculous lesion. In mesentery and mesenteric lymph nodes, greenish discharge was observed in the lesions. This method was appropriate for verification and checking for false positives as it is quick and easy to apply under field condition in

Malawi and Tanzania compared to culture preparation and use of PCR techniques (Kazwala *et al*., 2001*a*, 2001*b* and 2006; Katale *et al*., 2012).

However, post moterm lesions and isolation of the organisms from lungs and pulmonary lymph nodes of asymptomatic positive cases using selective culture media though laborious and time consuming, confirms the presence of BTB infection within the mixed herds and communal grounds.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The aim of this study was to demonstrate the potency of conventional CIT/BTB test and its efficiency over IFN-γ test in apparently healthy goats managed in mixed herd. The study has managed to answer the following specific objectives, (i) to determine the performance of conventional comparative intradermal BTB test in mixed herd goats, (ii) to assess the performance of interferon-gamma BTB test in mixed herd goat flocks, (iii) to evaluate the performance of conventional comparative intradermal BTB test over interferon-gamma BTB test in mixed herd goat flocks, (iv) to determine and compare location-dependent BTB prevalence in mixed herd goat flocks within Morogoro municipality i.e. Milela, Mlandizi, Bigwa, Magadu, Folkland, Mazimbu and Kihonda. Mixed herds and communal grazing grounds are pockets of BTB infection reserves and build-ups. These pockets, are points to consider for BTB screening as well as diagnosis in order to achieve BTB free herds. In this areas detecting the infection at an early stage using the IFN-γ test can lead to BTB free goat flocks and cattle herds. Therefore, all the animals reacted positive in this study has to be culled and destroyed.

Both tests have potential to detect BTB infected herds but the conventional CIT/BTB test has proven to have no significant better potency and efficiency over the IFN-γ test ($P<0.05$; 95% confidence interval). Our results demonstrated that IFN-γ test was an effective diagnostic tool, therefore, this assay could be used either in diagnosis or as a screening assay in goats. More importantly, it should be used as confirmatory tool for CIT when screen BTB in goats.

Prevalence of Bovine tuberculosis is relatively higher in periurban goat flocks compared to urban goat flocks. The study established that most of the cases are subclinical in nature with lesions either disseminated or localised in the lungs and pulmonary lymph nodes that need careful attention during meat inspection.

6.2 Recommandations

Recommandation is made that interferon gamma test should be used alongside comparative intradermal test in goats. The test has two major properties, that is quick and simple though expensive as such it should be used to screen out goats along side cattle during the BTB herd screening. Furthermore, NGOs should use it to screen goats before distributing to people living with HIV/AIDS and other vulnerable households. This is a good development in fighting against *M. bovis* in mixed herds of Malawi and Tanzania that are mostly managed in communal grounds throughout the year.

5.3 Further Studies

Further studies are required to elaborate expression of interferon gamma for different ranges of age groups in asymptomatic BTB goats using immunohistochemistry and westen blot techniques. The study should even correlate the pathological lesions observed in asymptomatic BTB goats and the degree of interferon gamma expression in relation to BTB disease resistance in goats. There is strong need to conduct study on molecular epidemiology of BTB disease to link the disease agent with the disease in susceptible species (cattle and goats) within the mixed herd.

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APPENDICES

Appendix 1: Sample size and sampling methods

The sample size was estimated by the formula $n = Z^2SD^2/e^2$ for the unknown population (Kothari, 2004), where *n* = size of sample, *z* = standard variate at 95% confidence level (1.96), SD = the standard deviation of population (0.12) and *e* = acceptable error (0.05). The sample $n = (1.96)^2\ (0.12)^2/\ (0.05)^2 = 22$. The obtained 22 represented number of mixed herds that had to be included in the study from both urban and periurbun herds. Thereafter, the mixed herds from urban and periurbun herds were listed and each was asigned a random number. Then, random sampling of random numbers was done using excell package to get the ones that participated in the study. After getting the 22 mixed herds, the study recruited 66 goats. The study targeted breeding buck and two does from each herd. Therefore, from each mixed herd three goats of not less than 2 years were sampled. The samples from mixed herd were 3 x 22 = 66 goats. The sample size was contributed equally from urban 33 goats and periurban 33 goats to campare prevalence. Five more old members Norwegian breeds from LITA Morogoro campus farm were included making the total of 71 samples. In addition, 10 cattle were included as standard for the efficiency of reagents. The sample frame ensured inclusion of different breeds and age groups to minimise effects arising from breed and age specific factors.

Appendix 2: Procedure for running comparative interadermal test

Animals were identified by their ID numbers and record identification. That was followed by shaving two sites in the middle third of the neck on one side (the right hand side was used in this study), one above the other, separated by about 12 cm. Then, at each site, a fold of skin was measured using a calliper and the measurement was recorded. After site preparation, PPD-bovine and PPD-avian (Prionics Deutschland GMBH, Netherlands) was injected (0.1ml) intradermally. The upper site was used for the avian PPD and the lower site for the bovine PPD). We re-visited after 72 hours for re-measurement of the same skin fold at each site using callipers and recording. We made sure that the same operator took measurements on both occasions. Standard interpretation was then applied to the results;

a. When the reaction to bovine PPD was > 4.0 mm and PPD was > 2.0 mm (for small ruminants) greater than to avian PPD the test was considered positive.
b. When the reaction to bovine PPD was between 1.0 and 4.0 mm greater than to avian PPD the test was considered inconclusive/doubtiful.
c. When the reaction to bovine PPD was < 1.0 mm greater that to avian PPD the test was considered negative (Tschopp, *et al*., 2011).

Appendix 3: Procedure for interferon gamma test

The Bovigam® immunoassay was used to run the gamma interferon assay (BOVIGAM γ, Prionics Deutschland GMBH, Netherlands). This procedure was done before conducting CIT procedure.

Blood samples (2 ml from each animal) were collected by jugular venepuncture into heparinised vacutainers and was stored in cooler box that had ice packs. Then, it was taken to SUA, FVM, Public health laboratory where it was incubated within 16 hours of collection. Blood sample was first evenly mixed before aliquot blood (250 µl from each animal) was transfered into three eppendorf into which one of three substances was added;

a. 25 µl Phosphate buffered saline (negative control)

b. 25 µl Bovine PPD antigen

c. 25 µl Avian PPD antigen

After adding, the samples were incubated at 37 ° C for 16-24 hours (whole blood). After incubations, the samples were centrifuged and plasma was harvested. Obtained plasma was tested by assaying each sample in duplicate and their controls in triplicate**.** The plasma was pipetted from the eppendorf into a 96-well tissue culture tray. The microplates coated with monoclonal antibody to interferon gamma were supplied for the assay by Prionics (Prionics Deutschland GMBH, Netherlands). All reagents (except conjugate) were brought to room temperature before been used.

Assay procedures involve reconstituting the freeze dried components, thereafter, transfering 50 µl of Green Diluent to the required wells of microplates, followed by addition of test and control samples from the eppendorf to the appropiate wells containing Green Diluent. The control samples were added last to each plate. Thorough mixing was

achieved by vortexing microplate for 1 minute. This was followed by covering the microplate plate with a lid and incubating at room temperature (25 °c) 60 minutes. After incubation, the contents of the wells were shake off and the microplate was washed 4 times with running water. Then, all wells were filled with 300 µl of Wash Buffer at room temperature for further washing. This was repeated four times. Thereafter, microplate was placed face down on clean filter paper to allow drain. This facilitated removal of as much as Wash Buffer as possible. Upon draining the Wash Buffer, 100 µl of freshly prepared Conjugate Reagent were added to wells. Thorough mixing was achieved by vortexing microplate for 1 minute. Thereafter, the microplate was covered with a lid and incubated at room temperature for 60 minutes. The wells were thoroughly washed for four times using Wash Buffer. Thereafter, 100 µl of Enzyme Substrate Solution were added to wells. Thorough mixing was achieved by vortexing microplate for 1 minute. Thereafter, the microplate was covered with a lid and incubated at room temperature for 30 minutes. The wells were thoroughly washed for four times using Wash Buffer. After incubation time, 50 µl of Enzyme Stopping Solution was added to each well and thoroughly mixed. Each well was subjected for reading by absorbance within 5 minutes from termination of reaction. Plates were read using a microplate reader with both 450 nm filter and 620-650 nm filters.

Parameters for validation of Bovigam®

Positive (indicates M. Bovis infection)	**Negative**
Bov.PPD.OD – nil.Ag.OD ≥ 0.1 And Bov.PPD.OD – Av.PPD.OD ≥ 0.1 Positive bovine IFNγ OD > 0.100 (positive must not deviate by more than 30% from mean OD)*	Bov.PPD.OD – nil.Ag.OD < 0.1 And Bov.PPD.OD – Av.PPD.OD < 0.1 Negative bovine IFNγ OD < 0.100 (maximum variation of ± 0.04)*

*If these conditions are not met then the test results are invalid and should be repeated.

Appendix 4: Test results of comparative intradermal and interferon gamma tests in goats

S/N	ID	CIT RESULTS PPD (B-A)	CIT INFERENCE	BOVGAM RESULTS PPD B-PPD A	PPD B-PBS	BOVGAM INFERENCE
1	Male	-1.83	Non Reactor	-0.0135	0.0285	Non Reactor
2	4763	2.7	**Reactor**	0.111	0.118	**Reactor**
3	1	0.02	Non Reactor	-0.1855	0.085	Non Reactor
4	4	-1.2	Non Reactor	-0.0195	-0.0155	Non Reactor
5	4908	0.18	Non Reactor	-0.0615	-0.009	Non Reactor
6	45/2	0.29	Non Reactor	-0.04	0.0195	Non Reactor
7	1665	1.12	Non Reactor	0.0125	0.002	Non Reactor
8	14	-0.64	Non Reactor	-0.1615	0.0775	Non Reactor
9	1434	0.08	Non Reactor	-0.0035	0.066	Non Reactor
10	4806	-0.6	Non Reactor	-0.0025	-0.0105	Non Reactor
11	4817	1.8	**Doubtful**	0.1295	0.13	**Reactor**
12	3	2.1	**Reactor**	0.1305	0.1005	**Reactor**
13	40/iii	2.2	**Reactor**	0.1375	0.147	**Reactor**
14	4849	-0.1	Non Reactor	0.0095	0.009	Non Reactor
15	482	-1.9	Non Reactor	-0.01	0.0065	Non Reactor
16	1651	-1	Non Reactor	0	0.0055	Non Reactor
17	5/v	-1.2	Non Reactor	-0.0265	-0.0165	Non Reactor
18	1432	-1.1	Non Reactor	-0.0435	0.0065	Non Reactor
19	6/iv	1	Non Reactor	-0.004	-0.0005	Non Reactor
20	7/vii	-3.7	Non Reactor	-0.003	0.0055	Non Reactor
21	4906	0.4	Non Reactor	0.004	-0.0045	Non Reactor
22	1663	-0.9	Non Reactor	-0.011	-0.0265	Non Reactor
23	8/viii	0.1	Non Reactor	0.005	0.0185	Non Reactor
24	485	1.6	Non Reactor	-0.097	0.027	Non Reactor
25	10/x	0.9	Non Reactor	0.008	0.027	Non Reactor
26	1	0.6	Non Reactor	-0.002	-0.0105	Non Reactor
27	2	1.8	**Doubtful**	0.147	0.16	**Reactor**
28	3	1.2	Non Reactor	0.006	0.016	Non Reactor
29	4	-0.7	Non Reactor	-0.0245	-0.2645	Non Reactor
30	5	0.1	Non Reactor	0.002	-0.003	Non Reactor

31	6	1.4	Non Reactor	0.042	0.0135	Non Reactor
32	7	1.7	**Non Reactor**	0.1	0.1002	**Reactor**
33	8	1.3	Non Reactor	0.0125	0.002	Non Reactor
34	9	-1.7	**Non Reactor**	0.1775	0.1775	**Reactor**
35	10	1.2	Non Reactor	0.0525	0.0615	Non Reactor
36	11	-0.4	Non Reactor	-0.0355	0.0805	Non Reactor
37	12	0	Non Reactor	0.016	0.02	Non Reactor
38	13	1.2	Non Reactor	0.0225	-0.068	Non Reactor
39	14	-0.6	Non Reactor	-0.097	0.027	Non Reactor
40	15	0.5	Non Reactor	0.008	0.027	Non Reactor
41	16	-1.4	Non Reactor	-0.002	-0.0105	Non Reactor
42	17	-0.4	Non Reactor	0.013	0.0025	Non Reactor
43	18	1.2	Non Reactor	0.006	0.016	Non Reactor
44	19	-0.6	Non Reactor	-0.0245	-0.2645	Non Reactor
45	20	2	**Reactor**	0.109	0.104	**Reactor**
46	21	1.2	Non Reactor	-0.0135	0.0285	Non Reactor
47	22	0.9	Non Reactor	0	-0.0055	Non Reactor
48	23	0.3	Non Reactor	0.002	-0.023	Non Reactor
49	24	-0.1	Non Reactor	0.0055	0.003	Non Reactor
50	25	-1.6	Non Reactor	-0.0615	-0.009	Non Reactor
51	26	0.3	Non Reactor	0.0015	-0.0005	Non Reactor
52	27	-0.9	Non Reactor	0.002	0	Non Reactor
53	28	0.4	Non Reactor	0.001	-0.0135	Non Reactor
54	29/3133	-0.2	Non Reactor	0.0015	0.0025	Non Reactor
55	30/3135	0.8	Non Reactor	0.0005	0	Non Reactor
56	1b	-0.7	Non Reactor	0.0015	-0.012	Non Reactor
57	2b	-2.1	Non Reactor	-0.0025	0.0105	Non Reactor
58	3b	0.3	Non Reactor	0.006	0.016	Non Reactor
59	4b	-0.5	Non Reactor	-0.0245	-0.2645	Non Reactor
60	5b	-0.1	Non Reactor	0.002	-0.003	Non Reactor
61	6b	0.1	Non Reactor	-0.0055	-0.0055	Non Reactor
62	7b	-0.5	Non Reactor	-0.004	-0.0005	Non Reactor
63	Buck/ hairly	-0.3	Non Reactor	-0.0435	0.0065	Non Reactor
64	8b	2	**Reactor**	0.118	0.135	**Reactor**
65	9b	-0.3	Non Reactor	-0.0055	-0.0085	Non Reactor
66	10b	0.5	Non Reactor	0.005	0.0185	Non Reactor

67	11b	0.1	Non Reactor	-0.011	-0.0265	Non Reactor
68	12b	0	Non Reactor	0.004	-0.0045	Non Reactor
69	13`b	0.5	Non Reactor	-0.002	-0.0105	Non Reactor
70	14 b	0.6	Non Reactor	0.008	0.027	Non Reactor
71	15b	0	Non Reactor	-0.097	0.027	Non Reactor

NOTE:

c. Cut off for CIT was 2 mm; positive ≥ 2 mm and negative ≤ 2 mm.

d. Cut off for Bovgam was 0.100 PPD OD value for both differences {PPD Bovine less PPD Avian as well as PPD Bovine less OD value of PBS}.

Appendix 5: Test results of comparative intradermal and interferon gamma tests in cattle

S/N	ID	CIT RESULTS	CIT INFERENCE	BOVGAM RESULTS		BOVGAM INFERENCE
		PPD (B-A)		PPD B-PPD A	PPD B-PBS	
1	1c	-0.7	Non Reactor	0.0015	-0.012	Non Reactor
2	2c	-0.6	Non Reactor	-0.0245	-0.2645	Non Reactor
3	3c	0.3	Non Reactor	0.006	0.016	Non Reactor
4	4c	-0.5	Non Reactor	-0.0245	-0.2645	Non Reactor
5	5c	-0.1	Non Reactor	0.002	-0.003	Non Reactor
6	I	0.1	Non Reactor	-0.0055	-0.0055	Non Reactor
7	ii	-0.5	Non Reactor	-0.004	-0.0005	Non Reactor
8	iii	0.5	Non Reactor	-0.002	-0.0105	Non Reactor
9	iv	-0.3	Non Reactor	-0.0055	-0.0085	Non Reactor
10	Bighorn/x	1.2	Non Reactor	0.006	0.016	Non Reactor

*CIT is Comparative Intradermal Test, Avian PPD is Avian Purified Protein Derivative, Bovine PPD is Bovine Purified Protein Derivative, PBS is Phosphate Buffer Saline

NOTE:

a. Cut off for CIT was 4 mm; positive $\geq$ 4 mm and negative $\leq$ 4 mm.

b. Cut off for Bovgam was 0.100 PPD OD value for both differences {PPD Bovine less PPD Avian as well as PPD Bovine less OD value of PBS}.