SCHOOL OF PUBLIC HEALTH

COLLEGE OF HEALTH SCIENCES

UNIVERSITY OF GHANA

BOVINE TUBERCULOSIS AMONG HERDSMEN, NORTH TONGU DISTRICT, VOLTA REGION, GHANA

BY

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY IN APPLIED EPIDEMIOLOGY AND DISEASE CONTROL DEGREE.

JUNE, 2012.

DECLARATION

I, **ESTHER AMA AMEMOR**, hereby declare that with exception of the references cited to other people's work which has been duly acknowledged, this thesis is the result of my own research work done under supervision and has neither in part nor whole been presented elsewhere for another degree.

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CANDIDATE

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DATE

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DR. SAMUEL O. SACKEY

ACADEMIC SUPERVISOR

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DATE

DEDICATION

This work is dedicated first and foremost to God Almighty for giving me divine strength and guidance to bring this work to a successful end. It is also dedicated to my dear husband Mr. Eric Amemor and our daughters Maud Makafui Amemor-Mensah and Inex Mawumenyo Amemor- Mensah. I again dedicate this work to my late mum Mrs Victoria Nuku and my father Mr. Gabriel Nuku and to all who contributed in one way or the other to the success of this work.

ACKNOWLEDGEMENT

My profound gratitude goes to my academic supervisor Dr. Samuel Sackey for his support, guidance and encouragement.

I am highly indebted to Dr Nathaniel Yebuah, Head of the Epidemiology Unit of VSD, for whole heartedly supporting and supervising this work.

My appreciation goes to Prof. Edwin Afari, Dr. Fred Wurapa, Dr. Chima Ohuabunwo of the Ghana Field Epidemiology and Laboratory Training Programme (GFELTP) and other faculties of the Department of Epidemiology and Disease Control.

I am indebted to Dr. Kwesi Addo, and Mr. David Mensah, all of Noguchi Memorial Institute, for their great support.

I owe a debt of gratitude to the Director, Dr Mark-Hansen and staff, especially Drs. J. Awuni, Boi Kikimoto, Ben Aniwa, Patrick Ziem as well as Mr. Emmanuel Gaglo, Mr. Patrick Addae, Mr. Felix Nyadiaya, Mr. Joseph Welbeck and other laboratory staff for their support during data collection and laboratory work.

To Dr. Frank Bonsu of the Ghana Tuberculosis Control Programme, thank you.

To Dr. William Tasiame, Dr. Fenteng Danso, and Dr. Sherry Johnson, thank you so much.

To Mr. Daniel Amedzovor and Mr. Daniel Nkunafa of the Ho Municipal TB Training Centre and Mr. Noble Dika of Volta Regional Hospital, thank you for your great support.

My colleagues from the School of Public Health, especially, Cohort IV, I appreciate your sense of togetherness and matching forward to reach this stage.

To all cattle farmers especially North Tongu Cattle Farmers Association, thank you so much.

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LIST OF ACRONYMS AND ABBREVIATIONS

AFENET	African Field Epidemiology Network
AIDS	Acquired Immune Deficiency Syndrome
APHIS	Animal and Plant Health Inspection Service
BTB	Bovine tuberculosis
CDC	Centre for Disease Control and Prevention
cELISA	Competitive enzyme-linked immunosorbent assay
CI	Confidence Interval
CIDT	Comparative intradermal tuberculin test
CIT	Comparative intradermal tuberculin
CuSum	Cumulative sum
DHMT	District Health Management Team
DNA	Deoxyribonucleic acid
DOTs	Directly Observed Treatment Short- course
ELISA	Enzyme-linked immunosorbent assay
EPTB	Extra- pulmonary tuberculosis
FAO	Food and Agriculture Organization
FNA	Fine needle aspiration
GFELTP	Ghana Field Epidemiology and Laboratory Training Programme
GHS	Ghana Health Service
GLMM	Generalized Linear Mixed Models
HIV	Human Immunodeficiency Virus
ID	Identification Number
IgG	Immunoglobulin G
IgM	Immunoglobulin M

IU	International Units
JSS	Junior Secondary School
M. *bovis*	Mycobacterium bovis
MDR-TB	Multi-Drug Resistance tuberculosis
MTB	Mycobacterium tuberculosis
OIE	Office International des Epizooties - World Organization for Animal Health
OR	Odds Ratio
PPD	Purified protein derivative
RS	Random Start
SCITT	Single comparative intradermal tuberculin test
SI	Sampling interval
SPSS	Statistical Package for Social Sciences
SSS	Senior Secondary school
STD	Standard Deviation
TB	Tuberculosis
UK	United Kingdom
USDA	United States Department of Agriculture
VSD	Veterinary Services Directorate
WHO	World Health Organization
ZN	Ziehl-Neelsen

ABSTRACT

Introduction: Human Tuberculosis (TB) is an infectious disease of man caused by Mycobacterium tuberculosis (MTB) complex. The rate of decline in incidence of the disease is low due to the emergence of Multi-Drug Resistance tuberculosis (MDR-TB). The numerical contribution of bovine tuberculosis (BTB) to the general tuberculosis burden is unknown. Herdsmen, livestock workers, and veterinarians are at high risk of contracting BTB. There are information about BTB infection in cattle and human in Ghana but zoonotic transmission is yet to be established. The aim of this study is to determine the burden of BTB among herdsmen and cattle in the North Tongu district of the Volta Region to enhance prevention and control of the disease.

Method: A cross- sectional study was conducted in the North Tongu District of the Volta Region between the period of October 2011- March 2012. Sputum samples from 68 herdsmen and blood samples from 200 cattle belonging to these herdsmen were collected. Sputum samples were analyzed using Ziehl- Neelsen staining for TB. Cattle blood samples were tested for BTB using Anigen Rapid BTB Test. A structured questionnaire was used to collect socio-demographic information and possible risk factor information on cattle from participants.

Results: A total of 200 cattle consisting of 14 bulls and 186 cows were selected for the study. The prevalence of bovine TB was 19% (38/200) and those affected were all females. Eighty-four percent (164/195) cattle were routinely treated with antibiotics. The median age group of respondents was 31-40years. All (100%) human sample tested negative for Acid-Fast Baccilli (AFB). Ninety percent (61/68) of respondents consume fresh milk and 84% (57/68) do not use protective clothing. Seropositivity of cattle and kraal density were statistically associated (p= 0.001).

Conclusion and Recommendations: Bovine TB is prevalent in cattle in North Tongu district. Although herdsmen indulge in risky lifestyles that expose them to BTB, we found a zero prevalence of BTB among them. The study should be replicated using a larger sample size in the two populations. Milk from cattle in the area should be tested for *M. bovis*.

Keywords: Bovine tuberculosis, Ghana, Herdsmen, North Tongu.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Tuberculosis (TB) is an infectious disease of warm-blooded animals caused by *Mycobacterium tuberculosis (MTB)* complex which includes *Mycobacterium tuberculosi*s (the primary agent of TB in humans), *Mycobacterium bovis* (M. *bovis*, responsible for TB in cattle and other mammals) and *Mycobacterium avium* (Kaneene and Theon, 2004).

Tuberculosis usually affects the lungs (pulmonary TB) but can affect other organs of the body like the central nervous system, lymphatic system, circulatory system, genitourinary system, bones and joints (Watts, 1996). The main symptoms of tuberculosis are chronic cough with blood-tinged sputum, fever, night sweats and weight loss (CDC, 2012). It is spread through droplet infection. Most infections in humans result in an asymptomatic, latent infection and about one in ten latent infections eventually progresses to active disease, which, if not treated, kills more than 50% of its victims (Mishra and Singh, 2012)

Human infection with *M. bovis* can also cause TB that is clinically and pathologically not different from that caused by *M. tuberculosis* (De La Rua-Domenech, 2006). Other symptoms of human infection with *M. bovis* include cervical lymph nodes involvement and the intestinal tract or the meninges (Amdekar, 2005).

Tuberculosis is one of the three primary diseases of poverty alongside HIV/ AIDS and Malaria (Mahmood, S.A.I., 2010).

Tuberculosis is distributed worldwide (Figure 1). Currently, about two billion (1/3) of world's population is believed to be infected with TB with Asia and Africa being the most

endemic continents (WHO, 2012a). According to WHO 18 out of 23 countries which had TB incidence rate of 300 or more cases per 100,000 population in 2010 were African countries with Swaziland, South Africa, Sierra Leone and Lesotho listed as the leading areas respectively (WHO, 2010).

Tuberculosis kills half a million people a year in Africa, a quarter of the global total with a case rate of 216 per 100 000. In 1995, the WHO Regional Director for Africa declared a tuberculosis emergency state in Africa and appealed for urgent and extraordinary action to prevent the situation from getting worse (WHO, 2005). Although Ghana is not listed among the World Health Organization's (WHO's) 22 high-burden tuberculosis (TB) countries (WHO, 2012b), the disease is a major health problem in the country. With an estimated 47,632 new TB cases in 2007, Ghana ranks 19th in Africa for the highest estimated number of new cases per year (Ghana Health Service, 2012).

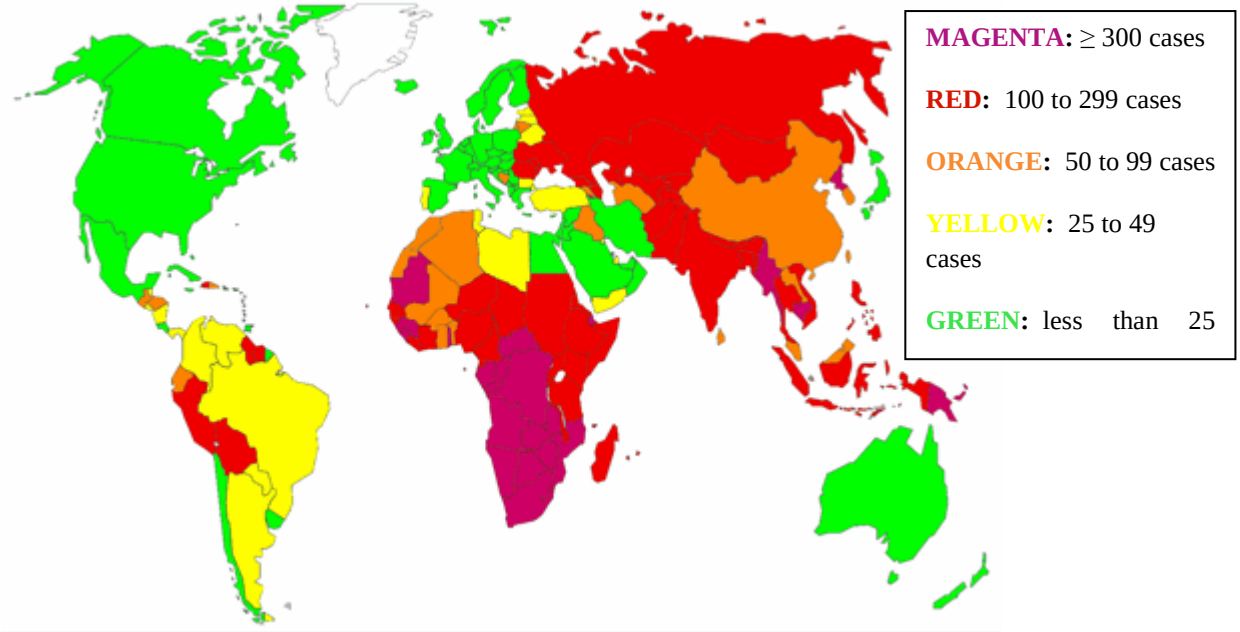

Figure 1: Global Distribution of tuberculosis. Incidence per 100,000 population.

Source: http://lgcpublishing.com/pop04.html. (WHO, 2010).

As part of the Millennium development Goal, WHO initiated the Global TB Control Programme to combat tuberculosis worldwide with an aim of halving the TB incidence and TB related Mortality by 2015 using the 2004 TB records as baseline (Chadha, 2009).

Despite the approaches to prevent and control tuberculosis the rate of decline in incidence of the disease is very slow due to the emergence of the Multi- Drug Resistance TB (MDR-TB) **(**Cosivi *et al*., 1998**)**. There is also the problem of bovine tuberculosis (BTB) whose numerical contribution currently to the general TB burden is unknown (Ayele *et al*., 2004). Human *M. bovis* infection was a major public health risk and an important source of TB in humans in the 1930s as a result of a high prevalence of bovine tuberculosis in cattle but the practice of the test and slaughter programme) as well as the introduction of pasteurisation of milk brought the incidence low (Shitaye et al., 2007, Davies, 1994). Currently bovine tuberculosis is mainly endemic in developing countries including sub- Saharan Africa where the test and slaughter policy has not been implemented (Cosivi *et al*., 1998). As a result bovine TB is either only partially controlled or not controlled at all.

People working with cattle such as herdsmen, veterinarians and livestock workers are at high risk of BTB infection (Georghiou et al., 1989).

Humans get exposed to *Mycobacterium bovis* the causative agent of BTB through direct inhalation from animals, consumption of uncooked infected meat or infected unpasteurized milk (Ayele *et al*., 2004). The close co-existence of farmers and animals is exemplified by the herdsmen, who live their entire lives with their animals, offering ample opportunity for zoonotic transmission of infection (Figure 2).

Figure 2: Close cattle- human relationship, North Tongu, October, 2010.

Source: **Picture taken by principal investigator during feasibility study. North Tongu, October 2010.**

Diagnosis of tuberculosis in Ghana relies mostly on sputum microscopy using Ziehl-Neelsen (ZN) staining, sputum culture and molecular typing. At the peripheral level of the health system in the Ghana, the Ziehl-Neelsen method is used for the diagnoses of tuberculosis. Detection of BTB in cattle in the country is carried out most commonly on the basis of tuberculin skin testing using the Purified Protein Derivative (PPD), abattoir meat inspection and rarely on bacteriological techniques (Abubakar *et al*., 2007). In recent years, there are many diagnostic methods for screening cattle for BTB. One of such methods is the Anigen Rapid BTB Ab Test Kit which saw a tremendous patronage over the years in other countries due to its specificity (98 %), sensitivity (85%), as well as being easy to use (Danbirni et al., 2010).

In Ghana cattle testing by the tuberculin skin test is sporadic. Between the period of 2005 to 2010, 516 TB cases were diagnosed at abattoirs and slaughter houses in Ghana (Veterinary monthly summary reports, 2010).

1.2 Problem Statement

Currently, the contribution of BTB to human tuberculosis is unknown in the country. In a study at the Korle-bu Teaching Hospital, 3% of TB positive human samples were M. bovis (Addo et al., 2007). Bovine tuberculosis cases were also reported in cattle at slaughter (2005-2009) according to the Veterinary monthly summary reports in 2010. An unknown proportion of cattle in the country is still infected with bovine TB because the test and slaughter policy for the control of the disease has not been implemented. The disease in cattle is therefore only detected at slaughter during meat inspection and occasionally during sporadic screening for the disease. People who attend to these animals such as herdsmen and veterinarians as well as the general public who consume fresh milk or infected meat are exposed to bovine TB from cattle (Bilal et al., 2010). The latter group gets exposed especially when animals are slaughtered in homes without veterinary supervision. Despite these facts zoonotic transmission of tuberculosis is yet to be established. There is therefore the need to establish zoonotic transmission of TB (Addo *et al*., 2007). This study takes the field approach (active search), rather than abattoir and hospital based approaches, to ascertain the burden of BTB in cattle and in human.

1.3 Justification

This study will lead to public health interventions, such as the establishment of zoonotic transmission of tuberculosis. The study will as well serve as basis for further studies which could culminate in formulation and implementation of the Test and Slaughter Policy for the control of BTB in cattle in the country. This study will also strengthen the "One Health" approach in the prevention and control of TB in the country. Again, the study will serve as a source of active surveillance data on the BTB as well as help target herdsmen as a high risk group for TB screening.

1.4 Objectives

1.4.1 General Objectives:

To determine the burden of BTB among herdsmen and cattle in the North Tongu district of the Volta Region.

1.4.2 Specific Objectives;

1. To determine prevalence of BTB in cattle.

2. To determine prevalence of BTB in herdsmen.

3. To determine the prevalence of risk factors of BTB in cattle.

4. To determine the prevalence of risk factors of BTB in herdsmen.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Prevalence of Bovine TB in Cattle

There are numerous literature that show the evidence of BTB infection in cattle across the globe. In a work done in Tanzania, the prevalence of BTB infection in cattle was investigated in pastoral and intensive production systems in the eastern zone of Tanzania. The prevalence of BTB infection as determined by single comparative intradermal tuberculin test (SCITT) was 1.3%. Similarly in another study, out of 143 herds tested, 28% of herds in the intensive systems and 88% of the herds in the extensive system or pastoral farms had BTB (Shirima *et al.*, 2003).

In a survey undertaken by Bonsu and others to determine the prevalence of tuberculosis infection in cattle in Dangme-West district of the Greater Accra region of Ghana, prevalence of a 13.8% infection in cattle in the district was detected. Cattle of all ages and both sexes were affected, but the prevalence in cows was twice as high as that in heifers or bulls. The study also established that there is a considerable lack of knowledge about BTB among cattle owners and herdsmen and that milk is often used untreated which exposes them to the risk of contracting BTB **(**Bonsu *et al*., 2003).

In a study conducted in the Southern Highlands of Tanzania to determine the prevalence of BTB and the risk factors associated with the occurrence of the disease in cattle an overall prevalence of 13.2% was found. It was found out that older cattle were more affected by the disease than yearlings and calves ($p<0.0001$). Sex, breed and lactating status of cattle came out as some of the risk factors of BTB in cattle (Kazwala *et al*., 2001).

In a prevalence study conducted in Nigeria on 22 herds consisting of 922 cattle, an individual prevalence of 1.08%) and a herd prevalence of 45.45% was found (Ibrahim *et al*., 2010).

Another study also conducted to determine the prevalence of BTB in cattle in central Ethiopia relative to the tuberculosis status of their owners showed that cattle owned by active TB patients had a higher prevalence of BTB than those owned by non- tuberculous owners. It was also found out that the prevalence of BTB was three times higher in cattle owned by farmers with active tuberculosis (24.3%) than in those owned by farmers who did not have active tuberculosis (8.6%). Regassa argued that the finding could suggest possible transmission of *Mycobacterium spp*. between cattle and their owners (Regassa *et al*., 2008).

In a study to determine the prevalence of BTB, 625 cattle were randomly selected from four districts in Ethiopia. Comparative intradermal tuberculin (CIT) test was used and an overall individual animal prevalence of 12.16% was found. Breed of animal was found to be a risk factor for BTB in cattle (Dinka and Duressa, 2011).

A study was conducted to determine the herd and individual animal prevalence of BTB (BTB) in zebu cattle in Boji district in western Ethiopia. A total of 62 herds consisting of 780 cattle were included in that study. A herd prevalence of 19%) and an individual animal prevalence of 1.6% were found. (Laval and Ameni, 2004).

In a cross-sectional study, conducted in Hawassa town and its surroundings in Ethiopia to estimate the prevalence of BTB based on comparative interadermal tuberculin test (CIDT) and abattoir survey, 39 herds comprising 413 cattle were subjected to CIDT, and the herd and individual animal prevalences of 48.7%) and 11.6% were found respectively. Age group and management system were found to be risk factors of bovine TB in cattle (Regassa *et. al*., 2010).

2.2 Risk factors of bovine TB in cattle

In a work done by Porphyre and others to investigate farm-level risk factors for confirmed BTB, a retrospective cohort study was conducted on a cattle population in New Zealand, it was realised that proximity to forest parks, breed of cattle, Herd size, and previous infection status came out as significant predictors of BTB in cattle (Porphyre *et al.*, 2008).

In another study to determine the risk factors of BTB in cattle, a representative stratified cluster sample survey was conducted using comparative intradermal tuberculin test in cattle from four regions in Ethiopia. None of the potential risk factors of disease transmission between cattle and human such as food consumption, husbandry system and presence of BTB-positive cattle were statistically significant (Tschopp *et al*., 2009).

In another survey carried out on households keeping livestock, it was found out that the only household level risk factor that significantly influenced the presence of BTB in cattle was the presence of animals coughing in the herd. (Boukary *et al*., 2011).

As a way of enhancing the Millennium Development Goals for control and eradication of the BTB worldwide, there was a review of the main risk factors for bovine TB in cattle based on a three-level classification; animal, herd and region/country level. A distinction is also made, whenever possible, between situations in developed and developing countries as the difference of context might have consequences in terms of risk of BTB. Recommendations were then made to animal health professionals and scientists directly involved in the control and prevention of BTB in cattle. Some of the main risk factors of bovine TB in cattle identified were sex, breed, age, genetics, immuno-suppression, malnutrition, milk, type of cattle industry, reduced veterinary services, and movement due to purchases, climate, soil, water and manure. (Humblet *et al*., 2009).

In a study conducted to identify risk factors for BTB in cattle in Zambia, a total of 106 herds of cattle were investigated for presence of bovine TB using the comparative intradermal tuberculin test (CITT), while an interviewer-administered questionnaire was used to gather epidemiological data on herd structure, management and grazing strategies. A prevalence of 49.8% at herd level was estimated. Area and husbandry practice came out as the possible risk factors of BTB (Munyeme *et al*., 2008).

A study was carried out in Belgium to assess of potential risk factors of BTB in cattle to enable surveillance measures. Risk factors such as history of BTB in the herd, proximity of an outbreak and cattle density were identified (Humblet *et al*., 2010).

2.3 Prevalence of Bovine TB in human

"The actual impact of animal BTB on human health is generally considered low in developing countries which may be based on the rare identification of *M. bovis* isolates from human patients" (Amanfu *et al*., 2006).

In Ethiopia, Kiros in 1998 cited in a review done by Shitaye and others demonstrated that out of 85 sputum samples taken from dairy farm workers and tuberculous patients 29.2% were M. *bovis* (Shitaye *et al*., 2007).

With a similar scenario, Regassa in 2005 cited in a review by Shitaye and others demonstrated that, out of 87 sputum and 21 fine needle aspiration (FNA) human samples 16.3% were *M. bovis* (Shitaye *et al*., 2007).

Mycobacterium bovis has been isolated from sputum and tissue samples from humans especially among the Fulani herdsmen with or without clinical signs of tuberculosis in Nigeria (Abubakar *et al*., 2011).

Furthermore, Camus and others in Nigeria indicated that a higher prevalence of bovine TB in cattle owned by tuberculous patients was found than in cattle owned by non-tuberculous owners, which suggests the significant role of *M. bovis* in the incidence of tuberculosis in humans (Cadmus *et al*., 2009).

"The occurrence of *M. bovis* in humans against the background of the soaring *HIV/AIDS* incidence in Eastern and Southern Africa implies that the risk of spill-over of zoonotic BTB to rural communities is rapidly increasing" Thus, the correlation between the prevalence of *M. bovis* infection in humans and that of local cattle populations highlights the potential threat of this disease for humans especially in developing countries, where drinking raw milk is a common practice in rural areas in particular (Cosivi *et al*., 2006).

According to a study conducted in Ghana at the Korle -bu Teaching Hospital on sputum samples from TB patients, 3% were BTB (Addo *et al*., 2007*)*. This result called for the need to conduct a nationwide survey using both conventional and molecular techniques to characterize various mycobacteria species causing TB in Ghana, to know the various strains circulating in the country (Addo *et al*., 2007).

2.4 Risk factors of BTB in human

Unpasteurised cow or goat milk and infected organs from slaughtered animals are routes of transmission of BTB to human. Cattle herders, many of whom are young boys, are at high risk of BTB as they stay in close proximity to cattle and indulge in risky lifestyle (Abubakar *et al*., 2011).

Lack of knowledge about BTB infection among cattle farmers exposes them to the disease as demonstrated by Kang'ethe and others in their study to determine the prevalence of BTB and possible risk factors for human infection in Kenya, it was found out that 57% of the respondents from dairy farms and 72% from non-farming households had limited knowledge

of BTB and this made them unable to take measures to protect themselves from contracting the disease. The main risk factors identified were being a milk processor, distance between cattle kraals and houses as well as the time spent attending to cattle (Kang'ethe *et al*., 2007). Proximity of humans to cattle farms and close interaction between humans and animals are potential risk factors of BTB in human (Regassa *et al*., 2008).

2.5 Zoonotic implication of bovine TB

It is estimated that 1.5–2million people die each year from tuberculosis of the approximately 2 billion infected person's worldwide (LoBue *et al*., 2010). *Mycobacterium bovis* infection currently accounts for only a small percentage of reported cases but it was a major public health problem in Europe and elsewhere, when this organism was transmitted to man in milk from infected cows, prior to the advent of pasteurization of milk and milk products (O'Reilly and Daborn, 1995).

According to McCrindle and Michel (2006) bovine TB, is a serious problem for a developing dairy industry in Africa as its main route of transmission is through milk products. Informal milk production with lack of adequate pasteurisation is very common in rural Africa and milk from infected cows is particularly dangerous for young children: those who most need the nutritive value of milk to supplement an inadequate diet. Bovine TB also poses an occupational risk for those working with dairy cattle. In cattle, bovine TB is almost incurable and valuable breeding stock may easily be lost through death or slaughter. This can be of considerable socio-economic importance in developing countries where replacement of equivalent breeding stock may be unaffordable. Literature reports indicate that a high number of cattle and human population of Africa are in areas where bovine TB is either partly controlled or not controlled at all due to lack of veterinary infrastructure required for the surveillance and control of the disease (Mccrindle and Michel, 2006).

Thoen and others and De La Rua-Domenech provide several reasons why *M. bovis* in humans is under-diagnosed even in developed countries (De La Rua-Domenech, 2006; Theon *et al*., 2006). "The consumption of unpasteurised milk or milk products still remains a risk for infection in countries where bovine TB has not been eradicated where ethnic populations present significantly different epidemiological profile or where HIV is prevalent" (Hlavsa *et al*., 2008; Doran *et al*., 2009; De Kantor *et al*., 2010). Zoonotic TB was originally considered primarily as a disease of children where the disease involved the cervical lymph nodes, the intestinal tract, or the meninges. It is now increasingly being recognised that infection in childhood is the precursor of disease in adults and that many infected children may remain asymptomatic, undiagnosed, and untreated (Amdekar, 2005). Zoonotic TB is of particular concern for developing countries, but where bovine TB controls are effective, human *M. bovis* isolates are uncommon and rare in countries where bovine TB has been eradicated (De La Rua-Domenech, 2006; Ingram *et al*., 2010, Davies, 1994). O'Reilly and Daborn also referred to a small outbreak of tuberculosis in human caused by *M. bovis* in the Netherlands in 1994 (O'Reilly and Daborn, 1995).

Tuberculosis diagnosis could go undiagnosed with many methods. According to Wilkins and others, a 74-year-old patient with symptoms of persistent fever, anorexia and severe unproductive cough was diagnosed with BTB with PCR after a tuberculosis (TB) skin test (TST) and a sputum smear staining for acid-fast baccilli (AFB) were negative (Wilkins *et al*., 2007).

Zoonotic transmission of BTB was also established in a case studies carried out on a 29-year-old hunter in 2004 who got injured with his knife when he was dressing an infected deer. The Bovine TB strain in both the deer and the hunter were the same. The investigation of the infection in the case patient provided strong evidence of transmission of *M. bovis*

infection from deer to human through percutaneous injection with a contaminated hunting knife (Wilkins *et al*., 2007).

Cosivi and others emphasised in a work done by McCrindle and Michel in 2006, that pastoral and traditional milk harvesting is far more common than the commercial system and probably constitutes about 85% of herds which are not being tested for tuberculosis.

A common practice of hand milking exposes humans to BTB due to the close contact which enhances droplet transmission of tuberculosis. Milk is not regularly pasteurised in pastoral societies and even if soured, can still contain infective levels of mycobacteria as argued by Kazwala. Animals in traditional African farming systems are rarely culled and there is a greater chance for chronic tuberculosis in old cows, especially those subjected to stress. The level of testing and control of BTB in Africa is also considerably restricted by the lack of infrastructure, both veterinary and transport related. (Kazwala, 1996).

2.6 Disease Description

In general, disease-causing mycobacteria cannot withstand adverse conditions such as heat; it survives longer and flourishes well under cold, dark, and moist conditions. Mycobacteria need a host for its growth except in cultured media, where they multiply approximately once every 20 hours. Because of this relatively slow rate of growth, the disease usually takes many months to develop. In some instances, the organisms lie dormant within the host's body for its lifetime, both in animals and in humans, without causing progressive disease.

Compared to other bacteria of the MTB complex, *M. bovis* has a very broad range of animal hosts. This complicates the control of bovine TB, particularly when wildlife species become reservoirs of *M. bovis* for domestic animals.

Bovine TB is a chronic disease, seldom becoming apparent until it has reached an advanced stage. Some infected livestock that seem to be in prime condition, show no evidence of

infection until they are slaughtered and many lesions (tubercule) found in internal organs during meat inspection (Figure 3). Human infection with *M. bovis* can also cause TB that is clinically and pathologically not different from that caused by *M. tuberculosis*. (De La Rua-Domenech, 2006).

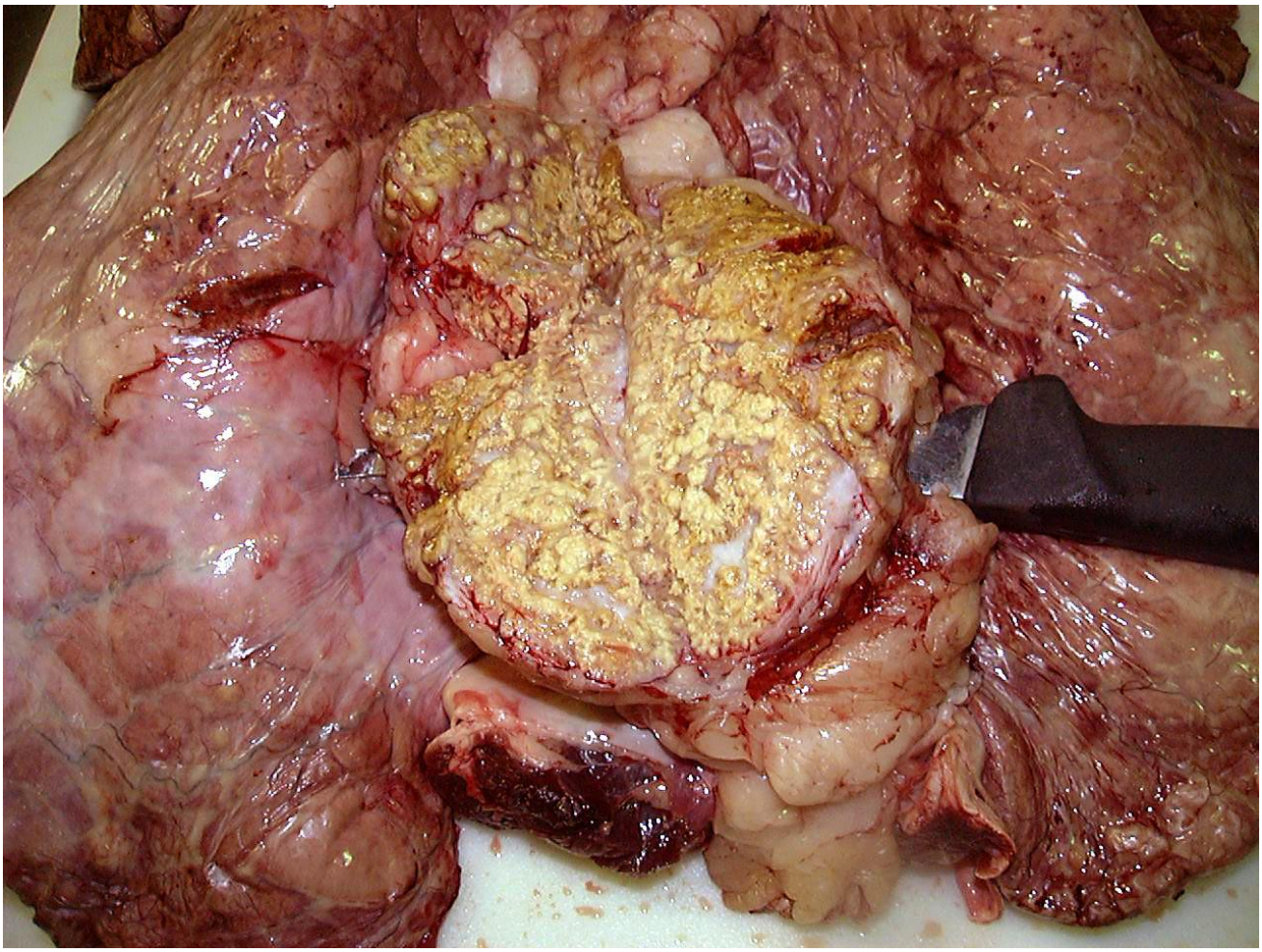

Figure 3: Tuberculosis lesions in the lungs of a two-year-old heifer

Source: http://www.michigan.gov/emergingdiseases/0,4579,7-186-25804-76372--,00.html

2.6.1 Symptoms of Bovine TB in Human

According to McCrindle and Michel, the symptoms of *M. bovis* infection in humans, which include swelling of the lymph-nodes of the neck, tubercles in the intestines and abdominal cavity, skin tuberculosis or even primary pulmonary TB in workers who inhale the bacillus, are not easily differentiated from human TB caused by *M. tuberculosis* (Mccrindle and Michel, 2006).

2.7 General Characteristics and Structure of *Mycobacterium bovis* (Same as *M. tuberculosis)*.

The MTB complex itself is part of a family of more than 80 different bacterial species known as *Mycobacteriaceae*. Most of these bacteria are non- pathogenic with the exception of others responsible for diseases such as Human TB, Bovine TB, and Human leprosy, Johne's disease in ruminants and avian TB. Pathogenic tuberculosis complex grows in warm-blooded animals and can reproduce within the phagocytic cells of humans including dendritic cells and macrophages.

There is no much difference between BTB and MTB; genome sequences of *M. tuberculosis* H37Rv and *M. bovis* 2122 are 99.95 % identical at the nucleotide level (Garnier *et al.*, 2003).

M. bovis which is very similar to *M. tuberculosis* is non-motile and rod shaped gram-positive and nonspore-forming. It has a size of 2-4 µm in length and 0.2-0.5µm in width (Todar, 2010). It is an obligate aerobe and requires a host typically for growth and reproduction. It is a facultative intracellular parasite that is mostly transferred by droplet infection and has a slow generation time, 15-20 hours. It has a cell wall made up of 60% lipid and contains peptidoglycan and is a Gram-positive bacterium although difficult to stain

(Figure 4) (Cole *et a*l., 1998). The lipid in M.TB cell wall is mycolic acids which determine the virulence of the bacteria. Mycolic acids are hydrophobic and help determine the permeability of the cell surface. The cell also contains cord factor which causes colonies of *M. tuberculosis* to grow in a serpentine like fashion. (Figure 5).

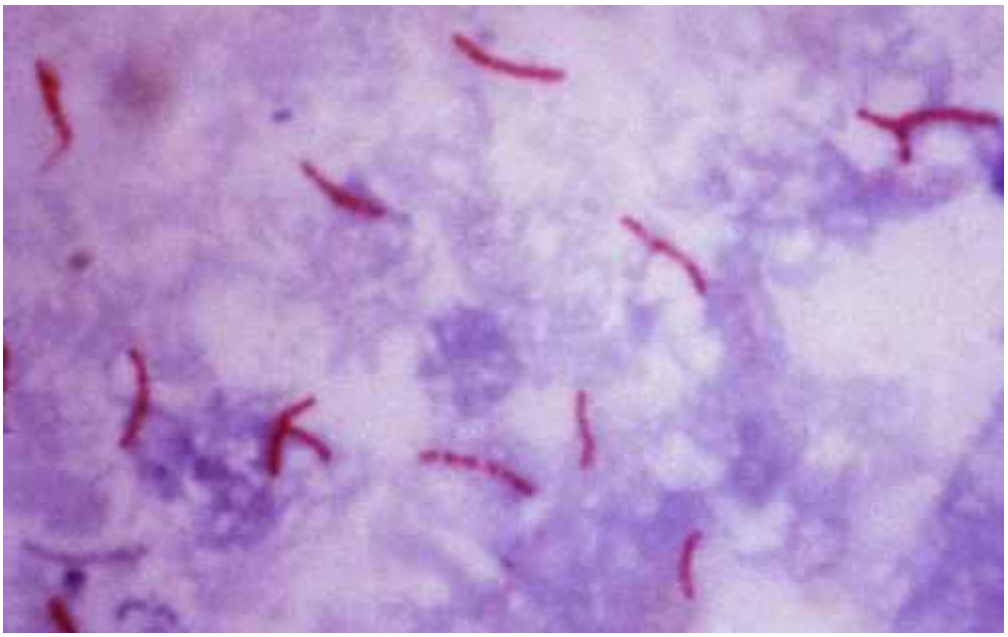

Figure 4: Gram Positive *Mycobacterium tuberculosis*. Acid-fast stain.

Source: http://lgcpublishing.com/pop01.html

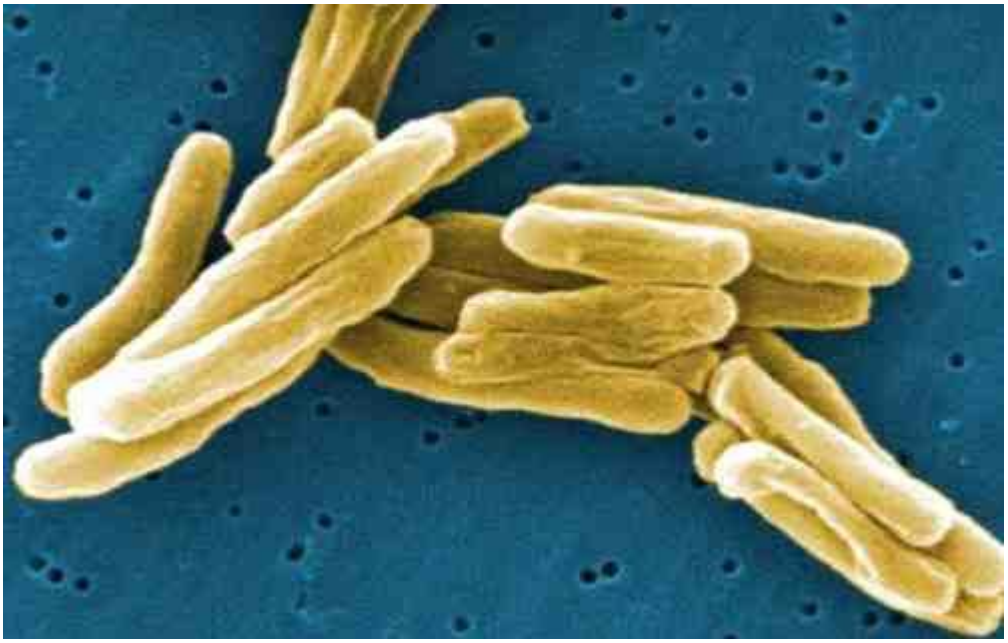

Figure 5: *Mycobacterium tuberculosis* scanning electron micrograph. Mag. 15000X.

Source: http://lgcpublishing.com/pop01.html

2.8 Pathogenesis of Bovine TB

Unlike the pathogenesis of tuberculosis in humans, pathogenesis of BTB has not been well understood (Mitchell *et al*., 2006). According to Neill and others cited in a work done by Mitchell and others, cattle become infected with *M. bovis* by either the oral or respiratory routes. The oral route occur most from tuberculous cows to calves, while the respiratory route is general in cattle (Mitchell *et al*., 2006).

BTB as well as *Mycobacterium tuberculosis* is a disease of the respiratory system. Pathological changes occur mainly in the lungs and associated lymph nodes. After infection, the macrophage is the primary host cell for intracellular growth of *M. bovis*. Phagocytosis then occurs leading to formation of lesions with caseous outline (tubercules). As the disease progresses necrosis, mineralisation and fibrosis occur (Pollock *et al*., 2006).

Upon entering a host organism, the pathogen may lie dormant for years or even decades depending on the immune status of the host cell. After the host response dies down, the

bacteria re-emerges and causes full blown infection within the host organism (Cole, 1998). *Mycobacterium tuberculosis* lives and replicates in the host cell macrophages and some dendritic cells. The bacteria initially grow and divide in the lung alveoli, alveolar ducts, and draining lymph nodes in addition to the phagocytic cells.

The infected macrophages form granulomas consisting of giant cells, T cells, some B-cells and fibroblasts (Flynn, 2001). Under aerobic condition, *M. tuberculosis* sustains itself through glycolysis and oxidative phosphorulation provides the necessary Adenosine Triphosphate. It then sustains itself on lipids while it replicates within the macrophage endosome. Through the process of β-oxidation, *M. tuberculosis* or *M. bovis* degrades and uses host-cell lipids as the precursors for many of its own metabolic processes leading to clinical infection.

Reactivation of latent TB after the primary infection may be triggered by various factors including HIV/AIDS, poor nutrition, old age and stress

2.9 Diagnosis

2.9.1 Diagnosis in animals

For animals, the comparative cervical tuberculin test, serological tests, Ziehl-Neelsen staining culture and post mortem examinations and other newly laboratory procedures such as the Anigen Rapid Ab Test are also currently used.

Bovine TB infection in cattle is usually diagnosed in the live animal on the basis of delayed hypersensitivity reactions. Infection is often subclinical; when present, clinical signs are not specifically distinctive and can include weakness, anorexia, emaciation, dyspnoea, enlargement of lymph nodes, and cough, particularly in the advanced stage.

After exposure to TB an immune response is develop, which can be detected by the tuberculin skin test. Tuberculin is a sterile laboratory product made by growing TB bacteria and killing them with heat. About 72 hours after tuberculin is injected into animals affected with TB, a characteristic swelling reaction appears at the point of injection indicating a positive reaction. A positive test result indicates exposure to one type of mycobacteria. Further diagnostic methods are necessary to confirm the presence of bovine TB.

2.9.1.1Diagnoses BTB during post mortem examination

TB lesions which are usually found in the lungs or any other organ of diseased animals are difficult to find during the early stages of the disease, even during post- mortem examination. Lesions in the forms of nodules or lumps are more evident in the lungs and associated lymph nodes and in the lymph nodes of the head and intestinal tract at the later stages of the disease. These lesions may also appear in the abdominal organs, reproductive organs, nervous system, superficial body lymph nodes, and bones.

Necropsy, histopathological and bacteriological techniques are used for the diagnoses of BTB in diseased animals. Rapid nucleic acid methodologies, such as the polymerase chain reaction (PCR) are also used although these are demanding techniques. The traditional mycobacterial culture remains the gold standard method for routine confirmation of infection.

2.9.1.2 Identification of the agent

Bacteriological examinations may consist of the demonstration of acid fast bacilli by microscopic examination, which provides presumptive confirmation. The isolation of mycobacteria on selective culture media and their subsequent identification by cultural and biochemical tests or DNA techniques, such as PCR, confirms infection.

2.9.1.2.1 Delayed hypersensitivity test

This method is the standard method for detection of BTB. It involves measuring the skin thickness, injecting bovine tuberculin intradermally into the measured area and measuring any subsequent swelling at the site of injection 72 hours later. The recommended dose of bovine PPD in cattle is at least 2000 International Units (IU).

2.9.1.2.2 Blood-based laboratory tests

Diagnostic blood tests are now available, e.g. the gamma interferon assay, which uses an enzyme-linked immunosorbent assay (ELISA) as the detection method for interferon, the lymphocyte proliferation assay, which detects cell-mediated immune responses, and the indirect ELISA, which detects antibody responses. The logistics and laboratory execution of some of these tests may be a limiting factor. (Cousins and Florisson, 2005).

2.9.2 Diagnosis in Human

In humans, although Acid Fast Bacilli staining and mycobacterial culture are still the primary diagnostic tests i.e. microscopy and culture on liquid or solid media in most laboratories. Molecular tests based on nucleic acid amplification techniques are also available for the detection of the *M. tuberculosis* complex in a variety of clinical specimens using PCR (Kiet et al., 2010) . Adoption of rapid TB tests could allow earlier treatment of active cases which would greatly help in preventing the spread of the disease.

In a study by Onubogu and others, patients were screened for AFB by both microscopy and culture. It was observed that more AFB was detected by culture than microscopy and was statistically significant ($p < 0.05$). There was decreased sensitivity of smear microscopy for the diagnosis of TB (Onubogu *et al*., 2012).

Kagu and Ayilara argued in a work done by Onubogu and others in Nigeria that the consequences for this low sensitivity of microscopy are several, including delayed or

misdiagnosed cases, contributing to delay in treatment and increased morbidity and mortality rates (Onubogu *et al.*, 2010). Culture of *Mycobacterium tuberculosis* remains the gold standard for TB diagnosis because it is sensitive and specific for TB in both HIV-positive and HIV-negative individuals (Onubogu *et al.*, 2012). Recent techniques are centred on the use of rapid molecular methods such as line probe assays example of which is the Hains Test in the diagnosis of TB infection (Kiet et al., 2010).

2.10 Control and Prevention

The control of bovine tuberculosis in cattle is key in preventing the disease in human.The control and eradication of zoonotic TB requires the early recognition of preclinical infection in animals and the prompt removal of any infected animals in order to eliminate a future source of infection for other animals and for humans. The treatment of BTB is difficult because *Mycobacterium bovis* is resistant to most antibiotics used for the treatment of human TB (Bilal *et al*., 2010).

M. bovis is innately resistant to pyrazinamide, therefore the standard treatment is isoniazid and rifampicin for 9 months. However, most cattle with BTB are culled. The test and slaughter policy control of BTB in cattle has not been implemented in most African countries due to its financial implications .According to Cosivi and others, of all African nations only seven applied test-and-slaughter policy as a control measure for bovine TB and consider it as a notifiable disease; the remaining control the disease inadequately or not at all. Only about 15% of the cattle population in Africa are found in countries where BTB is a notifiable disease and a test-and-slaughter policy is used. In effect, approximately 85% of the cattle and 82% of the human population of Africa are in areas where bovine TB is either partly controlled or not controlled at all (**Cosivi *et al*., 1998).**

Currently the gold standard for the diagnoses of BTB in cattle internationally is the test and slaughter method in which cattle are tested with the intra-dermal tuberculin test and those that react positive are culled and slaughtered under veterinary supervision.

Routine surveillance of abattoirs for positive BTB carcasses are also used for prevention of the disease by ensuring wholesome meat is delivered to general public (Michel *et al*., 2004). Vaccines are being developed and evaluated for use in bovine and wildlife species, but at this time are not routinely administered as they may compromise the use of the tuberculin skin test and other immunological tests to detect infected animals. There are standard methods for the production of bovine PPD tuberculins and vaccines in accordance with the WHO standard (OIE, 2009).

CHAPTER THREE

3.0 METHODS

3.1 Study Design

A cross- sectional study was conducted between the period of October 2011 to March 2012.

3.2 Study Area

North Tongu District is one of the 18 districts in the Volta Region. It lies within latitude 5^0 47′ North to 6^0 North and longitude 0^0 5′ East. The total area of the district is 1460 square km, which is about 7.1% of the Volta Region. Geographically, it shares borders with South Tongu to the south east, Dangbe East and West Districts to the southwest and with Asuogyaman District to the west. To the north and east, the district is bordered by Ho Municipality and Akatsi District respectively. The district lies within the Tropical Savannah Grassland zone. The total population of the area is130,388. Livestock productions in the district include large ruminants (cattle), small ruminants (sheep and goats) and monogastrics (pigs, rabbits) as well as poultry. Cattle production is mainly done by the extensive system where animals are herded by local cattle boys and Fulani herdsmen for grazing and watering in the mornings and return in late afternoon. Most kraals are situated in the communities and in close proximity to households to minimize theft. There are a few farms that practice semi-intensive system where cattle go for grazing but they are supplemented with hay and leguminous plants like cajanus and stylosanthes.

North Tongu was chosen for the study based on the fact that it reported 2% (9/516) of national bovine TB cases at slaughter (Veterinary monthly summary reports, 2010), According to the District profile of the area, TB is among the top five human diseases in North Tongu and also a high human and cattle interaction alongside risky lifestyle was

observed in the area. Health records show that the top five diseases of the District are Malaria, HIV/AIDS, Cardio vascular Accident, Anaemia and Tuberculosis (Figure 6).

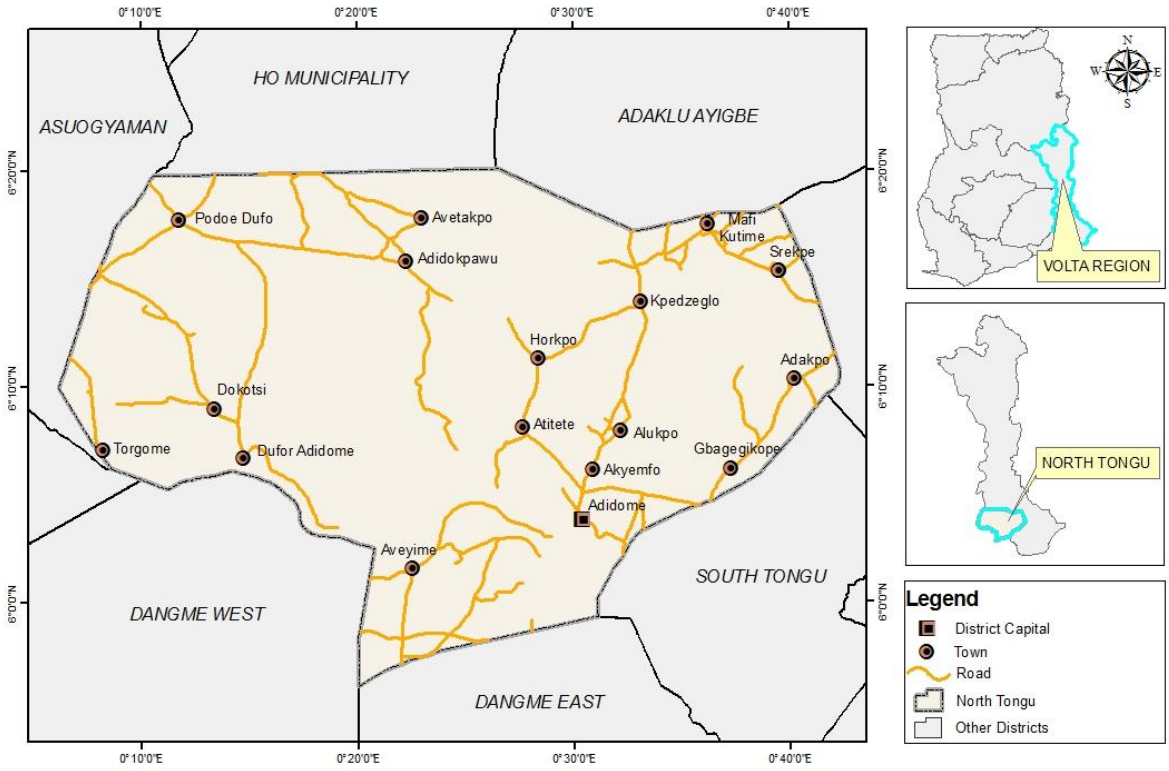

Figure 6: Map of North Tongu District

3.3 Variables

The dependent variables in the study were positive/ negative test result for bovine tuberculosis in both human and cattle.

The independent variables in the human group include socio-demographic characteristics such as age, sex, educational level, religion, ethnicity and occupation as well as occupational characteristics such as experience (years), consumption of raw milk and overcrowding.

The independent variables in cattle include age, sex, breed, herd size, antimicrobial usage, and husbandry practices.

3.4 Sampling Method

The district was purposefully chosen for the study because the district has a high cattle population and reported some percentage of the national figure of BTB cases diagnosed at slaughter in cattle. Also a high human-cattle interaction exist in the area which puts the human population especially those living with the cattle at high risk.

The study was conducted early in the morning, between 6:00am and 8:00am. This time was convenient for the participants since it was before they normally take their cattle for grazing. Sputum samples were more conveniently and effectively taken from participants during the selected period as well.

3.4.1 Cattle Population

3.4.1.1 Study population for cattle

The study population for cattle was all cattle reared in the North Tongu District which is 48, 564 (projected livestock census, 2010).

3.4.1.2 Inclusion criteria for cattle

The inclusion criteria for cattle were all cattle of age 6months and above in North Tongu between October 2011 and March 2012.

3.4.1.3 Exclusion Criteria for cattle

All cattle that were moved from elsewhere to the study location after the commencement of the study were excluded.

3.4.1.4 Sample size for cattle

The sample size for cattle which was 200 was computed from the following formula; $N=\underline{z^2\ p(1-p)/d^2}$,where N= sample size,

z= risk of Type 1 error (=1.96 at 95% confidence level)

p= prevalence of BTB =13.8%

d= precision (allowable error) = 5% = 0.05

Using an estimated prevalence of BTB in cattle of value 13.8 % found from a study that was conducted in Ghana (Bonsu *et al*., 2003) a minimum sample size of 183 was computed and rounded up to 200.

3.4.1.5 Selecting the cattle kraals

Probability proportional to size was used for sampling the animals (Cameron, 1999). The sample frame for cattle was 48,564 animals which came from 825 cattle kraals. Cattle kraals were selected through the following procedure:

- The cattle kraals were listed with their corresponding herd sizes.
- A cumulative sum (CuSum) for the animal population which equalled our sample frame was drawn up.
- A sampling interval (SI) was calculated by dividing the total population (sample frame) by our sample size (n) and got a value of 493.
- A random number between one and 493 was generated using a random numbers table to get a random start (RS) of value 113.
- The random start was located by searching down the cumulative total column to the number which was equal to or greater than the randomly selected number (113).
- The cattle kraal containing that number was selected. This was the random start.

- The random start and the sampling interval were used to compute the sampling series in the sequence: RS, RS+SI, RS+2SI, RS+3SI, RS+4SI.
- The sampling series was used to select the corresponding kraals until the sample size for the study was attained.

3.4.1.6 Selecting the individual animals from the kraals

The selected kraal was visited by the investigator together with the owner or herdsman. The animals were examined first without disturbing them. The animals were then counted aloud by the farmer. By this way, the owner was assigning a temporary identification number to each animal. We checked our list of sampling series and as he reached that number we noted the corresponding animal's number and consequently selected it. When the owner has finished, he was asked to catch the selected animals for examination and specimen collection. The process was repeated for each of the selected kraals until we got our sample size (Cameron, 1999).

3.4.2 Human population

3.4.2.1 Study population for humans

The study population for humans was the population of cattle herdsmen in the North -Tongu District which totalled 1182.

3.4.2.2 Inclusion criteria

All herdsmen of 10 years and above in North Tongu District between October 2011 to March 2012.

3.4.2.3 Exclusion Criteria for humans

People who are physically and mentally unable to give consent were excluded from the study.

3.4.2.4 Sample size for humans

The sample size for herdsmen which was 80 was computed using the formula; $\mathbf{N=\underline{z^2\ p(1-p)/d^2}}$, where N= sample size,

z = risk of Type 1 error (=1.96 at 95% confidence level)

p= prevalence of BTB in human = 3%

d= absolute precision = 5% = 0.05

Using a prevalence of BTB in human of 3% found in a study done by Addo and others in Ghana (Addo *et al*., 2007) a minimum sample size of 44 was computed and rounded up to 80.

3.4.2.5 Sampling of Humans

All persons living in households with the selected kraals who fall within the inclusion criteria and who consented were sampled by simple random sampling using the ballot method. 'Yes or No' were written on folded pieces of papers and those who pick 'yes' were sampled.

3.5 Ethical Considerations

The study protocol was reviewed and approved by the Ethical Review Board of the Ghana Health Service. The protocol was also approved by the Veterinary Services Directorate of the Ministry of Food and Agriculture.

The study protocol was followed and permission sought from the District Health Management Team (DHMT) and the District Assembly. Permission was granted by traditional rulers and the community members for interaction with community members. The subject of the study was discussed with the traditional authorities and community leaders for their ideas as well as their consent and ownership of the study. Verbal and written consent was duly sought from the study participants for voluntary participation in the study (Participant's Consent and Assent forms - Appendix 4 and 5 respectively). There were no potential risks involved in this study apart from the little discomfort that was experienced during sample taking. There was no conflict of interest in the study. Study participants benefited from this study by getting to know the status of their cattle and themselves as to BTB.

All sputum samples collected from humans had numbers assigned to and not names of study participants to ensure confidentiality. Data was stored in computer with password and used only by authorized persons.

3.6 Data Collection Methods/ Techniques and Tools.

3.6.1 Pre-testing of Questionnaire

The questionnaire was validated by pre-testing in Ho Zongo community which is similar to the selected areas. Repeated questions were checked, ambiguities removed and complex questions simplified. This exercise was also used to strengthen training for the research assistants who were recruited from both the Health and Veterinary sectors.

3.6. 2 Questionnaire survey

Structured questionnaires were administered to eligible and consenting participants for socio-demographic, risk (predicting) factors and to assess their knowledge on the occurrence of BTB and its control. The owners of the selected cattle were also interviewed on herd composition and risk (predicting) factors of the disease in their animals (See Appendix for Study Questionnaire).

3.6.3 Sample Collection Methods

3*.6.3.1 Sputum sample collection from Humans*

After carefully labelling sputum containers, participants were asked to cough up to produce about 3- 5ml of sputum into the containers and tightly closed the lid. Sputum samples were then transported to the Ho Municipal Tuberculosis Training Centre for laboratory analysis maintaining the cold chain. The sputum samples were worked on the same day.

3.6.3.2 Blood Sample Collection from cattle

For the identification of the animals, researchers relied on owners' experience. Owners have given names to each of their animals. These names are reliable and depict the colour, shape and other resemblances of the animal as to when it was born. These names were therefore used for those animals that do not have tags for identification and trace back.

After the area was disinfected with 70% alcohol about 5 ml of blood was collected from jugular vein of each cattle with sterile disposable syringe and needle and was kept undisturbed on a tray for at least 30 minutes at room temperature in a slightly inclined position to facilitate clotting and separation of serum. After this period, the clotted blood samples with sera were transferred to Ho Veterinary Laboratory where they were kept in a refrigerator at 4^0C overnight. The sera were centrifuged at 2500 rpm for 10 min. After

centrifugation, clear sera were formed and then poured into carefully labelled eppendorf vials for the analysis. The test tubes were labelled with the kraal labels and the name or tag number of the animal. The same labels were copied on the eppendorf vials with permanent marker just before pouring in the sera. The eppendorf vials were then stored in ice chamber at -20^0C until the time for testing.

3.6.4 Laboratory Method

For the diagnoses of tuberculosis in human samples Ziehl-Neelsen Staining was used because this is the currently used method for tuberculosis diagnoses in the country at the peripheral levels of the health system. For the diagnoses of BTB in cattle, Anigen Rapid BTB Ab Test Kits was used. Anigen Rapid Test Kit is a chromatographic immunoassay for the qualitative detection of *Mycobacterium bovis* antibody in plasma and serum which has many advantages over the tuberculin skin test. Whole blood or serum could be used for this method. For this study serum was used for the laboratory analysis.

3.6.4.1 Ziehl-Neelsen Staining(Human samples)

This is differential staining method was introduced by Ehrlich in 1882 and was subsequently modified by Ziehl and Neelsen independently. This staining method is useful in staining mycobacteria in clinical specimens. Mycobacterial cell wall is made up of a waxy material (mycolic acid) that normally does not allow ordinary stains to enter the cell. The staining technique comprises of a primary stain, a decolouriser and a counterstain. The primary stain, which is typically concentrated (strong) carbol fuchsin, is made by dissolving the dye basic fuchsin in phenol. Basic fuchsin dissolves better in phenol than in water. Heating the slide softens the waxy material of cell wall and phenolised dye readily enters the cell. Once stained by this method, these bacteria do not readily decolorize by weak mineral acids. Visualisation

is done by counterstaining with methylene blue solution. The acid fast bacilli appeared pink in colour.

Ziehl-Neelsen Staining Procedure:

A. Preparing the slide

Slides were correctly labelled with participants identification number (ID) and date of running the test.

The sputum container was carefully opened and the most mucopurulent part of the sample was picked with an applicator onto the centre of the slide.

The sputum was spread uniformly to get a size between 1-2 cm width and 2-3 cm length.

The wooden applicator was discarded into a discard jar with disinfectant.

The smear was allowed to dry completely.

The smear was fixed by passing it over a burner 2 -3 times.

B. Carbol Fuchsin Staining (*Ziehl-Neelsen method*):

1. The fixed slides were arranged on a staining rack leaving enough spaces between slides to prevent cross contamination.

2. Carbol Fuchsin was then poured onto the slides.

3. The slides were heated from beneath until steam comes out. It should be noted that the solution was not supposed to boil but only to steam.

4. The slides were left for 5minutes to cool down and then washed off excess carbol fuchsin with water.

C. Decolourisation with H_2SO_4

1. The slides were then covered with 20% H_2SO_4 for about 5 minutes as decolouriser and drained.

2. The slides were then washed with water.

D. Methylene blue counter staining

After the carbol fuchsin staining, the slides were covered with methylene blue for 1 minute, rinsed and drained.

The slides were then left to dry completely on a slanting rack.

3.7.3.1.2 Smear Examination

After slides were completely dried, a drop of immersion oil was placed on the smear. The slide was then viewed and graded according to the Grading standard for Ghana (WHO, STD).

The grading Standard for Ghana for Identification of Mycobacterium species by staining (Addo *et al*, 2001) is shown below in Table 1.

Table 1: Grading of AFB, Ghana, WHO standard.

Grading	**Number of AFB found in a field**
Negative	No AFB found in at least 100 fields
Scanty	1-9 AFB found in100 fields
1+	10-99 AFB found in 100 fields
2+	1-10 AFB found per in at least 50 fields
3+	More than 10 AFB per field in at least 20 fields

Source: (Addo *et al.*, 2001)

3.6.4.2 Anigen Rapid BTB Test (Cattle samples)

Anigen Rapid BTB Test is a chromatographic immunoassay for the quantitative diagnoses of *mycobacterium bovis* antibody in blood plasma and serum. This test has numerous advantages over the PPD skin testing for BTB (Appendix 1).

The Anigen Rapid BTB test kit has a 'T and C' line as Test and control Lines on the surface of the kit. Both the 'T and the C' lines in the result window are invisible before any sample is applied. The 'control Line' is used as the procedural control.

Procedure for using blood Serum for the Anigen Rapid BTB Test: The test kit was placed on a flat surface. One drop of serum was dropped with a capillary tube into sample hole located on the test kit and waited for 1min. Three drops of developing buffer were added into the developing hole and results read in 20 minutes (Figure 7). Two bands of purple colour within the result window indicated a positive result. A single purple band within the result window indicates a negative result. If there is no visible band within the result window the result is invalid and recommended for retesting.

Figure 7: Research Team Preparing work environment for running Anigen Rapid BTB Test on Animal samples, Volta Regional Veterinary Laboratory, Ho, February, 2012.

3.7 Data Storage, Processing and Analysis

All hard copy data were stored in a safety cabinet. Data was entered into Epi Data, coded and exported into SPSS version 16.0 for analysis. Data was cleaned and descriptive and statistical analysis was done.

3.7.1 Data Analysis: Data was entered and cleaned in Epidata. For socio-demographic categorical data (e.g. sex), summary tables of counts and percentages were presented. For socio-demographic continuous data (e.g. age), summary tables of means and mode were presented.

Pearson's Chi-Square test was used to test associations between demographic data and dependent variables. Tabulations of frequencies (and percentages) and graphical presentations were done using Statistical Package for Social Sciences (SPSS) software package (version16). All statistical tests were declared significant for p-value < 0.05.

3.8 Quality Control

The outcome of a research work needs to be reliable with minimal errors, hence in this study, we took steps to ensure the good quality of work in all the stages of the study.

To this end;

The interview questionnaire was translated into the local language to standardize the meaning so that there is a common understanding of the ways to ask questions during the interviews.

- Research assistants with adequate knowledge were selected from both the Veterinary Service department and the Ghana TB control programme for the study.
- A day's training on the administration of the interview questionnaire and all other related instructions such as communication skills when communicating to respondents was organised for investigating team.
- The Researcher was responsible for distribution of questionnaires before the interviews and collection after completion of the day's work.
- All questionnaires submitted at the end of the day were reviewed by team members before submission. Data was carefully checked for completeness and internal consistencies of each questionnaire. Questionnaires found to be incomplete and with inconsistent information were corrected by performing fresh interviews.
- Supervision was rigorously enforced in the field to ensure quality collection of data.
- Blood samples were processed and transported safely under cold chain facilities to the laboratory for testing to begin on the same day.

CHAPTER FOUR

4.0 RESULTS

4.1 Cattle Population

A total of 200 cattle were sampled. The prevalence of bovine TB in cattle was found to be 19% (Table 2). All cattle samples that tested positive came from cows.

Table 2: Laboratory result of cattle for bovine TB, North Tongu District, October 2011- March 2012.

Species	Status	Counts	%
Cattle	Positive	38	19
	Negative	162	81

Of the 200 cattle 14 were bulls and were186 cows. Ten were exotic breeds, 95 were local breeds, and 95 cross breeds. Ninety nine percent (193/194) of cattle were kept under the semi- intensive system and the rest under the intensive system. Eighty four percent (164/195) of cattle were routinely treated with antibiotics (Table 3).

Table 3: Possible Risk Factors of BTB in cattle, North Tongu District, October 2011- March 2012

Variable	Item	Counts	%
Husbandry System	Semi-intensive	193	99.5
	Intensive	1	0.5
	Total	**194**	
Breed	Local	95	47.5
	Exotic	10	5.0
	Crossbreed	95	47.5
Antibiotic Usage	Yes	164	84.1
	No	31	15.9
	Total	**195**	
Sex	Male	14	7.0
	Female	186	93.0
	Total	**200**	**100**

Kraal density is statistically associated with seropositivity of cattle to bovine TB (p=0.001) but all the other risk factors considered were not statistically associated (Table 4).

Table 4: Seropositivity of cattle to bovine TB and related possible Risk factors, North Tongu District, October 2011- March 2012.

Factor	P-Value	Odd Ratio	95% Confidence Interval	
			Lower	Upper
Kraal density	0.001	-	-	-
Age of cattle	0.936	-	-	-
Sex of cattle	0.235	0.306(male/female)	0.039	2.411
Type of breed	0.650	-	-	-
Type of husbandry	0.621	-	-	-
Cough/Running nose	0.552	0.778(yes/no)	0.339	1.784
Treated with antimicrobial	0.313	1.765(yes/no)	0.578	5.388

Cattle between the ages of 4-6years (97- 120months) were most infected with bovine TB (Figure 8).

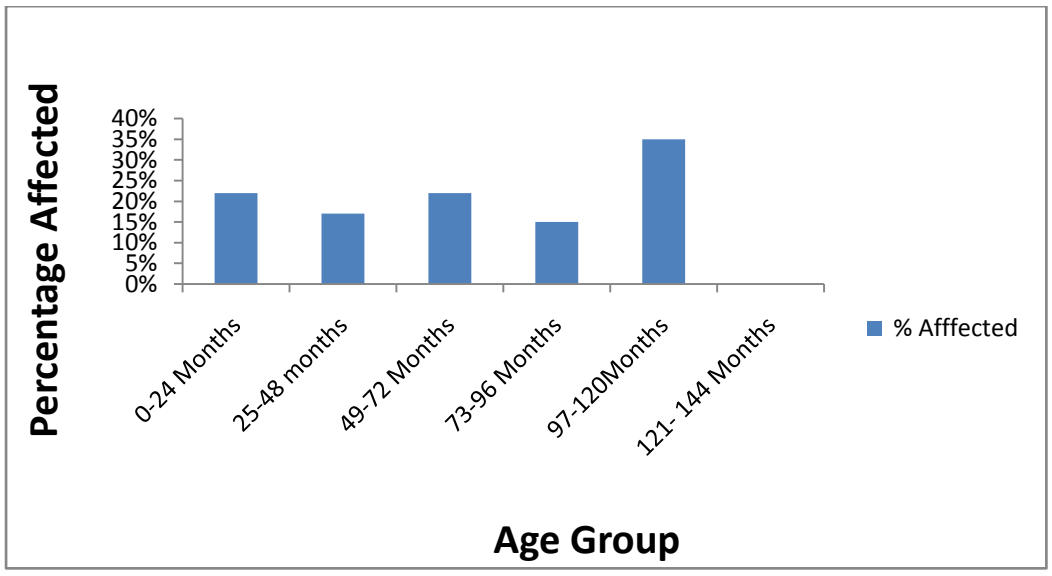

Figure 8: Seropositivity of BTB in cattle by age groups, North Tongu District, October 2011- March 2012.

4.2 Human population

A total of 68 herdsmen were sampled. Almost 68% (46/68) of participants were males. All human samples tested Negative for Acid Fast Bacilli (AFB) (Table 5). Majority of respondents were between the ages of 11- 20 years and the median age group of respondents was 31-40 years (Table 6).

Table 5: Laboratory result of humans for TB, North Tongu District, October 2011- March 2012.

Species	Status	Counts	%
Human	Positive	0	0
	Negative	68	100

Table 6: Characteristics of participants, North Tongu District, October 2011- March 2012.

Variable	Item	Count	%
Age	1-10	1	1.5
	11-20	18	26.5
	21-30	5	7.3
	31-40	13	19.1
	41-50	16	24
	51-60	7	10.3
	61-70	5	7.3
	71-80	3	4.0
Sex	Male	46	68
	Female	22	32

The majority of the respondents were illiterates. Among those who had education, middle school/JSS was the highest level of education attained (Table 7).

Table 7: Characteristics of participants, North Tongu District, October 2011- March 2012. Continued.

Variable	Item	Count	%
Educational Level	Primary	16	24
	Middle/JSS	17	25
	Secondary/SSS	2	2.5
	Tertiary	2	2.5
	None	31	46
Ethnic group	Mole Dagbani	13	19
	Ewe	49	72.1
	Krobo	1	1.5
	Other	5	7.4
Religion	Christian	28	41.2
	Moslem	20	29.4
	Traditional	8	11.8
	None	8	11.8
	Other	4	5.8

Most of the participants indulge in risky practices such as consuming fresh milk, sharing bulls for breeding as well work without protective clothing that could expose them to contracting bovine TB (Table 8).

Table 8: Possible risk factors of bovine TB in human, North Tongu District, October 2011- March 2012.

Variable	Response	Count	%
Fresh milk consumption	Yes	61	89.7
	No	7	10.3
Protective clothing usage during herding and milking	Yes	11	16.2
	No	57	83.8
Bull sharing with other farms	Yes	48	70.6
	No	20	29.4

CHAPTER FIVE

5.0 DISCUSSION

5.1 Prevalence of BTB in cattle from study

The 19% prevalence rate of BTB in cattle that was found from this study indicates the existence of the disease in the area. The prevalence found in this study is higher than what was found by Bonsu and others. This could mean that active search other than passive surveillance yielded more results as their work was bases on routine data from slaughter houses (Bonsu *et. al*., 2003). Ibrahim and others however found a higher prevalence of BTB in cattle than was found in this study. This might be due to the fact that they used a larger sample size (Ibrahim *et al*., 2010).

5.2 Risk factors of BTB in cattle from the study

The strong statistical association found between seropositivity and the kraal density demonstrates the effect of overcrowding on the degree of transmission of bovine TB within the flocks and agrees with the findings of Humblet and others who found that seropositivity of cattle to BTB and animal density were statistically significant (Humblet *et al*., 2010).

The increase in seropositivity of cattle to BTB with age which was though found to be statistically insignificant agrees with the fact that BTB manifests most at the later stage of the disease. It could be that the infection occurred some period in the past and as the years go by becomes more manifest. These cattle are potential reservoirs of the disease.

The finding from this study which shows that cattle between 4-6 years were mostly affected, agrees with the findings in a work done by Kazwala and others where it was found out that older cattle were more affected by the BTB than yearlings and calves (Kazwala *et al*., 2001).

The usual purpose of treatment of animals with antimicrobial is to reduce the level of infection in the herd. This was not the case in the study; our findings might be due to the fact that the antimicrobials that were used were not effective against *M. bovis* since *mycobacterium bovis* is resistant to many antibiotics (Bilal *et al*., 2010).

The study result which shows cows were thrice infected as bulls by BTB agrees with the findings of Bonsu and others who found from their study that cows were twice affected as bulls by BTB (Bonsu *et al*., 2003). In practice this could mean that cows are more at risk of contracting BTB especially during pregnancy when they have reduced immunity since tuberculosis flourishes well in immuno-compromised host. In addition, for the purpose of increasing production, most cattle farmers have more cows than bulls on their farms. This gives rise to more females being sampled.

5.3 Prevalence of BTB in human from study

In the human population, the zero prevalence of BTB that was found does not necessarily indicate the absence of the disease, as humans are exposed in diverse ways to bovine TB infection putting them at a very high risk. This can be seen in our results where 90% (61/68) of respondents consume fresh milk and 84% (57/68) do not use protective clothing coupled with a third factor that these people live in close proximity with the animals. It could also mean that milk may be infected with *Mycobacterium bovis* but there were other underling factors that made it impossible for us to detect the pathogen among humans or herdsmen might have strong immune systems that combat the disease.

5.4 Study Limitation

We could not perform regression analyses because all human samples tested negative for tuberculosis. If there were to have been positive TB cases in human, those positive samples

would have been subjected to molecular typing to see if the strains involved were the same as the strains in cattle. We could then have run a regression for possible associations.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

BTB is endemic in cattle in North Tongu District with a prevalence rate of 19%.

The total number of animals in a kraal (Kraal density) is a risk factor for cattle contracting bovine tuberculosis.

The prevalence of tuberculosis in herdsmen in the North Tongu District is zero; likewise we did not identify any risk factor of bovine TB among herdsmen.

6.2 Recommendations

Ghana Health Service/Veterinary Services/ TB Control Programme

Due to the restricted time of our study, we recommended that the study should be replicated using a larger sample size in both human and cattle to gather more understanding on the transmission of bovine TB from cattle to human.

Veterinary Services Directorate in North Tongu

A study should be carried to determine if milk from cattle in the area is infected with bovine TB or not since 90% of participants admitted consuming fresh milk but tested negative for tuberculosis.

REFERENCES

Abubakar, I.A. (Ed.). (2007). Molecular Epidemiology of human and BTB in the federal capital territory and Kaduna State, Nigeria, PhD Thesis, Plymouth University, UK.

Abubakar, U.B., Ameh, J.I., Abdulkadir, I.A., Salifu, I., Okaiyeto, S.O. and Kudi, A.C. 2011. Bovine tuberculosis in Nigeria: A review. *Veterinary Research*.**4**:24-27.

Abubakar, U.B., Shehu, S.A. and Mohammed, F.U. (2011). Retrospective Study of Tuberculosis in Slaughtered Cattle at Maiduguri Abattoir, Nigeria**.** *Veterinary Research*. **4**: 1-4.

Addo, K.K., Caulley, P., Yebuah-Manu, D.K., Owusu-Darko, K., Dan-Dzide, M., Minamikawa, M. and Akpedonu, P. (2001). Tuberculosis Microscopy. A laboratory Manual for Ghana. pp: 27-36.

Addo, K.K., Owusu-Darko, O.K., Yeboah-Manu, D., Caulley, P., Minamikawa, M., Bonsu, F., Leinhardt, C., Akpedonu, P. and Ofori-adjei, D. (Eds.). (2007). Mycobacterial species causing pulmonary tuberculosis at the Korle- bu Teaching Hospital, Accra. *Ghana Medical Journal*. **41**:52-57.

Amanfu, W. (2006). The situation of Tuberculosis and tuberculosis control in animals of economic interest. *Tuberculosis*. **86**:330-335.

Amdekar, Y. K. (2005). Tuberculosis—persistent threat to human health. *Indian Journal of Pediatrics*. **72**: 333–338.

Ayele, W.Y., Neill, S.D., Zinsstag, J., Weiss, G. M. and Pavlik, I. (2004). BTB: an old disease but a new threat to Africa. *The International Journal of Tuberculosis and Lung* Disease. **8**: 924-937.

Bilal, S., Igbal, M., Murphy, P. and Power, J. (2010). Human bovine tuberculosis- remains in the differential. *Journal of Medical Microbiology*. **59**: 1379-1382.

Bonsu O.A., Laing E. and Akanmori B.D. (2003). Prevalence of Tuberculosis in Dangme-West District of Ghana, Public Health implications. *Actna Tropica*. **76**:9–14.

Boukary A.R., Thys E., Abatih E., Gamatié D., and Ango I, (2011). BTB Prevalence Survey on Cattle in the Rural Livestock System of Torodi (Niger). *PLoS ONE* **6**: 24629.

Cadmus, S.I., Adesokan, H.K., Akinbowale, J.O., Soolingen, D.V. (2009). Mycobacterium bovis and M. tuberculosis in Goats, Nigeria. *Emerging Infectious Diseases*. **15**: 2066–2067.

Cameron, A. R. (Ed.). (1999). Survey Toolbox: A Practical Manual and software package for Active Surveillance of Livestock Diseases in Developing countries. *Australian Centre for International Agricultural Research (ACIAR*). pp. 54-61.

CDC, (2012). TB Elimination. Available at : http://www.cdc.gov/tb/publications/factsheets/general/LTBIandActiveTB.pdf. Accessed on 25 June, 2012.

Chadha, V.K. (2009). Progress Towards Millennium Goals for the TB Control in Seven Asian Countries. Indian Journal of Tuberculosis.**56**:41.

Cole, S.T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S.V., Eiglmeier, K., Gas, S., Barry, C.E., Tekaia, F., Badcock, K., Basham, D., Brown, D., Chillingworth, T., Connor, R., Davies, R., Devlin, K., Feltwell, T., Gentles, S., Hamlin, N., Holroyd, S., Hornsby, T., Jagels, K., Krogh, A., McLean, J., Moule, S., Murphy, L., Oliver, K., Osborne, J., Quail, M.A., Rajandream, M.A., Rogers, J., Rutter, S., Seeger, K., Skelton, J., Squares, R., Squares, S., Sulston, J.E., Taylor, K., Whitehead, S. and Barrell, B.G. 1998. Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. *Nature*. **396**: 190.

Cosivi, O., Daborn, C.J., Grange, J.M., Raviglione, M.C. and Fujikura, T. (1998). Zoonotic Tuberculosis due to Mycobacterium bovis and M. Tuberculosis in goats, Nigeria, *Emerging Infectious Diseases*. **4**: 59-70.

Cosivi, O., Grange, J.M. and Daborn, C.J. (2006). BTB in Dairy cattle farm as a threat to public health, *African Journal of Microbiology Research*. **3**: 446-450.

Cousins, D.V. and Florisson, N. (2005). A review of tests available for use in the diagnosis of tuberculosis in non-bovine species. *Scientific and Technical Review, International Office of Epizootics Epizooties*. **3**: 24.

Danbirni, S., Sackey, A.K.B.. Ayo, J.O., Baw, E.K., Kudi, A.C., Okaiyeto, S.O. and Pewan, S.B. 2010. Exposure and shedding in milk of *Mycobacterium tuberculosis* in Dairy herds using One-Step Anigen Rapid Bovine Tuberculosis Antibodies Test and Ziehl-Neelsen Stain. *Veterinary Research*. **3**:38-43.

Davies, P. D. O, (Ed.). (1994). Clinical Tuberculosis, Chapman & Hall, Boca Raton, Fla, USA, 1st edition.

De Kantor, I. N., LoBue, P. A. and Thoen, C. O. (2010). Human tuberculosis caused by Mycobacterium bovis in the United States, Latin America and the Caribbean. .*International Journal of Tuberculosis and Lung Disease*. **14**: 1369–1373.

De La Rua-Domenech, R. (2006). Human Mycobacterium bovis infection in the United Kingdom: incidence, risks, control measures and review of the zoonotic aspects of BTB. *Tuberculosis*. **86**:77–109.

Dinka, H. and Duressa, A. (2011). Prevalence of bovine tuberculosis in Arsi Zones of Oromia, Ethiopia. *African Journal of Agricultural Research*. **6** :3853-3858.

Doran, P., Carson, J., Costello, E. and More, S. J. (2009). An outbreak of tuberculosis affecting cattle and people on an Irish dairy farm, following the consumption of raw milk. *Irish Veterinary Journal*. **62**: 390–397.

Flynn, J. L. and Chan, J. (2001). Immunology of Tuberculosis. *Annual Review of Immunology*.**19**: 93-129.

Garnier, T., Eiglmeier, K., Camus, J.C., Medina, N., Mansoor, H., Pryor, M., Duthoy, S., Grondin, S., Lacroix, C., Monsempe, C., Simon, S., Harris, B., Atkin, R., Doggett, J., Mayes, R., Keating, L., Wheeler, P.R., Parkhill, J., Barrell, B.G., Cole, S.T., Gordon, S.V. and Hewinson, R.G. (2003). The complete genome sequence of Mycobacterium bovis. *Proceeding of the National Academy of Sciences of the United States of America*. **100**:7877-7882.

Georghiou, P., Patel, A.M. and Konstantinos, A. (1989). *Australian and New Zealand Journal of Medicine*. **19**: 409–410.

Ghana Health Service, (2012). Available at: http://www.ghanahealthservice.org/articles.php?nd=112&tt=Easter%20revellers%20asked%20to%20help%20curb%20the%20spread%20of%20TB. Accessed on18th June, 2012.

Hlavsa, M. C., Moonan, P. K. and Cowan, *L. S.* (2008). Human tuberculosis due to Mycobacterium bovis in the United States. *Clinical Infectious Diseases*. **47**: 168–175.

http://lgcpublishing.com/pop01.html. Accessed on 26th June, 2012.

http://www.michigan.gov/emergingdiseases/0,4579,7-186-25804-76372--,00.html Accessed on 25th May, 2012.

Humblet, M. F., Gilbert, M., Govaerts, M., Fauville-Dufaux, M., Walravens, K. and Saegerman, C. (2010). New Assessment of BTB Risk Factors in Belgium Based on Nationwide Molecular Epidemiology. *Clinical Veterinary Microbiology*. **48**: 2802-2808.

Humblet, M.F., Boschiroli, M.L. and Saegerman, C. (2009). Classification of worldwide BTB risk factors in cattle: a stratified approach. *Veterinary Research*. **40**: 50.

Ibrahim, S., Agada, C.A., Umoh, J.U., Ajogi, I., Farouk, U.M. and Cadmus, S.I.B. (2010). Prevalence of BTB in Jigawa State, north-western Nigeria. *PLoS ONE*. **42**: 1333-1335.

Ingram, P. R., Bremner, P., Inglis, T. J., Murray, R. J. and Cousins, D. V. (2010). Zoonotic tuberculosis: on the decline, *Communicable Diseases Intelligence*. **34**: 339–341.

Kaneene, J.B. and Theon, C.O. (2004). Tuberculosis. *Journal of the American Veterinary Medical* Association. **224**:685

Kang'ethe, E.K., Ekuttan, C.E. and Kimani, V.N. (2007).Investigation of the prevalence of BTB and risk factors for human infection with BTB among dairy and non-dairy farming neighbour households in Dagoretti Division, Nairobi, Kenya. *Academia. education*. **84:** 92-95.

Kazwala, R.R (1996). Molecular epidemiology of bovine tuberculosis in Tanzania. A PhD

Kazwala, R.R., Kambarage, D.M., Daborn, C.J., Nyange, J., Jiwa, S.F.H. and Sharp, J.M. (2001).Risk Factors Associated with the Occurrence of BTB in Cattle in the Southern Highlands of Tanzania. *Veterinary Research*. **25**: 609-614.

Kiet, V.S., Lan, N.T.N., An, D.D., Dung, N.H., Hoa, D. V., Chau, N.V.V., Chinh, N.T., Farrar, J. and Caws, M. (2010). Evaluation of the MTBDRsl Test for Detection of Second-Line-Drug Resistance in *Mycobacterium tuberculosis*. *Journal of Clinical Microbiology*. **48**: 2934–2939.

Laval, G., and Ameni, G., (2004). Prevalence of BTB in zebu cattle under traditional animal husbandry in Boji district of western Ethiopia. *RevMedVet*.**155**: 494-499.

LoBue, P. A., Enarson, D. A. and Thoen, C. O. (2010). Tuberculosis in humans and animals: an overview, *International Journal of Tuberculosis and Lung Disease*.**14**: 1075–1078.
Mahmood, S.A.I.2010. TB and HIV/AIDS in Bangladesh. *Journal of AIDS and HIV Research*. **2**: 68.

McCrindle, C.M.E. and Michel, A. (2006). Status for controlling BTB in Africa. Available at : http://www.pathobiologics.org/ivphc/ref/MCCRINDLE_SHANGAI_2006.pdf. Accessed on 17th June, 2012.

Michel, A.L.; Meyer, S.; McCrindle, C.M.E. and Veary, C.M. (2004). Community based veterinary public health systems, current situation, future trends and recommendations. In: FAO expert consultation on community based animal health systems (FAO Animal Production and Health Proceedings). *Food and Agricultural Organization.* pp. 71-79.

Mishra, N.C. and Singh, K. (2012). Comparative Computational Analysis of Mycobacterium species by Different Techniques in study. Advances in Life Sciences and Technology. **5**: 58

Mitchell, V. Palmer, M.V. and Waters, W. R. (2006). Advances in BTB diagnosis and pathogenesis: What policy makers need to know. *Veterinary Microbiology*. **112**: 181–190.

Munyeme, M., Muma, J.B., Skjerve, E., Nambota, A.M., Phiri, I.G.K., Samui, K.C., Dorny, P. and Tryland, M. (2008). Risk factors associated with bovine tuberculosis in traditional cattle of Livestock/Wildlife interface areas in the Kafue basin of Zambia. *Preventive Veterinary Medicine*. **85**:317-328.

OIE Terrestrial Manual, (2009). Available at:
http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.07_BOVINE_TB.pdf.
Accessed on 28/5/2012.

Onubogu, C. C., Kunle-Ope, C. N., Onyejepu, N., Nwokoye, N. N., Raheem, T. Y., Igbasi, U. T., Tochukwu, N. E., Omoloye, R. M., Ejezie, C. O., Musa, A. Z., Odunukwe, N. N., Onwujekwe, D. I. and. Idigbe, E. O. (2010). Prevalence of tuberculosis and human immunodeficiency virus (TB/HIV) co-infections amongst patients with bronchopulmonary disorders in Lagos. *African Journal of Microbiology Research*. **4**: 1904-1908.

Onubogu, C.C., Nwokoye, N.N., Kunle-Ope, C.N., Raheem, T.Y., Igbasi, U.T., Tochukwu, N., Ejezie, C.O., Onyejepu, N., Omoloye, R., Onwujekwe, D. and Idigbe, E.O. (2012). Sensitivity of Direct Smears Microscopy for the diagnosis of TB in high HIV prevalent population. Scientific Research and Essays. **7**:593-597.

O'Reilly, L.M. and Daborn, C.J. (1995). The epidemiology of Mycobacterium bovis infections in animals and man: a review. *Tubercle and Lung Disease*. **76**: 1–46.

Pollock, J.M., Rodgers, J.D., Welsh, M.D. and McNaire, J. (2006). Pathogenesis of Tuberculosis. The role of Experimental models of infection. *Veterinary Microbiology*. **112**: 141- 150.

Porphyre, T, Stevenson, M.A. and McKenzie, J. (20080. Risk factors for BTB in New Zealand cattle farms and their relationship with possum control strategies. *Preventive Veterinary Medicine*. **86**:93-106.

Regassa, A., Tassew, A., Amenu, K., Megersa, B., Abunna, F., Mekibib, B., Macrotty, T. and Ameni G. (2010). A cross-sectional study on BTB in Hawassa town and its surroundings, Southern Ethiopia**.** *Tropical Animal Health and Production*. **42**: 915-920.

Regassa, A., Medhin, G. and Ameni, G. (2008). BTB is more prevalent in cattle owned by farmers with active tuberculosis in central Ethiopia. *Veterinary journal London*. **178**: 119-125.

Shirima, G.M., Kazwala, R.R. and Kambarage, D.M. (2003). Prevalence of BTB in cattle in different farming systems in the eastern zone of Tanzania. *Preventive Veterinary* Medicine. **57**: 167–172.

Shitaye, J.E., Tsegaye, W. and Pavlik, I. (2007). Bovine Tuberculosis infection in animal and human populations in Ethiopia: a review. Veterinarni Medicina. **52**: 317-332.
thesis submitted to the University of Edinburgh (UK).

Thoen, C., LoBue, P. and De Kantor, I. 2006. The importance of Mycobacterium bovis as a zoonosis, *Veterinary Microbiology*.**112**: 339–345.

Todar, K. (Ed.). (2010). Todar's online Textbook of Bacteriology- Mycobacterium tuberculosis and Tuberculosis. pp.1-4.

Tschopp, R., Schelling, E., Hattendorf, J., Aseffa, A. and Zinsstag J. (2009). Risk factors of BTB in cattle in rural livestock production systems of Ethiopia. *Preventive Veterinary Medicine*. **89**: 205–211.

Veterinary monthly summary reports. Veterinary Services Directorate, Accra. (2010).

Watts H.G. (1996). Current Concepts Review- Tuberculosis of Bones and Joints. *The Journal of Bones and Joint Surgery*. **78**: 288-299.

WHO, 2005. WHO declares TB an emergency in Africa. Available at:
http://www.who.int/mediacentre/news/releases/2005/africa_emergency/en/index.html.
Accessed on 26 June, 2012.

WHO, 2010. Incidence of Tuberculosis Around the World. Available at:
http://lgcpublishing.com/pop04.html. Accessed on 25th June 2012.

WHO, 2012a- Tuberculosis.. Available at :
http://www.who.int/mediacentre/factsheets/fs104/en/. Accessed on 23rd May, 2012a.

WHO, 2012b- Tuberculosis Country Profiles. Available at: http://www.stoptb.org/countries/tbdata.asp. Accessed on 26June, 2012.

WHO. 2006. The Global Plan to Stop TB, 2006–2015. Actions for life: towards a world free of tuberculosis. *International Journal of Tuberculosis and Lung Diseases*. **10**: 240–241.

Wilkins, M.J., Meyerson, J., Bartlett, P.C., Spieldenner, S.L., Berry, D.E., Mosher, L.B., Kaneene, J.B., Robinson-Dunn, B., Stobierski, M.G. and Boulton, M.L. (1994–2007). Human *Mycobacterium bovis* Infection and BTB Outbreak, Michigan. *Emerging Infectious Diseases*. ***14***: 657–660.

APPENDICES

Appendix1: Main advantages of Anigen BTB Ab Rapid test over PPD

Characteristic	PPD	Anigen BTB Ab Rapid test	Anigen,s advantage
Principle	Delayed Hypersensitivity with a purified protein derivative	Chromatographic immunoassay- Humoral Immunity	
Specimen	Intradermal Direct injection	Serum, Plasma	
Method	1.Intradermal injection of PPD 2. The subsequent swelling at the injection site 3 days later	1.Adding 3 drops of Specimen 2. Reading results at 20 minutes	Easy test procedure
Reading method	By observation of increase in skin-fold thickness -No more than 2mm: Negative -2-4mm: inconclusive-repeat test after - More than 4mm: Positive	By observation of Line appearance - 2 Lines: positive - 1 Line; Negative	Simple
Testing Time	3days	20 minutes	Rapid Testing
Sensitivity	65% vs. Culture of *M. bovis*- High false positives due to cross reaction of *M. avium* and others	90% vs. Culture of *M. bovis*	High accuracy
Early infection Stage	False negative responses may occur	Less false negative responses may occur; by detection of IgM	Early Detection
Late infection stage	False negative responses may occur.	Less false negative responses may occur; by detection of IgG	

Source: ***www.bionote.co.kr***

Appendix 2: Herd Prevalence of cattle, North Tongu, October2011- March 2012.

Farm	Total sample	NO. +ve	Herd prevalence
A	10	7	70
B	30	7	23
C	10	4	40
D	20	2	10
E	10	3	30
F	15	0	0
G	23	3	13
H	10	3	30
I	10	1	10
J	42	5	12
K	15	2	13
L	15	1	7

Appendix 3: Seropositivity of Cattle by Age , North Tongu, October 2011-March 2012.

Age group	Total sampled	No. +ve
0months-24 months;	9	2
25-48months	46	8
49-72months	65	14
73-96months	47	7
97-120months	23	8

Appendix 4: Participants Consent form

Study title: Bovine Tuberculosis among Herdsmen, North Tongu District, Volta Region, 2011-2012.

Principal investigator: Dr. Esther Ama Amemor

Organization: Veterinary Services Directorate of Ministry of Food and Agriculture/ GFELTP, School of Public Health, Legon.

Dear Farmer,

My name is .....I am doing a study on Tuberculosis among herdsmen and cattle in the North Tongu District of the Volta Region. The study is focused on BTB. This is a study being done in collaboration with the Veterinary Services Directorate, Noguchi Memorial Institute for Scientific Research, GFELTP of Department of Epidemiology and Disease Control, School of Public Health, University of Ghana, Legon and the Tuberculosis Control Programme

.

Purpose of the study: The purpose of this study is to determine the burden of Tuberculosis among Herdsmen and cattle as well as to determine the strain of Tuberculosis that is circulating among herdsmen and cattle. Is cattle infecting humans with Bovine TB or is human infecting cattle with Mycobacterium Tuberculosis?

Study Procedures If you agree to participate, you will be asked questions regarding your usual husbandry practices, your sputum sample will be taken for analysis; we will also take blood from your cattle to test for BTB.

Risks and discomfort : There are hardly any risks associated with your participation in this study.

Benefit: The benefit of taking part in this study is that if you happen to be TB positive you will be referred to the nearest health facility for treatment.

You would also be contributing to knowledge that is useful for the successful control of Tuberculosis in Ghana.

Incentives: ***You will not be given any incentives to take part in the study.***

Confidentiality: Any information about you that will be collected during the study will be confidential and will be stored in a file which will have only a number assigned to it and not your name.

Right to refuse or withdraw: Your participation in this study is purely voluntary and you are free to withdraw at any point in the study. You would not suffer any penalty for refusing to participate or for withdrawing from the study at any point

Who to contact:

For general questions about the study you can call the Academic Supervisor Dr. S.O. Sackey, Department of Epidemiology and Disease Control, School of Public Health, University of Ghana, Legon (0242216542) or the Principal Investigator Dr. Esther Ama Amemor, on phone number 0244122314

Consent to participate: Signing this consent form indicates that you have read/been read to you and understand what will be expected of you and are willing to participate in this survey.

CONSENT FOR STUDY PARTICIPATION

I have read the foregoing information, or it has been read to me. I understand that the purpose of the study is to determine the burden of Tuberculosis among Herdsmen and their cattle as well as to determine the strain of Tuberculosis that is circulating among herdsmen and cattle.

With the view to providing knowledge that would be useful for the successful control of Tuberculosis in Ghana. I have had the opportunity to ask questions. I consent voluntarily to participate in this study and understand that I have the right to withdraw with no consequent penalties.

Name	Signature/thumb print
Participant……..…….	…….
Investigator…….……..	…….
Witness…… ….	……

Date Place

Appendix 5: Assent Form For Juveniles

Participants Assent form
Study title: Bovine Tuberculosis among Herdsmen, North Tongu District, Volta Region, 2011-2012.

Principal investigator: Dr. Esther Ama Amemor

Organization: GFELTP, School of Public Health, Legon. Veterinary Services Directorate of Ministry of Food and Agriculture.

Dear Miss/Master,

My name is .....I am doing a study on Tuberculosis among herdsmen and cattle in the North Tongu District of the Volta Region. The study is focused on BTB. This is a study being done in collaboration with the GFELTP of Department of Epidemiology and Disease Control, School of Public Health, University of Ghana, Legon, Veterinary Services Directorate and the Tuberculosis Control Program

Purpose of the study: The purpose of this study is to determine the burden of Tuberculosis among Herdsmen and cattle as well as to determine the strain of Tuberculosis that is circulating among herdsmen. Are cattle infecting humans with BTB?

Study Procedures : If you agree to participate, you will be asked questions regarding your usual husbandry practices, your sputum sample will be taken for analysis.

Risks and discomfort: There are hardly any risks associated with your participation in this study.

Benefit: The benefit of taking part in this study is that if you happen to be TB positive you will be referred to the nearest health facility for treatment.

You would also be contributing to knowledge that is useful for the successful control of Tuberculosis in Ghana.

Incentives: You will not be given any incentives to take part in the study.

Confidentiality: Any information about you that will be collected during the study will be confidential and will be stored in a file which will have only a number assigned to it and not your name.

Right to refuse or withdraw: Your participation in this study is purely voluntary and you are free to withdraw at any point in the study. You would not suffer any penalty for refusing to participate or for withdrawing from the study at any point.

Who to contact: For general questions about the study you can call the Academic Supervisor Dr. S.O. Sackey, Department of Epidemiology and Disease Control, School of Public Health,

University of Ghana, Legon (0242216542) or the Principal Investigator Dr. Esther Ama Amemor, on phone number 0244122314.

Consent to participate: Signing this assent form indicates that you have read/been read to you and understand what will be expected of you and are willing to participate in this survey.

ASSENT FORM FOR STUDY PARTICIPANTs UNDER 18YEARS

I have read the foregoing information, or it has been read to me. I understand that the purpose of the study is to determine the burden of Tuberculosis among Herdsmen and their cattle as well as to determine the strain of Tuberculosis that is circulating among herdsmen.

With the view to providing knowledge that would be useful for the successful control of Tuberculosis in Ghana. I have had the opportunity to ask questions. I consent voluntarily to participate in this study and understand that I have the right to withdraw with no consequent penalties.

Name	Signature/thumb print
Participant……..…….	…….
Investigator…….……..	…….
Witness…… ….	……
Date	Place

Appendix 6: Study Questionnaire

MODULE 1: DEMOGRAPHICS

Q. No.	Questions	Response	Code
1.1	Location (house number)		D1
1.2	Description of location (to facilitate call-back)		D2
1.3	Name of Household Head		D3
1.4	Respondent's First name		D4
1.5	Respondent's Family name		D5
1.6	Contact phone number		D6
1.7	Sex	Male ……1 Female …….2	D7
1.8	What is your date of birth?	☐☐ ☐☐ ☐☐☐☐ dd mm yy (If Don't know, code 77-77-7777 and go to Q1.9. If known, go to Q1.11)	D8
1.9	How old were you at your last birthday?	Age (in complete years)…….	D9
1.10	If you don't know your age, please estimate how old you are? Interviewer should estimate maximum age and narrow down until respondent agrees appropriate age range	<1……1 1-5……2 6-10……..3 11-15……....4 16-20……....5 21-30……....6 31-40……....7 41-50……....8 >50…….…..9	D10
1.11	What is the highest level of school you attended?		D11

		Primary……..….1 Middle / JSS……..2 Secondary / SSS……3 Voc./Comm./Tech./……4 Post Secondary……..5 Tertiary……..6 None ……7 Other (specify)……..96 Don't know……..…77	
1.12	What is your religion?	Catholic……..1 Protestant……..….2 Pentecostal/Charismatic.……3 SDA/Jehovah Witness etc…....4 Moslem……..……5 Traditiomnal religion...……..6 Spiritual church…...……..7 No. Religion……..……8 Other (specify)_____ 96	D12 D12other
1.13	To which ethnic group do you belong?	Akan……1 Guan……2 Ga/Dangme…….……3 Ewe…….……4 Krobo……..……5 Mole Dagbani…….……6 Other (specify)_____ 96	D13 D13 other
		Unemployed……1 Student……2 Milk processor ……..3	

1.14	Occupation	Butcher……4 Livestock Farmer…...……...5 Crop Farmer…….……6 Cattle herdsman ……7 Salary worker…….....8 Community livestock worker…….9 …….10 Other (specify)_____96	D14 D14 other
1.15	What is your main source of income and livelihood?	Salary/wages ……1 Cash crop ……2 Food crop ……3 Livestock/poultry ……..4 Agro-processing…….5 Non-agric enterprise ……..6 Gifts/remittances……...7 Others (Specify) ……..…96	D15
1.16	What are your supplementary sources of income?	Salary/wages ……1 Cash crop ……..2 Food crop ……3 Livestock/poultry ……..4 Agro-processing…….5 Non-agric enterprise ……..6 Gifts/remittances……...7 Others (Specify) ……..96	D16 D16other

MODULE 2: Risk factors

Q. No	Question	Response	Code
2.1	Do you consume fresh milk?	Yes …1 No …2	R1
2.2	Do you boil milk before drinking?	Yes -1 No -2 sometimes-3	R2
2.3	If yes/sometimes, how many times in a week?	……..	R3
2.4	Do you milk cattle?	Yes …1 No …2	R4
2.5	if yes do you wash hands before and after milking?	Yes …1 No …2	R5
2.6	Are there any cattle that have been experiencing cough, running nose?	Yes…1 No…	R6
2.7	Do you share bulls for breeding?	Yes… No…	R7
2.8	Where does the family obtain drinking water	communal watering point/dam …… 1 tap water……2 well……..3 river……...4	R8
2.9	Do you boil water before drinking?	Yes…1 No…2 Sometimes…3	R9
2.10	Do you assist cow during delivery?	Yes…1 No…2	R10
2.11	Do you use gloves?	Yes…1 No…2	R11

2.12	Do you experience cough, night sweats, and weight loss?	Yes…1 No…2	R12
2.13	If YES, was the problem diagnosed?	Yes…1 No…2	R13
2.14	What was the diagnosis?	Malaria……..…1 Tuberculosis……..2 Other (specify)……3	R14

MODULE 3: Knowledge

Q. No	Question	Response	Code
3.1	Have you ever heard of BTB?	Yes …1 No …2	K1
3.2	Do you know the cause of BTB?	Yes …1 No …2	K2
3.3	Do you know the symptoms of BTB?	Yes …1 No …2	K3
3.4	If Yes in Q2.3 then mention symptoms	…… …… ……	K4
3.5	Do you know man can get tuberculosis from cattle?	If Yes in Q 2.5 then specify	K5
3.6	Do you know the symptoms of human tuberculosis	Do you know boiling of milk can prevent human tuberculosis	K6

3.7		Do you know how to prevent BTB?	K7
3.8		Yes…1 No…2	K8
3.9		Yes…1 No…2	K9
3.10	If Yes in Q 2.6 then specify	…… …….. ……	K10
3.11	Do you tuberculosis is curable	Yes…1 No…2	K11

Thank you very much for agreeing to participate in the study.

MODULE 4: Individual cattle data

Q. No	Question	Response	Code
4.1	Name of kraal owner	…….	C1
4.2	Cattle name/number	…….	C2
4.3	Total number of cattle in kraal	……..	C3
4.4	Age of cattle	6months-2years……..1 2-4yearsmonths…….2 4-6years…….3 6>…..……4	C4
4.5	Sex of cattle	Male…1 Female…2	C5
	Type of breed		

4.6		Local …1 Exotic …2 Crossbreed 3	C6
4.7	Type of husbandry system	Intensive…1 Semi-intensive …2 Extensive....3	C7
4.8	Has it emaciation history?	Yes.....1 No.....2	C8
4.9	Has it got a cough history?	Yes …1 No …2	C9
4.10	Has it got/ had running nose?	Yes…1 No …2	C10

SAMPLE COLLECTION

24. Samples collected ☐ Sputum

THANK YOU VERY MUCH FOR YOUR TIME AND PATIENCE