

**DIAGNOSIS OF BOVINE TUBERCULOSIS AT SLAUGHTER-
HOUSES**

**A THESIS SUBMITTED TO
THE BOARD OF GRADUATE STUDIES
UNIVERSITY OF GHANA, LEGON
GHANA**

**IN FULFILMENT OF THE REQUIREMENT FOR THE
DEGREE OF MASTER OF PHILOSOPHY (M.PHIL)
(PUBLIC HEALTH)**

BY:

**OSEI-AGYEMANG BONSU
M.Sc (Vet.), MPH**

**SCHOOL OF PUBLIC HEALTH
UNIVERSITY OF GHANA
LEGON**

SEPTEMBER 2001

370445

SP% 1>TZ £>£<+
hJ-+c

C'I

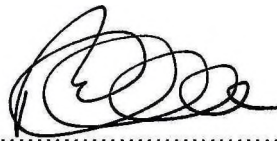
k₁-o_{iy}^ ^

DECLARATION

I do hereby declare that except for investigations by others which have been cited and duly acknowledged, this thesis is the result of my own field research, and that this thesis, either in whole or part, has not been presented for another degree here or elsewhere.



.....
OSEI-AGYEMANG BONSU
(Student)



.....
DR. B. D. AKANMORI
(Supervisor)

DR. G. K. ANING
(Co-supervisor)

DEDICATION

THIS WORK IS DEDICATED

TO

MY BELOVED CHILDREN

ELVIS AND EUNICE

ACKNOWLEDGMENT

It is the one who climbs a good tree that gets a good push; I am therefore grateful to the good Lord for providing the opportunity, the strength and wisdom to see me through this Master of Philosophy course. I am indebted to all my lecturers for providing me with knowledge, which helped me during my data collection for the production of this thesis. I wish to place on record the enormous support the workers and management of the Accra abattoir and Amasaman slaughterhouse accorded me during data collection. I am most grateful to my academic supervisors: Dr. B D, Akanmori, Head of the Immunology Unit, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Dr. G. K Aning of the Animal Research Institute, CSIR, Accra and Dr. Peter Cripps, Faculty of Veterinary Medicine and Animal Husbandry, University of Liverpool, for their direction, supervision and constructive contribution during the preparation and implementation of the research proposal as well as writing of this work. I am also very grateful to the Director and staff of the School of Public Health, University of Ghana, Legon for their assistance.

Finally, I express my profound gratitude for material, moral and prayer support from my family especially my two children, Elvis and Eunice and my sister Philomina Prempe.

TABLE OF CONTENTA

	PAGE
Declaration	i
Dedication	ii
Acknowledgement	iii
Table of Content	iv
List of Tables and Figures	vii
List of Abbreviations	viii
List of Appendices	ix
Abstract	x
Chapter One	1
1.1 Introduction	1
Chapter Two	5
2.1 Causative agent of bovine tuberculosis (BTB)	5
2.1.1 Properties of the causative agent	5
2.1.2 Pathogenesis of BTB	6
2.1.3 Variation in host resistance in cattle	8
2.1.4 Variation in host resistance in humans	10
2.1.5 Variation in host resistance in developed countries	12
Epidemiology	14
2.2.1. Species affected	14

2.2.2 Source of infection	16
2.2.3 Route of transmission	18
2.2.4 Distribution of bovine tuberculosis in Africa	22
2.2.5 Zoonotic transmission of BTB in humans	22
2.2.6 <i>Mbovis</i> in AIDS patients	26
2.3 Economic importance of BTB	27
2.4 Diagnosis of BTB	28
2.4.1 Ante mortem (clinical diagnosis of BTB)	28
2.4.2 Ante mortem (meat inspection)	29
2.4.3 Ante mortem (tuberculin test)	31
2.4.3.1 The single intradermal test	32
2.4.3.2 The short thermal test	32
2.4.3.3 Intravenous tuberculin test	32
2.4.3.4 Stormont test	33
2.4.3.5 The intradermal comparative tuberculin test (ICTT)	33
2.4.4 Serological test for the diagnosis of bovine tuberculosis	34
2.4.5 DNA fingerprinting of <i>Mbovis</i> strains	34
2.4.6 On farm risk assessment: an ante mortem solution	36
2.4.7 On farm risk factors	37
2.4.8 Diagnosis on BTB at slaughterhouses (P M Inspection.)	38
2.4.9 Microscopical examination	46
2.5.1 Control of BTB	47
2.5.2 HACCP	48

2.5.3	BCG vaccination of cattle	54
Chapter Three		57
3.1	Materials and methods	57
3.1.1	Study site	57
3.1.2	Accra abattoir	58
3.1.3	Amasaman slaughterhouse	58
3.1.4	Ashaman cattle market	59
3.1.5	Individual farms/ranches	60
3.2	Study period	61
3.3	Study population	61
3.4	Tuberculin test	62
3.5	Meat inspection	63
3.6.1	Laboratory investigations	64
3.6.2	Bacteriological examination	64
3.6.3	Parasitological examination	65
3.7	Knowledge attitude beliefs and practices of meat inspectors	65
3.8	Limitations	66
3.9	Ethical considerations	66
Chapter Four		67
Results		67
4.1	Results of meat inspection	67

4.2	Microscopic exam, of smears for acid fast bacteria/helminths	68
4.3	Cases of BTB at slaughterhouses	69
4.4	Strength of staff at slaughterhouse Greater Accra Region	69
4.5	Knowledge attitude belief and practices of meat inspectors	69

List of Figures

Fig 1	Microscopic exam, of smears of pimply gut-like lesions	72
-------	--------------------------------------------------------	----

List of Tables

Table 1	Results of tuberculin test for cattle from farms and cattle market	71
Table 2	4x4 table showing the relationship between tuberculin test result on cattle and the presence of pimply gut-like lesions at P.M	71
Table 3	Microscopic examination of lesions for Mycobacteria/Oesophagostomum eggs/larvae	71
Table 4	Monthly incidence of suspected BTB cases, Accra abattoir	72
Table 5	Monthly incidence of suspected BTB case at Amasaman Slaughterhouse	73
Table 6	Batch submission of cattle to slaughterhouses	74
Table 7	Strength of staff of meat inspectors at Accra and Amasaman slaughterhouses	74

Table 8	Knowledge attitude beliefs and practices of meat inspectors	
Table 9	Condition at Accra abattoir and Amasaman Slaughterhouse	75
Chapter Five		76
5.1	Discussion	76
5.2	Conclusion/Recommendation	86
5.3	References	88

List of Abbreviations

ANDR.....	Approximate Non-Detection Rate
AQIS.....	Australian Quarantine Inspection Services
AIDS.....	Acquired Immune Deficiency Syndrome
BCG.....	Bacille Calmette-Guerin
BIV.....	Bovine Immunodeficiency like Virus
BTB.....	Bovine Tuberculosis
CBPP.....	Contagious Bovine Pleurupneumonia
CCP.....	Critical Control Point
FAO.....	Food and Agriculture Organization
HAS.....	Hygiene Assessment System
HACCP.....	Hazard Analysis Critical Control Point
HIV.....	Human Immunodeficiency Virus
KABP.....	Knowledge, Attitude Beliefs and Practices

NSS.....	National Surveillance Scheme
NGSP.....	National Granuloma Submission Programme
NVL.....	No Visible Lesion
PPD.....	Purified Protein Derivative
REA.....	Restriction Endonuclease Analysis
RFLP.....	Restriction Fragment Length Polymorphism
SSNIT.....	Social Security and National Insurance Trust
SID.....	Single Intradermal
TB.....	Tuberculosis
WHO.....	World Health Organization

List of Appendices

Checklist questions for an interview

ABSTRACT.

Cattle infected with *Mycobacterium bovis*, the causative agent of bovine tuberculosis (BTB), an important zoonotic disease, can be identified by a range of tests on live animals as well as at post mortem. Post mortem inspection often fails to detect early cases where lesions have not yet developed, cases where lesions are present in organs or parts of the carcass, which are not routinely examined, or in cases where the lesions are confused with those due to other infectious agents. The present study was designed to test the specific hypothesis that “pimply gut-like lesions found in the intestines of cattle at meat inspection are largely due to BTB and not the gut worm, *Oesophagostomum*, and are missed at meat inspection, posing substantial risk to human infection. In a prospective, double-blinded experiment, 150 cattle were sent for slaughter and meat inspection, after they had tested for BTB using the BTB-specific comparative tuberculin test (CTT). Seventy-five out of the one hundred and fifty cattle (50%) were tuberculin test positive, with an equal number negative.

At slaughter, meat inspection was performed as recommended by the Ghana meat inspection act, 1962. and records of post mortem findings for individual animals were carefully recorded. Tissue samples were also taken to the laboratory for detailed examination. Detailed examination involved slicing of organs at 2-mm interval and examination for the presence of tuberculosis lesions. Smears from the lesions were stained and microscopically examined for the presence of acid-fast bacilli. The detailed post-mortem meat inspection investigation carried out revealed the presence of small, round nodular (raised) lesions, of diameter 0.01-to 0.5cm, often referred to as “pimply

gut” by the meat inspectors, exclusively in the intestines of all the seventy-five cattle that tested positive to tuberculin test prior to slaughter. Microscopic examination of these lesions in the laboratory revealed the presence of acid-fast bacilli in 77.7% of the cattle.

Of the one hundred and fifty animals with known tuberculin status (seventy five tuberculin positive and seventy five tuberculin negative) slaughtered, only one was suspected of having bovine tuberculosis by meat inspectors. Seventy-four out of the seventy-five (98.7%) cattle that tested positive by CTT were passed as bovine tuberculosis negative and so fit for human consumption.

The causative organism of BTB, *M bovis* is an acid-fast bacillus. Although the acid fast organisms present in the “pimply gut-like” lesions were not specifically identified, taken together with strong association between tuberculin positive reaction and the presence of these lesions, it can be concluded that animals that tested positive to tuberculin test were indeed infected with the bovine tuberculosis. The high percentage of lesions found in the intestines of BTB positive cattle confirms that, in Africa, including Ghana, water and feed for cattle are likely to be contaminated with *M bovis*, making the oral route of infection a most important means by which BTB is spread. In the developed countries, where water and feed are almost free from *M bovis*, aerosol may be the common route by which BTB is spread. Hence the lungs and associated organs are considered to be the points for BTB lesions.

The results of the present study provide explanation for the very large percentage of tuberculin positive test animals without lesions and. reveals that meat inspection may not

be effective in diagnosis of BTB in Ghana. Lack of training and poor facilities further makes meat inspection less effective. Further work is required to isolate and type the acid-fast bacilli identified and to help formulate programmes for the control of BTB in Ghana. The study further recommends the training of Meat Inspectors and provision of logistics.

CHAPTER ONE

1.1 INTRODUCTION

Tuberculosis (TB) is caused by acid-fast bacteria of the genus *Mycobacterium*, and is characterized by slow progressive development of tubercles in almost any organ, perhaps with the exception of skeletal muscles. TB is an infectious disease of worldwide distribution, affecting both man and animals. It is also ancient and in certain part of Africa, notably in Egypt, tuberculosis has been reportedly present since 3700BC, as evidenced by studies on mummies (Morse *et al.*, 1964). The etiological agents of mammalian tuberculosis, classified as members of the *Mycobacterium tuberculosis* complex are *M. tuberculosis*, *M. bovis*, *M. microti* and *M. africanum*, the last consisting of rather heterogeneous group of strains isolated from man in equatorial Africa (Collins and Grange, 1987). *M. bovis*, otherwise known as the bovine tubercle bacillus, is the cause of bovine tuberculosis (BTB). The latter term should strictly only be used to refer to tuberculosis in cattle, but it is often used to denominate tuberculosis caused by bovine strains of the tubercle bacillus irrespective of the host.

Human tuberculosis is still the single most important cause of human morbidity and mortality in many developing countries. During the last decade, 1990-1999, an estimated 88.2 million new cases of tuberculosis occurred in the world, of which 8 million were attributable to Human Immune-deficiency Virus (HIV) infection. Thirty million people were predicted to die of tuberculosis during the last decade. (WHO, 1993). A major factor, which has fueled the re-emergence of TB, is the emergence of drug-resistant strains of the causative agent mainly in

immuno-compromised individuals, especially those with Acquired Immune Deficiency Syndrome (AIDS).

Recent studies indicate that tuberculosis is present in Africa within animals and humans (Cosivi, 1998). In Tanzania, a prevalence range of 13.1 to 40% has been reported in cattle (Kazwala et al., 1997). The prevalence of bovine tuberculosis in Egypt ranges between 6.9 and 16%, whilst prevalence range of 2.5 to 5.4% have been reported in humans (Ghobashy, 1997). In Ghana, a prevalence range of 2 to 13.8% has been reported, with prevalence as high as 50% among cattle in individual kraals in the coastal plains (Bonsu et al., 2000).

The report of the WHO (1993) reported that, in developing countries, especially Africa, where *M. bovis* infection appears to be present in a number of animal species, there is a substantial lack of knowledge of the distribution, epidemiological patterns and zoonotic implications of this important disease. In addition its contribution to human TB infection, especially in Ghana is also unknown. So there is a need for more information on the distribution, epidemiology and the zoonotic importance of BTB in these countries, taking into account also the veterinary public health aspects of human *M. bovis* infection, particularly populations at greatest risk.

The report indicated that at the end of 1992, 50% of bovine carcasses and approximately 10% of sheep and goat carcasses for instance were subjected to condemnation in Cote D' Ivoire due to tuberculosis. In Mali, 237 cattle and 124 sheep were found infected with *M. bovis* during 1992, and their carcasses were fully condemned at slaughter. In some districts in Morocco, slaughtered cows were found to be heavily infected with tuberculosis. The isolation rates were 30% for *M.*

bovis and 9.7% for *M. tuberculosis*. The report also indicated that in South Africa the prevalence of *M. bovis* infected herds varied between 3 to 33%, whilst in Somalia it ranges from 4.5 to 10.2%. (WHO, 1993). Even though, there is little information on the number of bovine carcasses condemned at slaughterhouses in Ghana, the figure could be very high. So it is evident that, apart from the public health importance, BTB is also important economically.

In 1996, the government of Ghana instituted a programme to promote dairy' cattle development and the use of local milk and milk products in southern Ghana. It therefore became necessary that, the prevalence of BTB in cattle in the programme areas be known and measures instituted to prevent human infection with the disease. A survey was therefore conducted to establish the prevalence of BTB in the project areas. The study established a prevalence of 13.8% of bovine tuberculosis among cattle of two years old and above in the project areas (Bonsu *et ai*, 2000). Ten out of the five hundred and seventy of the cattle, which reacted positive to the tuberculin test, were removed and slaughtered. Although none of the ten cattle that tested tuberculin positive showed any of the typical lesions of BTB upon inspection by meat inspectors, all of them showed small nodular circumscribed round lesions, 0.01-0.5 cm in diameter and containing yellowish cheesy material upon incision in the intestines, which were often termed "pimply gut" lesions by meat inspectors. These lesions were attributed to the gut worm, *Oesophagostomum* and were often dismissed as of little public health significance. During the same period, prevalence of BTB reported from slaughter houses/slabs in the project areas ranged from 0.001 to 0.1% (Monthly Reports, Veterinary Services (1997)). Also several researchers have reported instances when cattle have reacted positive to tuberculin test but without lesions of tuberculosis

at post mortem (Turk son and Boadu, 1999 (Unpublished Report), Stumpff *et al.*, 1985, Kazwala *et al.*, 1997). It therefore became necessary to establish the risk of infection with *M. bovis*.

It was therefore hypothesized that; *“Pimply gut like lesions found in the intestines of cattle at meat inspection are largely due to tuberculosis and not the gut worm (oesophagostomum) and are often missed at meat inspection, posing a significant risk to human infection ”* The general objectives of the study therefore was to test this specific hypothesis and to show that pimply gut like lesions found in the intestines of cattle at post mortem are largely due to tuberculosis, but often confused with oesophagostomosis.

The Specific Objectives of the study were:

- To identify TB infected cattle using the comparative tuberculin test prior to slaughter.
- To subject the carcasses to routine meat inspection.
- Carry out detailed inspection for the carcasses for any evidence of BTB
- To determine the presence of Mycobacterium in “pimply gut” like lesions in the intestines of slaughtered cattle.
- To determine the proportion of BTB lesions that can be confused with pimply gut lesions (gut worm).

CHAPTER TWO

LITERATURE REVIEW

2.1 Causative Agent of bovine tuberculosis (BTB).

Robert Koch first isolated mycobacterium, the causative agent of tuberculosis, in 1882 (Bakulov *et al.*, 1987). It is an acid fast, slender organism, which is microaerophilic.

2.1.1 Properties of the Causative Agent:

Mycobacterium bovis grows slowly in artificial media with a doubling time of 18 hours, in contrast to most bacteria that can double in number in 1 hour or less. Since growth is slow, cultures of clinical specimens must be held for 6- 8 weeks before being recorded negative. *M. bovis* is cultured on bacteriologic media, which contain complex nutrients (e.g. egg yolk) and dyes (e.g. malachite green). The dyes inhibit the unwanted normal flora present in the pathological sample. Although the organism does not form spores, it is relatively resistant to acids and alkalis. It is also resistant to dehydration and so survives in dried expectorated sputum, a property that is important in its transmission by the aerosol route (Bakulov *et al.*, 1987).

The early literature reviewed suggests that *M. bovis* is a highly resistant organism, capable of surviving in cattle faeces for at least 5 months in winter, 4 months in autumn, 2 months in summer and in soil for up to 2 years. Maddock (1936) showed that direct sunlight killed the bacilli in cultures within a few hours, whereas when the bacilli are present in pus and morbid discharges, remained viable for several weeks (Bakulov *et al.*, 1987). In experiments with artificially infected faeces exposed on pasture in the south of England under ordinary conditions,

virulent *M. bovis* organism were demonstrated to survive for at least 5 months during winter, for 2 months during spring and 4 months during autumn (Stenhouse and Hoy, 1930). Maddock (1936) found that *M. bovis* on growing grass, treated with a fine emulsion of tuberculous bovine lungs containing an estimated 120 million (1.2×10^8) living virulent bacilli per square foot, remained viable and virulent for at least 49 days in summer. On plots of grass similarly treated with 1.2 million (1.2×10^7) living virulent bacilli per square foot, the survival times for *M. bovis* were 28 days and 14 days respectively.

More recent evidence suggests that *M. bovis* disappears from the environment much more quickly (Duffield and Young, 1985). In the summer months in England, *M. bovis* could not be recovered from grass contaminated with infected badger urine after 3 days or from naturally infected badger faeces after period of 1 or 2 weeks. The activity of sunlight and of other bacteria, protozoa and fungi, which normally contribute to the breakdown of faeces, appears to destroy tubercle bacilli. Similarly, the decomposition of a carcass will destroy *M. bovis*. In a carcass left on pasture, the level of infection had dropped sharply after 2 weeks, and after 4 weeks, *M. bovis* could not be recovered. In 3 buried badger carcasses, *M. bovis* could not be recovered after 2, 3 and 6 weeks respectively. Thus, while *M. bovis* deposited on sterile faeces and soil and stored away from sunlight may survive for several months, under natural conditions *M. bovis* appears to die out more quickly. Contaminated pasture therefore constitutes an important source of infection with BTB.

2.1.2 Pathogenesis of BTB

Following infection, tuberculosis spreads in the body by two stages, primary complex and post-primary dissemination. The primary complex consists of the lesion at the point of entry and in

the local lymph node. Post primary dissemination from the primary complex varies considerably in rate and route. It may take the form of acute milliary tuberculosis, discrete nodular lesions in various organs, or chronic organ tuberculosis cause by endogenous or exogenous re- infection of tissues rendered allergic to tubercule protein (Blood and Radostits, 1989). In cattle, horses, sheep and goats, the disease is a progressive one and although generalized tuberculosis is not common in pigs; localization as non-progressive abscesses in the lymph nodes of the head and neck is the most common finding. (Blood and Radostits, 1989).

According to Crofton (1992), abdominal tuberculosis is common in developing countries, especially in females, but it is rare in industrialized countries. It may arise in the gastrointestinal tract with spread to the mesenteric nodes. He identified three forms of abdominal tuberculosis: primary, secondary and hyper plastic ileo-caecal tuberculosis. According to him, clinically, the primary and secondary forms may be very similar. In the secondary form, patients with pulmonary tuberculosis may swallow their sputum, the tubercle bacilli in the sputum infects the wall of the intestines and cause ulceration. Fistula may occur; Infection may spread into the abdominal cavity and cause acites. Bovine tuberculosis is suspected in any patient who is losing weight, has fever, and has vague abdominal pain. Be even more suspicious if there is an abdominal mass or fluid in the abdomen. In the advanced stages of the disease, lesions may be found in many organs and tissues that are seldom affected primarily, thus infection of the udder, uterus, lymph nodes, kidneys and the meninges occurs with varying frequency (Crofton, 1992). The skeletal muscles are very seldom affected, even in advanced cases. Tuberculosis of the udder is of special significance because of contamination of milk with infective organisms which can be transmitted through drinking of the infected milk.

In Ghana, there is very little information on the pathogenesis of bovine tuberculosis. Although it is probable that, most primary lesions of tuberculosis in animals may occur in the intestine as the oral route of infection seems to play a major role, there is therefore the need for more information on the pathogenesis of bovine tuberculosis in cattle in Ghana, in order to institute appropriate control measures for the reduction of the risk of human infection.

2.1.3 Variations in Host Resistance in Cattle.

The origin and taxonomic status of domesticated cattle, which historically have been the principal reservoir and host species for *M. bovis*, is controversial. Zebu and Taurine breeds are differentiated primarily by the presence or absence of a hump and are recognized as separate species (*Bos indicus* and *Bos taurus*). Examination of mitochondria (mt) DNA sequences from representatives of 6 European (taurine) breeds, 3 Indian zebu breeds and 4 African (3 zebu 1 taurine) breeds, demonstrated that the sequences fell into 2 very distinct geographical lineages: all European and African breeds were in 1 lineage, and all Indian breeds in the other (Radostits *et al.*, 1994). Clustering of all African zebu mt DNA sequences within the taurine lineage was attributed to ancestral crossbreeding with the earlier *B. Taurus* inhabitants of the continent. It has been stated that Zebu (Brahmin) type cattle are thought to be much more resistant to tuberculosis than European taurine cattle, and the effects of the disease on these cattle are much less severe, but under intensive feedlot conditions a morbidity rate of 60% and a depression of weight gain can be experienced in tuberculous zebu cattle (Radostits *et al.*, 1994).

Carmichael (1939) commented on the very low incidence of tuberculosis in zebu (*B. Indicus*) cattle in India and tropical Africa. Indian workers reported on a survey of necropsy findings in a total of 1268 bovine animals, including 308 exotic, 733 crossbred and 227 indigenous (zebu) cattle, above 6 months of age slaughtered during the period from January 1974 to July 1984. Gross tuberculous lesions were confirmed in 25.97% of the pure-exotic breeds (Jersey, Holstein-Friesian and Brown-Swiss), in 9.69% of the crossbreeds and in only 7.05% of the indigenous (zebu) animals. These workers stated that the higher distribution of tuberculosis in pure exotic breeds ($P < 0.05$) as compared to Indian breeds (zebu cattle) simulated previously reported findings (Ram and Shama, 1955).

Experimental and epidemiological studies in East Africa showed the short-homed, zebu cattle to be much more resistant to bovine tuberculosis than the less common local Ankole cattle, or any of the European breeds. The Ankole cattle with enormous horns and no hump are much longer in the leg than the zebu animals. The zebu and Ankole breeds were kept under similar conditions in Uganda but the incidences of tuberculosis, judged on meat inspection figures, were: ankole 16%, zebu 0.93%, and on tuberculin testing 54.9% and 4.6% respectively (Carmichael, 1941).

Carmichael (1941) inoculated 7 Zebu calves and 3 Ankole calves subcutaneously with 50 mg of a virulent *M. bovis* strain. Only moderate to slight lesions were observed in Zebu cattle when they were slaughtered at intervals of 3-12 months after inoculation. The 3 Ankole calves died 21, 59 and 154 days respectively after inoculation. From both the epidemiological and experimental evidence, it was concluded that the Ankole cattle were as susceptible to *M. bovis* infection as European breeds, whereas the common Zebu cattle were much more resistant.

In an experiment in Malawi, where 3 breeds of cattle (zebu, zebu crossbreeds and Sussex) vaccinated with live bacillus Calmette-Guerin (BCG) were subsequently challenged by subcutaneous injections of virulent *M. bovis*, it was concluded that the zebu vaccinates derived most protection from the vaccination (Elwood and Waddington, 1972).

Very little information on host resistance of breeds of cattle (the West African Short Horn, the Zebu, the White Fulani, the Shanga, and their crossbreeds) to tuberculosis exists in Ghana. Recent tuberculin survey, conducted in Southern Ghana did not show any significant difference between breeds and how they reacted to tuberculin test (Bonsu *et al.*, 2000), but more studies are required to determine any differences in clinical progression, distribution of lesions and outcome of BTB in the major breeds of cattle in Ghana

2.1.4 Variation of Host Resistance in Humans.

Under apparently identical conditions in the United States Army during a 15- year peacetime period (1922-1936), tuberculosis mortality rate was 24 per 100 000 for white and 99 per 100 000 for black soldiers. Furthermore, the average duration of a fatal infection in a black soldier was one fourth that of a white soldier. The morbidity rates were 210 per 100 000 for white soldiers and 256 for black soldiers (Roth, 1940).

According to Rakower (1953), the Jewish race is most resistant and the black race most susceptible to clinical disease and death from human type tuberculosis. The chances of a black man becoming tuberculous in a given environment are no greater than those of a white man, but

the chances that the black man will die of tuberculosis are 3 times greater. In the United States, members of the Jewish race had no increased resistance to tuberculous infection, but had a definite resistance to tuberculous disease.

In contrast, 1st and 2nd generations of Irish and Italian emigrants were quite susceptible to tuberculous disease. Among the 4 ethnic Jewish groups, which were in Israel, the Yemenite Jews were exceptional because of their lack of resistance to tuberculosis. Presumably due to the process of natural selection, the Ashkenazi, Sephardi and Oriental Jews who were for centuries exposed to tuberculosis in urban areas, acquired a greater degree of resistance (Rakower, 1953). In contrast, the Yemenite Jews lived under desert conditions, such as are found in Yemen, far from the rest of the people

Whether the fact that the prevalence of tuberculosis among blacks, which was about twice that among whites, could be due in part to racial differences in susceptibility to tuberculosis infection, was investigated (Stead *et al.*, 1990). On repeat skin testing of 25398 initially tuberculin-negative residents of racially integrated nursing homes in Arkansas, it was found that 13.8% of the blacks and only 7.2% of the whites had evidence of a new infection. Data collected from 3 outbreaks of tuberculosis in 2 prisons also showed that blacks have about twice the relative risk of whites of becoming infected with tuberculosis. It was concluded that blacks are more readily infected by tuberculosis than are whites, but that susceptibility to tuberculosis infection varies independently of the factors governing the progression to clinical disease. There was no evidence of a difference between blacks and whites with respect to the percentage of infected persons in whom clinical tuberculosis developed. Blacks with pulmonary tuberculosis were

significantly more likely than whites to be highly infectious (with positive sputum smears). However there was an interesting paradox in that a single black person with a positive smear infected about 30% fewer persons of both races than a comparable white person. This was probably due to the more chronic nature of the disease in whites (Stead *et al.*, 1990).

The literature on the effect of war on tuberculosis in humans is reviewed and the results of a tuberculin survey among displaced persons in El Salvador were reported. It was concluded from the literature review that the adverse conditions of war have been consistently demonstrated to result in increased morbidity and mortality in the civilian populations affected, presumably due to reactivation of prior tuberculous infection.

During its 1980-92 civil war in El Salvador, 1 million civilians (i.e. 20% of the population) who were displaced, experienced a drop in calorific intake, disruption of housing, and a diminution in availability of medical services. These conditions resulted in a significant decrease in host resistance with an increased reactivation of tuberculosis and a rapid increase in morbidity and mortality from tuberculosis. Overall, 21.2% of the non-BCG vaccinated had significant tuberculin reactions. The estimated incidence of smear positive pulmonary tuberculosis was 125 per 100 000 or 3 times the previously reported rate for El Salvador. The immunologically activated macrophage induces a persistent bacteriostasis which is usually sufficient to protect the host, although it will not eliminate the infection altogether. So reactivation can occur whenever the cellular defenses are depleted.

2.1.5 Variation of Host Resistance in Developed Countries.

In developed countries, it is the general experience that only about 10% of humans infected with the tubercle bacillus develops clinical disease in their lifetime (Bates, 1986). About half of these

clinical cases are patients with primary disease, the vast majority of whom have smear negative sputa. The remainder consists of highly infectious patients with post-primary pulmonary tuberculosis and direct smear positive sputa. In post-primary disease, which by definition occurs more than 5 years after primary infection, extra cellular multiplication of very large numbers of tubercle bacilli is facilitated by liquefaction of the solid caseous lesions in which the growth of the tubercle bacillus is normally inhibited. Bronchioles and larger airways are frequently eroded with resultant cavitations and spread of bacilli through the bronchial tree to other parts of the lung. Erosion of arterial vessels resulting in the escape of blood into the air passages and the repeated coughing of sputum stained with bright red arterial blood (haemoptysis), sometimes with fatal Consequences, is a common feature of advanced post-primary pulmonary disease in humans (Bates, 1986). Bad housing, poor nutrition and overcrowding are the main impediments to tuberculosis control in human populations. Reactivation of old primary foci infection with consequent clinical and/or open disease in humans may be triggered by immuno-depression resulting from old age, intercurrent infections and diseases, poor nutrition or stressful life events (Sauret *et al.*, 1992).

It has been reported that clinical tuberculosis preceded the diagnosis of acquired immunodeficiency syndrome (AIDS) in 9 of 14 patients with human immunodeficiency virus (HIV) infections by a mean of 7 months and occurred within the same month in the remaining 5. In both cases it is possible to be infected, but clinically healthy-overt disease may not commence until many years after the initial infection. During the interval before illness commences, the only signs of infection are, a positive tuberculin test and circulating antibodies (HIV positivity) (Festenstein and Grange, 1991).

Extra pulmonary tuberculosis among HIV-infected intravenous drug users is the most common expression of AIDS in Barcelona (Cayla *et al.*, 1991). In a review of the HIV-TB syndrome, it is concluded that unlike healthy tuberculosis carriers with their 1-in-10 lifetime odds of illness, HIV-infected tuberculosis carriers develop active contagious disease at a rate of 10% a year. If they do not die of something else first, virtually 100% of AIDS patients infected with tuberculosis develop active contagious disease at a rate of 10% a year. AIDS patients infected with tuberculosis are 30 times more likely to develop clinical tuberculosis than HIV negative people infected with tubercle bacillus (Shimao, 1995). In 1992, it was concluded that, the increase in clinical tuberculosis cases in the United State was an unmistakable symptom of broader social ills ranging from poverty, homeless, HIV infection and AIDS to collapse of health care system.

All the conditions reviewed, which predispose humans to tuberculosis, exist in Ghana. These conditions, coupled with close association with animals, especially cattle, in most communities in Ghana makes bovine tuberculosis a very significant risk to human health.

Epidemiology

2.2.1. Species affected

It has been confirmed that the bovine tubercle bacillus has one of the broadest host ranges of all known pathogens. The species, other than domesticated and feral cattle, in which the disease has been reported include the goat, pig, sheep, horse, cat, dog, ferret fox, deer, bison, buffalo, badger, possum, hare, ferret, wild and feral pig, Arabian oryx, antelope, camel, llama, alpaca, man, and

non- human primates (Collins and Grange, 1987; Anon, 1989). Collins and Grange (1983 and 1987) demonstrated that tuberculosis due to *M bovis* is a zoonotic disease with complex epidemiological pattern, which includes the transmission of infection within and between farm animal and wildlife populations.

Morris and others (1994) are of the opinion that difficulty in controlling the disease adequately in domestic animals generally result from administrative problems since the necessary technical procedures are available and have been shown to be effective, and think that, where there is interplay between infection in wild life and domestic animals, eradication of the disease becomes impractical.

According to Collins and Grange (1987), although *M. tuberculosis* infection in cattle have been reported, the disease is not progressive and lesions are usually small and self limiting, and that there are no recorded cases of cow to cow or cow to man transmission of human tubercle bacilli. They however cautioned that it is nevertheless important to bear in mind the possibility of man to cow infection with *M. tuberculosis* in herds where reactors with no gross lesions are repeatedly detected. Especially in those herds with long history of freedom from infection and where reactions are confined mainly to young stock. Kleeberg (1960) indicated that in cases of *M. tuberculosis* infection in cattle, a marked temporally reactivity to mammalian tuberculin develops which invariably disappears with the removal of the human source of infection. This temporary reactivity may persist for up to 9 months and occasionally for as long as 4years.

transmission is a principal cause. In a study, 12 patients had infected 114 cattle in 16 different herds: 9 of the 12 had genito-urinary tuberculosis and 1 such patient had infected 48 cattle in 4 different herds (Schliesser, 1992).

Bovine tuberculosis was thought of primarily as a disease of children where the disease either involved the cervical lymph nodes, the intestinal tract or the meninges. The clinical entity, characterized by swollen and discharging tuberculous lymph nodes in the neck, was referred to as scrofula. According to Collins and Grange (1987), the introduction of pasteurization of milk and milk products helped eliminate this problem.

The public health significance of consuming milk containing *M. bovis* was well illustrated in an outbreak of tuberculosis, mainly affecting children, which occurred in Horred, Sweden, in the autumn of 1936. A herd of 22 cows supplied milk to a small village. Some of the villagers had their own supply. 1 cow in the herd had a tuberculous mastitis and large numbers of tubercle bacilli were present in the milk. In school children, 29 of 32 who drank this infected milk were tuberculin test positive compared with 8 of 102 children who did not drink milk from this herd. Among 29 pre-school children, 11 who did not drink the infected milk were tuberculin negative and 16 of 18 who drank the milk were tuberculin positive (Zukeman, 1980).

Some researchers are of the opinion that, in cattle, the source of infection is usually other infected cattle, although in some European countries pulmonary or genitourinary tuberculosis of man caused by bacilli of the bovine type is a source of infection in up to 60% of re-infected herds. Adult animals are infected by inhalation of air borne dust particles as well as contaminated feed and water. Young calves are infected through milk (Blood and Radostits, 1989). Under

natural conditions, stagnant drinking water may cause infection up to 18 days after its use by a tuberculous animal. Viable *M. bovis* can be isolated from faeces of infected cattle and the ground in contact with the faeces for 6-8 weeks after the faeces are dropped (Radostits *et al.*, 1994).

Stagnant drinking water may play a very important role in the spread of bovine tuberculosis in Ghana because in the dry season, one pond could be a source of water for several thousands of cattle from many kraals several kilometers away. These ponds often become almost dry, leaving very little water that could easily be contaminated with tubercle bacilli, increasing the chance of animals getting infected after drinking from such ponds.

2.2.3. Route of transmission

In cattle grazing the open range, in wild pervade and feral cattle, the prevalence of tuberculosis is only about 1-5%, whereas in dairy cows and farmed deer, which are housed or penned in small padlocks and for much longer, the rate of infection may range from 25- 50% (Francis, 1947, Himes *et al.*, 1980). Intensive as opposed to extensive livestock systems facilitate close contact between animals, and favour the spread of respiratory diseases including tuberculosis (Steele and Ranney, 1958).

However, even under extensive pastoral conditions, husbandry factors such as the congregation of livestock from different sources at watering points or the gathering together of animals overnight may lead to increase respiratory transmission of infection including tuberculosis, and individual herds with high disease prevalence may be encountered (Francis. 1972).

Francis (1947) cites figures for Great Britain, which show the effect of herd size on transmission of infection. When herds consisted of an average of 17.2, 27.7, and 52.8 animals, the percentages of tuberculin test reactors were 4.3, 10.3, and 20%. Sigurdson (1945) cites a report on inhalation infection in tuberculin negative heifers, which were involved in non-tuberculous experiments at the Royal Veterinary College Copenhagen. The heifers were frequently stabled together with cattle with open pulmonary tuberculosis. When after some months the heifers were slaughtered, they invariably had tuberculous lesions in the lungs and/or pulmonary lymph nodes.

A national tuberculosis study carried out in Denmark during a 2-year period (1941-1943) investigated inter alia the way humans contracted *M. bovis* infection (alimentary or erogenous route), and the possible protective effect of this infection on the development of pulmonary disease due to *M. tuberculosis* (Holm and Holm 1945). The methodology used in this investigation was to compare chest X-ray examinations and Mantoux test results from the residents of districts in South Jutland, where tuberculosis among the cattle was still very common, with those of the residents of districts in Zealand where the cattle herds were almost tuberculosis-free. It was found that the transmission of tubercle bacilli from cattle to children was almost exclusively by the alimentary route and due to ingestion of infected milk. Among the children infected with tuberculosis (the Mantoux -positive), roentgenographic changes were 8 times more frequent in Zealand than in South Jutland. This finding was interpreted as signifying that, while aerogenous infection with *M. tuberculosis* was the most frequent infection for the children in Zealand, it occurred only exceptionally in South Jutland. Comparison between the towns in South Jutland and Zealand showed that, where tuberculous infection through the milk

was common, the occurrence of tuberculous changes in the lungs of infected children was only one-twentieth the frequency with which such changes were encountered in districts free from tuberculosis among the cattle and only one fifth of the frequency recorded for the total number of children.

It was found that while bovine tuberculosis through milk-borne infection to some extent protected young adults against pulmonary infection, in the rural districts of Denmark it caused a lot of inconsiderable number of cases of pulmonary tuberculosis among the adults through direct transmission of the tubercle bacilli from the cattle. The alimentary infection with *M. bovis* did not give rise to a subsequent appearance of the bovine pulmonary tuberculosis. Attention was drawn to the grave danger to which adolescents from tuberculosis-free areas are exposed when they meet respiratory infection in the towns, and the wide use of BCG vaccination particularly in adolescents, was advocated (Holm and Holm, 1945).

Francis (1949) was of the view that when pasteurization of milk is carried out in a country where *M. tuberculosis* infection in humans is common, the loss of specific immunity formerly produced in most children by ingesting tuberculous milk could be offset by vaccination with BCG, a procedure capable of reducing tuberculosis morbidity by 60-80%.

According to Collins and Grange (1987), nowadays, in developed countries, the incidence of bovine tuberculosis in children is almost zero due to the universal pasteurization of milk and milk products and the low prevalence or absence of *M. bovis* infection in the cattle population of these countries.

In recent years, epidemiological reports indicate that, humans principally contract *M. bovis* infection via the respiratory route following close contact with tuberculous cattle or humans (Ritacco and de Kantor, 1991. Collins and Grange, 1987). In Argentina, where the tuberculosis in the cattle population is relatively high, and there is pasteurization of milk and milk products combined with a high standard of meat inspection, most of the patients infected with *M. bovis* are slaughterhouse or rural workers (Collins and Grange, 1987). Pulmonary disease was the only manifestation in 24 of 48 adults (22 Women and 26 men) diagnosed with tuberculosis due to *M. bovis* in San Diego, USA (Dankner *et al.*, 1993). Women accounted for 58% of the patients with pulmonary disease.

In the South East of England, human-to-human erogenous spread of *M. bovis* has been postulated (Collins and Grange, 1987). The age and sex distribution of patients of both European and Afro-Asian ethnic origin infected with bovine strains were similar to those infected with human strains of the tubercle bacillus. In patients of European ethnic origin, infected with classical pyrazinamide resistant *M. bovis* strains, there was pulmonary and renal involvement in 44% and 26% respectively. This compared with 40% with pulmonary and 10% with renal involvement in patients of Afro-Asian ethnic origin. Even though several studies have proved that aerogenous spread of tuberculosis seem to be the most important, the situation may be different in developing countries including Ghana, where animals are most often left in the open, and are fed on contaminated grazing grounds and drink from contaminated water sources. In such situations, the oral route of infection may be very significant, but so far, little information is available to indicate how contaminated grazing grounds could be involved in the transmission

of BTB in Ghana. There is therefore the need for more information on how much grazing grounds and sources of drinking water are contaminated with *M. bovis*.

2.2. 4. Distribution of Bovine Tuberculosis in Africa

Of 55 African countries, 25 reported sporadic/low occurrence of bovine TB. 6 reported enzootic disease, 2, Malawi and Mali, were described as having a high occurrence, 4 did not report the disease; and the remaining 18 countries did not have data. Ghana was among the countries that reported tuberculosis as enzootic disease. Of all nations in Africa, only 7 apply disease control measures as part of a test-and-slaughter policy and consider bovine TB a notifiable disease. The remaining 48 countries control the disease inadequately or not at all. Ghana does not control the disease at all. Only 15% of the cattle population is found in countries where bovine TB is notifiable and a test-and-slaughter policy is used. Thus, 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)

2.2.5. Zoonotic Transmission of *M. bovis* in Humans

Data on the past prevalence of tuberculous infection in cattle and the more recent prevalence of tuberculin reactions among school children were studied for rural districts in England (Lesslie *et al.*, 1972). The percentage of tuberculin reactors among 13-year-old school children in the years 1959-60 and 1967-68, and the percentage of attested tuberculosis-free cattle 12 years earlier (1947, 1951 and 1955 respectively) in 41 rural districts of England were analyzed. It was found that the less exposure there was to cattle tuberculosis the lower was the infection.

According to Hardie and Watson (1992), 20 to 40 cases of *M. bovis* infection in humans are confirmed each year in England and Wales and this figure has continued to fall over the last 30 years. He concluded that reactivated (post-primary) disease is likely to account for most isolates, but some isolates are from patients born after control measures were implemented and may represent a primary infection acquired from cattle or humans, either in the United Kingdom or abroad. As the age group in the human population with a high prevalence of previously acquired *M. bovis* infection decreases in size, the number of *M. bovis* isolates from tuberculosis patients is likely to continue to fall.

It was reported that of 1002 consecutive culture positive cases of tuberculosis diagnosed at Peamount Hospital, Newcastle, Co. Dublin, Republic of Ireland, from January 1982 to December 1985, 9 (0.9%) were *M. bovis* (Collins and Grange, 1987). All but one of the patients with bovine tuberculosis had pulmonary disease and *M. bovis* was isolated from sputum. Only 4 of the patients had definite contact with livestock, one of these was with cervical lymph node involvement, 6 of the 9 patients with confirmed *M. bovis* infections had other major diseases as well as tuberculosis. The history and clinical features in the 4 cases of bovine tuberculosis in which there was definite contact with farming suggested reactivation of disease contracted decades earlier.

Ten cases of bovine type tuberculosis were diagnosed in Barcelona hospital during the period 1986-1990 (Sauret *et al.*, 1992). The incidence, in relation to the cases at the same period was 0.9%. The patients had an average age of 32 years (range 5-68 years). Pulmonary disease was observed in 5 patients (50%); lymphadenitis in 1. The most significant of the epidemiological

features was that 2 of the patients were Veterinary Students. An 18-year-old student had peritoneal disease and a 22-year old student a pleural effusion. 1 patient was a 5-year-old boy with sub maxillary lymph node involvement who did not live in a rural area and it was impossible to discover the source of infection despite epidemiological investigation. 2 patients of 48 and 52 years respectively, had chest X-rays showing undiagnosed residual tuberculosis. 2 other patients were diabetic and in 1 of these, the infection was associated with an ovarian carcinoma.

At the National Tuberculosis Reference Laboratory in Bulawayo, Zimbabwe, 3 (0.65%) of 462 mycobacterial isolated from clinical specimens were identified as *M. bovis* (Mason *et al.*, 1993). A total of 443 isolates were identified as *M. tuberculosis*.

In 1987, Collins and Grange listed the following observations in relation to the zoonotic transmission of *M. bovis* to humans:

1. As tuberculosis is spread among cattle primarily by the erogenous route, those working with cattle are more likely to develop pulmonary disease than alimentary disease.
2. An apparent decline in the incidence of bovine tuberculosis in man is the result of under-reporting, as few clinical laboratories distinguish between the bovine strain and the human strain.
3. There is evidence from the literature that the bovine bacillus is transmitted from man-to-man and from man-to-cow and that man is a potential reservoir and source of bovine tubercle bacilli.

According to Ritacco and de Kantor (1991), there is scarce epidemiological information on the impact of BTB on human health in Latin America, mainly because the bacteriological diagnosis of human tuberculosis is generally limited to the sputum smear examination, and even when

cultures are performed, the glycerol-containing Lowenstein-Jensen medium on which *M. bovis* strains are difficult to grow is the only one available. Acha and Szyfres (1987) confirms that in countries where milk is routinely boiled, such as those of Latin America, the incidence of human infection by *M. bovis* had always been low. Nevertheless, pulmonary as well as non-pulmonary disease due to *M. bovis* continues to be a problem in areas where the prevalence of infection in cattle is high.

In Peru, a study of 853 strains of tubercle bacilli from cases of pulmonary tuberculosis in humans identified 38 (4.45%) as *M. bovis* (Acha and Szyfres, 1987). In Argentina, where a relatively high prevalence of tuberculosis in cattle coincides with a reliable bacteriological diagnosis of human disease, the percentages of human tuberculosis due to *M. bovis* range from 0.4 to 6.2%, and most of the patients infected with *M. bovis* are slaughterhouse or rural workers (Ritacco and de Kantor, 1991).

In Santa de province, during the period 1984-1989, *M. bovis* was responsible for 2.4 to 6.2% of human cases of tuberculosis and 64% of the patient with tuberculosis were slaughterhouse or rural workers (Latini *et al.*, 1990).

In Ghana there is no information on the prevalence of *M. bovis* in the human population. This is primarily because routine diagnosis of TB is based on sputum examination, and not isolation and characterization of etiological agent. The impact of bovine tuberculosis in the population can therefore not be determined

2.2.6. *M. bovis* in AIDS Patients

M. bovis has been isolated from HIV infected persons in developed countries

In the case of *M. bovis* infections in man, it has been stated that it is extremely likely that any host factors affecting the development of post-primary disease or preventing man-to-man transmission would be abrogated in HIV/AIDS patients (Daborn and Grange, 1993. Bouvet *et al.*, 1987). Serious concern was expressed that the HIV pandemic would result in an increase of human tuberculosis due to *M. bovis* infection and that a greater degree of transmission of the infection to other human beings and to domestic and farmed animals could well occur. Two cases of *M. bovis* disease in HIV positive patients diagnosed by the Public Health Laboratory Service in Southeast England are cited. One was a 32-year-old female who developed pulmonary tuberculosis in 1989. The other was a 49-year-old male who presented with cervical lymphadenopathy in 1991. Both patients had English names and given that about 15 cases of tuberculosis due to *M. bovis* are diagnosed annually in Southeast England, with most cases in elderly people, this small number (6-7%) with HIV infection was not considered to be significantly different from the estimated 4-6% of tuberculosis cases in that region which are HIV related (Daborn and Grange, 1993).

Bovine Tuberculosis has been reported in 94 (69%) of 136 countries in the tropic. In a retrospective multicentre study in France, it was reported that 2 (1.6%) of 1223 tuberculous HIV-positive patients were infected with *M. bovis*. As *M. bovis* strains are intrinsically resistant to pyrazimide, it is of particular significance that these French *M. bovis* isolates were resistant to isoniazid. Accordingly, 2 of the first-line anti tuberculosis drugs were ineffective for these patients (Daborn and Grange, 1993).

In a clinico-epidemiological study of *M. bovis* infections in San Diego, California, over the 12-year from 1980-1991 inclusive, 12 (25%) of 48 infected with *M. bovis* were AIDS patients. Overall, *M. bovis* infections accounted for almost 3% of all tuberculous diseases reported in San Diego County during the study period (Dankner *et al.*, 1993). Although there are no reports of *M. bovis* infections in HIV infected or AIDS cases in Africa, HIV seroprevalence rates of over 40% have been found in tuberculous patients in various countries (Narin *et al.*, 1992). In 1992, 33 (80%) of 41 African countries reported the presence of bovine tuberculosis ((Daborn and Grange, 1993).

2.3. Economic Importance of BTB

Apart from its Public Health importance, bovine tuberculosis is important for its detrimental effects on animal production. Housing predisposes to the disease as zero grazing. So the disease is more common and serious where these forms of husbandry are practiced. In spite of the low overall incidence of the disease in countries where cattle are at pasture all the year round, individual herds with 60-70 % morbidity may be encountered (Blanco and Radostits, 1989; Radostits *et al.*, 1994). Among beef cattle, the degree of infection is usually much lower because of the open range condition under which they are kept. However, individual beef herds may suffer a high morbidity if infected animals are introduced and large number of animals have to drink from stagnant water holes, especially during dry season (Blood and Radostits, 1989).

Due to the chronic nature of tuberculosis, detection of the disease is often delayed whilst productivity goes down. At meat inspection, carcasses of animals with tuberculosis are often

condemned. In countries like Ghana where no compensation is paid for condemnation of classes, it becomes a big economic loss to the farmer. Animals suffering from tuberculosis often die eventually, which is also of economic loss to the farmer. Condemnation of carcasses and deaths of animals due to tuberculosis also leads to reduction of protein which otherwise could have been made available to the population. Proper diagnosis of tuberculosis is therefore not beneficial to only the cattle owner but to the nation as a whole.

2.4 DIAGNOSIS OF BTB:

In order to identify animals infected with *M. bovis*, the causative agent of bovine tuberculosis, a range of diagnostic methods have been developed which can be utilized on the live animal as well as at post-mortem.

2.4.1. Ante mortem /Clinical diagnosis of BTB

Clinically, the disease is difficult to diagnose as cows with extensive miliary tubercular lesions are clinically normal, but progressive emaciation unassociated with other signs should always arouse suspicion of tuberculosis. Because of the marked variation in symptoms, it is sometimes impossible to identify the disease with certainty. Bovine tuberculosis may be confused with Contagious bovine pleuropneumonia (CBPP) when the lungs are involved, and the two diseases are sufficiently similar to require the application of the tuberculin test, and complement fixation test for CBPP, for accurate diagnosis to be made.

Tuberculous mastitis may be distinguished from most other forms of mastitis by the fibrous induration, which occurs at the base of the udder, rather than nearer the teat. A capricious

appetite and fluctuating temperature are also commonly associated with the disease. Pulmonary involvement is characterized by a chronic cough due to bronchopneumonia. The cough is never loud or paroxysmal, occurring only once or twice at a time and is low, suppressed and moist. It is easily stimulated by squeezing the pharynx or by exercise and is most common in the morning or in cold weather. In the advanced stages when much lung has been destroyed, dyspnoea with increased rate and depth of respiration becomes apparent. The most common sign of alimentary involvement are caused by pressure of enlarged lymph nodes on surrounding organs. Retropharyngeal lymph nodes enlargement causes dysphagic and noisy breathing due to pharyngeal obstruction (Blood and Radostits. 1989).

In cattle, other chronic pulmonary diseases, which may be confused with tuberculosis pneumonia, are:

1. Lung abscess due to aspiration pneumonia,
2. Pleurisy and pericarditis following traumatic reticulitis
3. Chronic contagious bovine pleuropneumonia.

Snoring respiration is relatively common in cattle, and some differentiation of the cause is necessary and practicable. But the final differentiation is by tuberculin test.

2.4.2 Antemortem (meat inspection)

The objectives of meat inspection are twofold:

- a) To ensure that only apparently healthy, physiologically normal animals are slaughtered for human consumption and that abnormal animals are separated and dealt with accordingly.

b) To ensure that meat from animals is free from disease, wholesome and no risk to human health.

These objectives are achieved by ante mortem and postmortem meat inspection procedures and by hygienic dressing with minimum contamination. Whenever appropriate, the Hazard Analysis Critical Control Point (HACCP) principles should be used: The inspection procedures should be appropriate to the spectrum and prevalence of diseases and defects present in the particular class of livestock being inspected using the principle of risk assessment (Herenda *et al.*, 2000).

Some of the major objectives of ante mortem inspection are therefore as follows:

- To screen all animals destined to slaughter
- To ensure that animals are properly rested and that proper clinical information, which will assist in the disease diagnosis and judgment, is obtained.
- To reduce contamination on the killing floor by separating the dirty animals and condemning the diseased animals if required by regulation.
- To ensure that injure animals or those with pain and suffering receive emergency slaughter and that animals are treated humanely.
- To identify reportable animal diseases to prevent killing floor contamination.
- To identify sick animals and those treated with antibiotics, chemotherapeutic agents, insecticides and pesticides.
- To require and ensure the cleaning and disinfection of trucks used to transport livestock.

Ante mortem examination should be done within 24 hours of slaughter and repeated if slaughter has been delayed over a day. Unfortunately, most of the above objectives cannot be achieved in

Ghana due to the conditions under which Meat Inspectors have to operate. For example, there is no meat inspection law in the country. Secondly, a significant number of animals including cattle, slaughtered in the country for human consumption are slaughtered at non-authorized places where there are no Meat Inspectors. These and many other factors make it very difficult to ensure meat safety in the country.

During ante mortem, Cattle with BTB may show:

1. Low grade fever
2. Chronic intermittent hacking cough and associated pneumonia
3. Difficult breathing
4. Weakness and loss of appetite
5. Emaciation
6. Swelling superficial body lymph nodes.

2.4.3. Ante mortem Tuberculin Test

Tuberculin test has been the most widely ante mortem test used. Tuberculin is a sterile liquid containing the growth products of a specific substances extracted from the tubercle bacillus used in various forms in the diagnosis of tuberculosis. This technique detects cell-mediated immune response to a challenge by antigens of the causative agent. There are different types of tuberculin tests (Monaghan and Hannan 1983, Blood and Radostits, 1989). These tests are discussed below:

2.4.3.1 The Single Intradermal (SID) Test:

This test is applied by the intradermal injection of tuberculin into the anal fold of the test animal. The reaction is read between 72 and 96 hours after the injection and a positive reaction is characterized by a diffuse swelling at the injection site (Blood and Radostitis, 1989). The main disadvantage of the SID test is its lack of specificity and the number of No Visible Lesion reactors (NVL) which occur.

2.4.3.2 The Short Thermal Test.

Intradermal tuberculin is injected subcutaneously into the neck of cattle with rectal temperature of not more than 39 degrees Celsius at the time of the injection and two hours later. If the temperature at 4, 6, and 8 hours after the injection rises above 40 degrees, the animal is classed as a positive reactor. The test is simple, as it requires only one injection. But the disadvantages are that, it requires a lot of labour and also there are occasional deaths due to anaphylaxis.

2.4.3.3. Intravenous Tuberculin Test:

Intravenous tuberculin test has been used experimentally but requires a special research tuberculin. As in the short thermal test, a positive reaction is a febrile one at 4-6 hours after the injection, continuing for at least 8 hours and the elevation of temperature to exceed 1.7 degrees Celsius.

There is difficulty in interpreting the test and hematological changes may have to be considered to avoid false negative test results (Blood and Radostitis, 1989).

2.4.3.4, Stormont Test:

This test has been devised to select those animals, which are poorly sensitized for any reason. The test is performed in similar fashion to the SID test in the neck with further injection at the same site 7 days later. An increase in skin thickness of 5mm or more, 24 hours after this second injection is a positive result. Cattle injected with *M. avium* do not give positive reaction but skin tuberculosis cases do.

A practical difficulty is the necessity for three visits to the farm. Special purified protein derivative tuberculin of a specified potency must be used to fulfill the requirements of the test (Blood and Radostits, 1989).

2.4.3.5. The intradermal Comparative tuberculin Test (ICTT).

The single intradermal comparative test is used in an area where Johne's disease, avian tuberculosis or skin tuberculosis is suspected. The comparative test depends on the greater sensitivity to homologous tuberculin. Avian and mammalian tuberculin are injected simultaneously into two separate sites on the same side of the neck, 12 cm apart and one above the other, and the test is read 72 hours later. The greater of the two reactions indicates the organism responsible for the sensitization. The test is not intended for primary use in detecting reactors but only to follow up known reactors to determine the infecting organism (Blood and Radostitis, 1989).

The advantages of this test over the other tuberculin tests are that, even though there is the need for two visits and the thickness of the skin of the test animal must be measured two times, the test is able to differentiate reactions of *M. bovis* and *M. tuberculosis* from reactions caused by other mycobacterium which are not harmful to the animals or humans. This differentiation

becomes very important especially when the test is used for control of tuberculosis when positive reactors are slaughtered. The other advantage is that the injections are carried out at one time.

2.4.4. Serological Test For Diagnosis Of BTB:

The principle of serological test is based on the fact that when animals are infected by mycobacterium, they develop antibodies, which are specific for the organism. When such antibodies come into contact with challenge antigen, a specific reaction takes place. The serological tests include complement fixation, fluorescent antibody technique (FIT), direct bacterial agglutination and precipitation test, and haemagglutination test. To detect the reaction mentioned above, a secondary antibody conjugated to a fluorescent dye or an enzyme can be used. The disadvantage of the test is that, it is not able to differentiate the types of the mycobacteria causing the reaction (Blood and Radostitis, 1989).

2.4.5. DNA fingerprinting of *M. bovis* strains

Grange and Collins (1987) found out that the cultural and biochemical properties of 150 *M. bovis* strains isolated from tuberculous badgers, deer and cattle in the Republic of Ireland were homogeneous. According to them, one of the major setbacks in the epidemiological study of the spread of *M. bovis* in animals and man and particularly amongst cattle or from wild animals to cattle and vice versa, has been the lack of a reliable system for differentiating strains or sub-species of *M. bovis*.

According to Grange and Collins (1987), neither bacteriophage typing nor biotyping has proved useful but hopes were raised by the successful application of Restriction Endonuclease Analysis

(REA) of extracted DNA to epidemiological studies in New Zealand. However, initial reports by Grange and Collins (1987) indicated that this technique did not differentiate between 40 strains of *M. bovis* from 8 herds in the Republic of Ireland, and neither did the more discriminatory technique of Restriction Fragment Length Polymorphism (RFLP) analysis of 1 *M. bovis* isolate from each of another 8 herds.

Despite the disappointing RFLP findings, it was believed (Cooper *et al.*, 1989) that the use of additional endonucleases and probes might reveal RFLP patterns that would permit a subdivision of the strains for epidemiological purpose. More recently, REA has been used to type 20 isolates of *M. bovis* recovered from cattle (12 isolates) and badgers (8 isolates) in 9 separate herd breakdowns in 4 counties (Cork, Cavan, Offaly, Limerick) in the Republic of Ireland (Collins *et al.*, 1994). 3 endonucleases (Bst ELL, PvuII and BclI) were used. Each of the 8 isolates recovered from badgers was from a different herd breakdown and belonged to different restriction type; however, 6 of these badger isolates to the same restriction type as at least 1 isolate from cattle involved in the same herd breakdown in the course of a 5-month period of investigations. 10 different restriction types of *M. bovis* were identified among the 12 isolates recovered from cattle. In 1 of the 9 herds, the restriction type of 1 of the cattle *M. bovis* isolates was identical to that of a badger isolate. Although the restriction of a further 3 *M. bovis* isolates from cattle in the same herd was different, it was so closely related to that of the badger isolate and yet so different from the restriction types of all other isolates from cattle and badgers in the other 8 herds, that it is likely that either the restriction type of the 'badger' strain or the predominant cattle *M. bovis* isolate from this herd was the result of a very recent molecular transposition.

It was concluded that REA and related technologies were likely to provide valuable epidemiological information concerning the pattern of tuberculosis in Ireland, and could be of considered importance in determining the nature and spread of *M. bovis* infection in individual herd breakdowns (Collins et al., 1994).

2.4.6. On Farm Risk Assessment: an ante mortem solution

In a risk assessed meat inspection programme, where the origin and health status of slaughtered livestock are known, high-risk groups of livestock would receive additional attention in the abattoir at increased cost to the producer. Such a system would certainly encourage the producer to improve the health status of their slaughter animals.

In addition to studies on the feasibility of on-farm, ante mortem inspection, there have been others who have considered improvement in animal health monitoring and the collection of farm data for regional or national databases. The development of monitoring systems is necessary since reliable information on diseases is vital in protecting a nation's agricultural system and its potential for production (Glosser, 1988). Such systems assist the data gathering and handling practices essential for a longitudinally integrated quality assurance programme, and also familiarize the industry with the benefits to be gained from greater knowledge of the herd/flock health status.

Monitoring the health status of animals' calls for good records on the animals. This is something, which is lacking in the livestock industry in Africa and in Ghana in particular. In the Netherlands, for example, pigs are individually ear-tagged on the breeding or farrowing farms before the age of 12 weeks and, at the end of the period on the finishing farms, each pig is

earmarked for a second time. Consequently, each carcass and its herd of origin can be identified at the slaughterhouse (Elbers *et al.*, 1992).

This, and the document accompanying the pigs to the abattoir, enables identification of the rearing pen as well as the farm and has obvious benefits for on-farm assurance schemes. This approach is in line with the recommendation of the Codex Alimentarius Commission (1991) that the health of animals should be monitored so that information that is relevant at the abattoir to assist in dressing, inspection and judgments can be made available. Obtaining the full benefit of this information require an effective recording and transfer system, as well as identifying animals with their place of production.

The absence of a user and animal welfare friendly method of livestock identification is a major stumbling block to the promotion of a comprehensive data retrieval and feedback service. Progress is being made in the identification of farm livestock with the use of electronic transponders, with many different designs currently being tested. Despite moves towards standardization, both on a European and worldwide scale there is a vast range of different tags and databases, not all of which are compatible (Gracey and Collins, 1992).

2.4.7. On-Farm Risk Factors

According to Curtis (1990), environmental medicine is concerned with all the factors on the farm that impinge on the animals. Animals kept in a controlled environment can be protected to a large extent from reservoirs and vectors. However, with extensive animal husbandry systems, where there is little or no insecurity, the water supply may be a source of microorganisms and

potable water, preferably from a mains supply, provides the safest source of water for food animals. Ground water or streams are often contaminated so that access to such water by livestock should be prevented. Wells and free-flowing springs may be suitable as long as the water quality can be satisfactorily monitored for microbial contamination or other pollution. In Africa, including Ghana, it is not only difficult to provide a safe source of drinking water for animals, but for humans as well. Water therefore serves as potential source of infection of many livestock diseases including BTB.

2.4.8. **Diagnosis of BTB at slaughterhouses (P .M. Inspection)**

Routine postmortem examination of carcass should be carried out as soon as possible after the completion of dressing in order to detect any abnormalities so that products only conditionally fit for human consumption are not passed as food. All organs and carcass portions should be kept together and correlated for inspection before they are removed from the slaughter floor (Herenda *et al.*, 2000). Postmortem inspection should provide necessary information for scientific evaluation of pathological lesions pertinent the wholesomeness of meat. Professional and technical knowledge must be fully utilized by:

1. Viewing, incision, palpation and olfaction techniques.
2. Classifying the lesions into one or two major categories-acute or chronic.
3. Establishing whether the condition is localized or generalized, and the extent of systemic changes in other organs or tissues.
4. Determining the significance of primary and systemic pathological lesions and their relevance to major organs and systems, particularly the liver, kidneys, heart, spleen and lymphatic system.

5. Coordinating all the components of ante mortem and postmortem findings to make a final diagnosis.
6. Submitting the samples to the laboratory for diagnostic support, if abattoir has holding and refrigeration facilities for carcasses under detention (Herenda *et al.*, 2000)

Monitoring by post-mortem examination of cattle viscera and carcasses at abattoirs is a critical element of tuberculosis eradication. Effective monitoring requires diligence by meat inspectors and acceptance that the cause of granulomas cannot be determined macroscopically. Detection of bovine tuberculosis also required an effective identification and tracing system to identify herds of origin.

As the prevalence of tuberculosis declined, concern was raised that the efficiency of abattoir detection of tuberculosis lesions might also decline. These concerns were addressed in 1992 by the implementation of the National Granuloma Submission Program (NGSP), following the report on tuberculosis monitoring post 1992. Abattoir monitoring for tuberculosis can only be relied upon where the inspection procedures are thorough, requiring inspectors to be well trained, examine the correct tissues and have the cause of all granulomas diagnosed by laboratory examination.

The NGSP emphasizes the importance of laboratory confirmation for a diagnosis of tuberculosis and aims to maximize the detection of tuberculous granulomas in abattoirs. All granulomas are required to be submitted to a laboratory for histopathology and/or culture examination. A submission rate of one granuloma being submitted for every 2000 cattle slaughtered was seen as ideal (NGSP 1996).

Tuberculosis in cattle and humans is said to be mainly a pulmonary disease (Francis, 1947). Madlar (1940), who studied 520 pairs of tuberculous lungs from cattle slaughtered at meat plants in the USA and Canada, stated 'Of all species so infected, there is only one, the bovine, that naturally develops a chronic progressive tuberculosis of the lung that is comparable to the disease in man.

The haphazard distribution of lesions in the lung parenchyma and their location convinced Madlar (1940) that the infection was air-borne. Single tuberculous lesions, although more common in the dorso-caudal areas, were found in all regions of both lungs. Multiple lesions showed a similar haphazard distribution.

In Britain, it was reported that the pathological examination of cattle over 6 months old showed that the primary complex is in the lungs and their associated lymph nodes on at least 60% of cases (Stamp and Wilson, 1946). An Irish study in 13 depopulated herds yielded similar results (O'Reilly and Castello, 1988). Of 376 cattle slaughtered, tuberculous lesions were found in 116 (31%). Lesions were confined to the pulmonary and retropharyngeal lymph nodes respectively in 60% and 16% of the lesion positive animals. No mesenteric lymph node lesions were found. Lung substance lesions were found at meat inspection on only a single animal in each of 2 herds.

Even when *M. bovis* infection in cattle has been attributed to contact with grass or hay heavily infected with badger excreta, it has been conceded that in the great majority of cases, the distribution of lesions in the respiratory tracts of the infected cattle indicated that aerosol inhalation was the predominant route of infection (Gallagher *et al*, 1980, Wilesmith and Williams, 1986) equally in the calf, the respiratory route of infection is most important. A

Danish study found lesions confined to the respiratory tract in 62.5% of 144 tuberculous calves, to the intestinal tract in 16% and in 7% the disease was probably congenital in origin (Plum, 1939).

The post-mortem evidence regarding the frequency of tuberculosis of the mammary glands in tuberculous cows is somewhat conflicting, but the biological testing of milk samples indicates that *M. bovis* is excreted in the milk of about 1 to 2%(Francis. 1947). Although biological testing is a very sensitive technique, it is possible that due to sampling errors and/or intermittent excretion of *M. bovis* in the milk of individual cows, this may be an underestimate of the potential public health risk.

In a retrospective study in India, based on necropsy findings, tuberculosis lesions were recorded in the mammary glands in 9 (5.4%) of 168 (29 males and 139 females) tuberculous cattle over 6 months of age (Shama *et al.*, 1985). Accordingly tuberculosis of the udder was found in 9 (6.47%) of the 139 female tuberculous animals. If it is assumed that the 14 tuberculous females aged 2 years or less were free of gross tuberculous lesions in their mammary glands, 9(7.2%) of the remaining 125 cows had tuberculosis of the udder. In discussing the relatively high percentage of cases of tuberculous mastitis observed, the authors state that this was only slightly higher than previously reported in a similar study carried out in Calcutta (Guha and Sarkar, 1970).

Tuberculosis of the central nervous system is usually confined to young animals and is probably the result of congenital infection. Tuberculous lesions were reported in the central nervous systems of 9 out of 11 congenital infected calves (McKay. 1943). If one excludes congenital

tuberculosis in the calf in which bacilli pass directly from the placenta into the blood stream, there is no evidence that haematogenous dissemination (characteristic of fatal tuberculosis in children) is more common in the calf than in adult cattle (Francis, 1947).

Francis (1947), in a review of the literature, concluded that only 12 to 50% of the calves from cows with tuberculous metritis were congenitally infected. He also concluded that the average incidence of congenital tuberculosis in calves from cows, 30-40% of which were tuberculous, was 0.35%. Accordingly approximately 1% of calves from tuberculous cows are congenitally infected. Animals are classed as congenitally infected when lesions, apart from those in various carcass lymph nodes, are confined to the liver or the liver and lungs (Stamp and Wilson, 1946).

In the absence of liver lesions, when the possibility of congenital infection is almost certainly excluded, haematogenous dissemination is rare. In a study of 104 calves with congenital tuberculosis, the portal lymph nodes were affected in all and in 37 (36%) calves, lesions were confined to the portal nodes and liver; in 14 (13%) the disease had extended to the mediastinal nodes and in 22 (21 %) to both the mediastinal and bronchial nodes (Francis, 1947).

In one British study, it was found that 22 (71%) of 31 naturally infected wild badgers had gross lesions in the lungs and/or thoracic lymph nodes, but that where haematogenous spread had occurred, the kidney was the preferred site (Gallagher *et al.*, 1980). There was renal involvement in 6 (19%) of 31 badgers with gross tuberculous lesions. In a subsequent study it was recorded that the distribution of lesions was consistent with respiratory infection in 23 (82%) out of 28 tuberculous badgers examined, and that the other 5 (18%) could have been infected from bites. It was reported that *M. bovis* infection had been introduced via bite wounds in 15

(56%) of 27 badgers, which had died of the disease. In an Irish study, it was reported that tuberculous lesions were found in the lungs and/or pulmonary lymph nodes of 13 (46%) out of 28 tuberculous badgers with gross evidence of the disease.

A review of the literature indicates that detailed post-mortem examinations of tuberculous deer have frequently revealed lesions in the retro pharyngeal, thoracic and abdominal lymph nodes. As the distribution of lesions indicates, the principal routes of infection are the respiratory tract and alimentary canal. As with cattle, the lesions in the retropharyngeal lymph nodes could indicate either route of infection.

Post mortem, as a method of diagnosing tuberculosis is a cheap method as it requires no chemical or much labour. However, it requires skilled personnel. In addition, there should be a facility to ensure proper disposal of the carcasses. The other disadvantage is that the animal must be dead before the test can be applicable.

In developed countries, classical epizootics such as tuberculosis were largely eradicated in the 1960s, resulting in major improvements in animal and human health and a dramatic reduction in the likelihood of discovering relevant lesions at meat inspection (Grossklaus, 1987). Modern farm livestock practices are more intensive and earlier slaughter of animals reduces the time available for exposure to microbiological and parasitic agents.

Exposure is also reduced in units operating with a biological barrier to wildlife and vermin, as is the case with intensive systems. Unfortunately, raising animals intensively results in the

occurrence of sub-clinical infections, including those of zoonotic agents such as tuberculosis, which are important in meat hygiene.

It has been recognized for several decades that reservoirs in livestock of tuberculosis and other meat-borne bacterial pathogens result in contamination of carcasses at meat inspection (Grau, 1986; Grossklaus, 1987). In recent years research has been aimed at reducing the spread of any meat-borne pathogens by minimizing carcass handling, and the number of incisions made during traditional, organoleptic red meat inspection (Grau, 1986).

The importance of ante- mortem inspection in the abattoir has long been recognized in the attempt to avoid the introduction of clinically diseased animals into the slaughter hall. However, the disease and treatment history of slaughter animals, while being reared on the farm, are arguably far more important in determining suitability for slaughter than a brief inspection at the abattoir. This is especially true with regard to 'invisible' meat safety hazards such as chemical residues and certain microbial pathogens, e.g. *M.bovis*, *Escherichia coli* 0157; H7, *Salmonella typhimurium* DTI 04 and *Campylobacter jejuni*.

An important function of meat inspection is to assist in monitoring disease in the national herds and flocks by providing feedback of information to the producer. An example is the recent outbreak of foot and mouth disease in Europe, which was first detected at the abattoir.

In order to reduce handling of meat at meat inspection there by reducing contamination, there is a need for good data on animals going for slaughter as stated earlier so that more attention is paid on animals which are believed to come from disease endemic areas. Unfortunately records on

animals in the developing countries, including Ghana, are almost not in existence. Therefore the only way to ensure safe meat is to resort to traditional meat inspection so as to reduce public health risks associated with the consumption of meat and meat products.

The main criticism of traditional meat inspection, the need for palpation and incision of organs and lymph nodes, is not only that it is of doubtful sensitivity, but the very nature of the procedures, e.g., incision of lymph nodes can have a detrimental effect on the safety and quality of meat by posing a risk from tire spread of bacterial pathogens (Berends *et al.*, 1993), especially in situations where Meat Inspectors use only one knife through out the day's inspection as done in Ghana. This is probably most clearly highlighted with Salmonella, localized within the mesenteric lymph nodes, as a potential source of contamination following detailed examination by multiple incisions (Archer, 1981, Murray, 1990).

Hathaway and Mckenzie (1991) claimed that inadvertent contamination with microbes during slaughter and dressing is the most important source of meat-borne public health hazards, and that a number of traditional meat inspection procedures were without any scientific basis when applied to the viscera of lambs. There is an increasing awareness that traditional, labour intensive, organoleptic inspection procedure for macroscopic abnormalities contribute far less to the safety and wholesomeness of the laboratory surveillance for microbial and chemical contaminants. Sensitivity and specificity in meat inspection are usually inversely related.

Madie (1992) found that with high disease frequencies where prevalence rates of more than 80% existed, the predictive value for detecting defect-free carcass dropped dramatically. The test falsely classified the majority of healthy animals as diseased. Similarly low prevalence rates

(10%) significantly decreased the predictive ability of Meat Inspectors to detect diseased animals. When traditional meat inspection was developed at the end of the last century, Ostetag (1999) had already reported that the lungs and mesenteric lymph nodes were the main sites of tuberculous lesions.

Concern has been expressed that a reduction in the incision of lymph nodes, even the mesenteric lymph nodes, could lead to carcass infected with tuberculosis being missed at meat inspection. In an Australian study comparing old and revised post mortem inspection procedures, there was only one occurrence of a single-site involvement of mesenteric lesions. This represented 0.4% of infected carcasses, equivalent to 0.0003% of cattle slaughtered (Murray, 1990).

2.4.9 Microscopical Examination

Mycobacterium bovis grows slowly (it has a doubling time of 18 hours, in contrast to most bacteria which can double in number in 1 hour or less). Because growth is slow, cultures of clinical specimens must be held for 6-8 weeks before being recorded negative. The above properties coupled with the very rich media required to grow *M bovis* have made the use of culture as a routine method for the diagnosis difficult. The method is therefore often employed when typing of the organism is required or in experimental procedures especially in the developing countries (Warren, 1994).

Even though this method is essential for diagnosis and confirmation of diagnosis of tuberculosis, the method is only used in research and experiments in Ghana. Diagnosis of tuberculosis as it has been said before is only based on sputum examination under the microscope. Any acid-fast organism can therefore be mistaken to be tubercle bacilli.

In most countries where culture is performed, the special media on which *M. bovis* grow may not be available. Even though the test is reliable and very important, most countries do not use it for routine diagnosis because it requires very skilled and dedicated personnel. The work involved is also big, but above all there is a need for complex facilities for the test to be conducted. These facilities are often beyond the means of developing countries including Ghana.

2.5.1 **Control** of BTB

Eradication of bovine tuberculosis has been virtually achieved in many countries.

The test and slaughter policy has been the only one by which effective eradication has been achieved (Kleeberg, 1960; Bakulov *et al*, 1987). The programme rests on the following principles:

- Removal of infected animals.
- Prevention of spread of infection.
- Avoidance of future introduction of the disease.

All the three points are of equal importance and neglect of one may result in breakdown of the control programme.

Detection of infected animals depends largely upon the tuberculin test.

Hygienic measures to prevent the spread of infection should be instituted as soon as the first group of reactors is removed.

It is most important that calves being reared as herd's replacement are fed on tuberculosis free milk either from known uninfected animals or that milk is pasteurized to eliminate mycobacterium if present.

Even though the programme have proven to be effective in many countries, it's implementation in Africa, especially Ghana, may be difficult due to lack of payment of compensation to farmers whose animals may be slaughtered (Otopri *et al*, 2000). As a result of this, bovine tuberculosis may continue to be a problem in some African countries.

2.5.2 HAZARD ANALYSIS CRITICAL CONTROL POINT (HACCP) CONCEPT IN MEAT INSPECTION.

A specific HACCP concept tailored to each abattoir and the class of animal should be developed to ensure the most efficient and effective concept of sanitary control.

The introduction of specific HACCP concept involves the following:

- a) Identifying hygienic hazards
- b) Ranking these hazards
- c) Defining the critical limit
- d) Identifying the critical control point
- e) Recommending necessary control
- f) Record keeping
- g) Verification procedures to ensure efficiency
- h) Tests to ensure that the concept is working

The Hazard Analysis Critical Control point concept was introduced in the food industry in 1971 to ensure that there would be effective control of the quality of processed food. The World Health Organization (WHO) recommends that this concept also be applied to Meat Inspection and Meat Hygiene to reduce bacterial contamination during slaughtering and dressing and to ensure quality control in meat inspection.

The practice of meat inspection has gradually changed over the last three decades. The classical ante mortem and postmortem procedures were designed to detect disease in an animal before slaughter and the lesions produced by the disease after slaughter respectively. This was done by the use of senses (organoleptic tests) such as the use of touch (palpation), sight (inspection and observation), smell (gangrenous smell) and taste (only in cooked products). Zoonotic diseases, particularly tuberculosis received high priority.

According to Wilson and Crouch (1987), meat inspection should be based on the analysis of risk. This involves the identification of risk so that they may be avoided, reduced or otherwise managed. According to them, the word 'risk' implies uncertainty; so, for veterinary public health purposes, the assessment of risk requires qualitative or quantitative estimation of the likelihood of an adverse effect resulting either from exposure to a defined health hazard or from the absence of a beneficial influence. One recognized method of risk analysis for meat production and processing is by use of the Hazard Analysis Critical Control Point (HACCP) system (ICMSF, 1988) which, since 1985, has been recommended by the World Health Organization.

According to Wilson and Crouch (1987), to be effective, the HACCP system would require a detailed analysis of the whole process from the farm through to the abattoir. The hazards should be scored according to the magnitude of risk to the consumer and a judgment is then made as to the necessary control points needed to eliminate or minimize the hazards. Once the critical control points (CCP) are in place, a monitoring system to ensure that the CCP are working should be maintained. Such a system requires the

cooperation and motivation of everyone involved in the chain and independent auditing can help to ensure that problems are not overlooked. Although the HACCP system is intended as a means of eliminating or minimizing microbial hazards, other hazards such as residues contaminants and parasitic infections are all open to the same approach. The evidence that traditional meat inspection, with certain procedures itself, provides a source of major cross-contamination does not alter the fact that the Meat Inspector acts as a critical control point for macroscopic hazards.

Therefore, meat inspection, while in need of modification, should still play an important part in meat safety and quality assurance for the consumer. Gracey and Collins (1992) wrote that, achieving these ideals would involve ante mortem examination, post mortem inspection, and where necessary, laboratory investigations, along with a close link between the abattoir and livestock production. Van Logtestijn (1993) considered the shortcomings of the present system to be a failure to identify pathogens undetected in the live animals by end-product inspection and meat inspection only.

To achieve an improvement in meat inspection using this risk analysis approach requires a number of considerations:

Identification of hazards. A list needs to be developed of all conditions of public health, animal health and aesthetic importance that are unacceptable to the consumer and which may cause macroscopic changes in the tissues to be examined (Hathaway and Mckenzie , 1991, Hathaway *etal.*, 1988;).

Hazard characterization, Ranking of macroscopic abnormalities based on the likely degree of consumer reaction, could be applied to aesthetically objectionable defects. In contrast, diseases

that are solely of animal health importance could be ranked according to an economic baseline. The optimal use of inspection resources will not eliminate all hazards, but should remove all major ones and ensure that any remaining hazards are minor in nature and exist at a level that does not constitute a risk to the consumer. *Exposure characterization.* Consumer exposure can be equated with a macroscopic condition that escapes the inspection procedure under investigation. The accuracy of the procedure at a known prevalence of the condition becomes the quantitative statistic.

Risk assessment model. The logistics of full evaluation of tissues to determine the true prevalence and the interaction between various conditions preclude whole-system comparisons. It is important to realize that formal risk assessments require a numerical base and the difficulties of defining public health objectives in these terms can result in meaningless comparisons between inspection systems that have not been individually evaluated with respect to sensitivity or specificity, and the hazards or benefits to human health (Hathaway and McKenzie, 1991).

It was concluded that many abnormalities would be detected just as well as by traditional post mortem inspection procedures, but the need to change current *post mortem* procedures to achieve a decrease in the number of false-negative findings should be carefully considered. Further study is required to determine whether it would be possible to introduce visual inspection into current meat inspection practices.

(1) Procedures that put the product at risk from microbiological contamination require detailed evaluation, e.g. the incision of mesenteric lymph nodes, tonsils, and the umbilicus and the parenchyma of the liver:

- (2) A number of organs and parts are treated as inedible. So, should all parts of a carcass be inspected or at least be made available for inspection?
- (3) The requirements to open the trachea and routinely incise most lymph nodes are open to challenge:
- (4) The use of the same inspection procedures for animals of different ages (apart from calves less than 6 weeks of age) cannot be supported; for example, the spectrum and frequency of disease in calves is very different from that in adult cattle. The same is true of lambs and sheep.

More recently, a comparative study of visual and traditional post mortem meat inspection procedures was carried out in Denmark in which more than 183 000 pigs were inspected using an electronic data capture system (Kyrval *et al.*, 1995). For this purpose an approximation procedure was developed to determine differences in non-detection rates for meat inspection procedures without having to determine the true status of the carcass. The traditional meat inspection method has a higher detection frequency for 52 out of 58 lesions. The visual method was better than the traditional method for detecting abnormal odour, abscesses in lungs, contusions, scabies, stomach and aspiration of scalding water, although only for the latter two were statically significant differences observed.

In absolute terms, however, the differences in approximate non-detection rates (ADNDR) were small (only seven lesions had ADNDR values five per 1000 pigs). Visual inspection recorded significantly less faecal and bile contamination than the traditional inspection. Faecal and bile contamination were the only lesions missed that posed a potential hazard to human health, with visual meat inspection failing to detect four pigs per 1000 carcasses, potentially contaminated

with salmonellas or Y. Enterocolitica. However, the bacterial cross-contamination that occurs in traditional meat inspection is likely to pose an even greater hazard.

It concluded that visual inspection procedures could replace traditional meat inspection without compromising the detection of most lesions. Similarly, Hathaway and others (1988), who devised a model for comparing organoleptic post mortem meat inspection procedures using palpation of the spleen of lambs, concluded that the risk assessment model overwhelmingly supported the case for organoleptic inspection being limited to visual examination.

However, in an evaluation of post mortem meat inspection procedures for the viscera of lamb in New Zealand, it was found that routine incision of the kidney was a scientifically justified inspection procedure for lambs. Palpation was also found to be a necessary procedure to accompany viewing of the liver. With the results of this study, it was recommended that Visual inspection must be an alternative to the traditional system, based on the statistical differences alone. However, such statistical differences could be used to argue either for retention of the old system of meat inspection or implementation of the new system (Hathaway and Mckenzie, 1991). Statistical differences alone do not necessarily indicate the superiority of one method over another. This can only be determined by a formal risk assessment

In Ghana the HACCP concept has not been introduced, neither is there any HAS. In fact over a quarter of animals slaughtered for human consumption are slaughtered at illegal slaughtering sites. There is no monitoring at the production levels to make sure that animals are produced under good conditions. Ante- mortem is also often not conducted and even when it is conducted, it is done under very poor conditions. Animals are often slaughtered without rest after traveling long distances. All these problems make it impossible to apply modern methods of meat

inspection. Carcasses are therefore over handled, which may lead to more contamination. Even when Meat Inspectors do their best to reduce contamination, the methods by which meat is transported, renders the meat contaminated. Meat is often transported in taxis and trucks.

2.5.3. BCG vaccination of cattle for the Control of Bovine Tuberculosis

In 1947, Francis reviewed the then available literature reports on the use of BCG as a vaccine in cattle, and concluded that a striking immunity can be demonstrated when vaccinated calves are exposed to a single *M. bovis* infecting dose 2-3 months after vaccination. 14 Results were however, disappointing when animals were exposed to infection in controlled trials under semi-natural conditions for a number of years. Under these conditions, re-vaccination did not appear to maintain immunity at a high level and little difference in the extent of the disease had been demonstrated in vaccinated and control animals.

Favorable results had however been reported in uncontrolled field trials. 14 BCG vaccines has been tried as a means of controlling bovine tuberculosis in many countries in Europe and also in North America and Africa, but it has been found neither practical or effective. In the Second Report of the Joint WHO/FAO Expert Committee on Zoonoses (1956), it was stated: “The committee is of the opinion that, generally speaking, vaccination has no place in the eradication of tuberculosis in cattle. The method has never been shown to lead to the eradication of the disease; vaccines such as those composed of BCG or the vole strain of bacilli create a sensitivity to tuberculin and therefore interfere to a marked extent with schemes for the eradication of the disease in which tuberculin testing forms the basis. Many difficulties are inherent in the application offering vaccination scheme, one of the most important being the essential condition that only animals free from the disease are submitted to vaccination”.

The maximum intradermal reactivity to tuberculin is attained 5 weeks post vaccination and it persists for up to 28 months (Modie, 1977). However, this impediment has significantly less validity in programmes such as those suitable for the tropics in which control rather than eradication of tuberculosis is the objective (Daborn and Grange, 1993). In 1958, British research workers described the use of BCG vaccination in a heavily infected herd of approximately 150 cattle in England (Doyle and Stuart, 1958). In view of the heavy infection existing in this herd, it appeared that repeated vaccination was advisable. Each calf was vaccinated within 10 days of birth, again at 6 months of age then once a year. Vaccination of calves was started in 1993 and in addition tuberculin negative stock, which were 2 years of age or under at that time were vaccinated also. Vaccination was carried out regularly until 1949 when it was discontinued in the milking herd. For a further 2 years, newborn calves were given a dose of vaccine. In some animals, post-vaccination allergy lasted for 5 years and it became difficult to detect tuberculosis animals. It was stated that this allergy may have been induced either by multiple doses of vaccine or by repeated re-infection.

It was concluded that the animal losses from bovine tuberculosis, both direct and indirect, are so continuous that, when practical it is more economical to eradicate the disease quickly by testing and disposal of reactors than by the slower method of control by vaccination (Doyle and Stuart, 1958). There is a serious public health objection to retaining known tuberculous cows for their economic life. Even where it is considered that a BCG vaccination policy for cattle is advisable in a particular environment, there is no necessity to check the skin sensitivity of animals 6-8 weeks after. It has been demonstrated that an adequate number of viable organisms induces a tuberculin-

positive state with great regularity and, that a relatively small number of viable organisms is required to induce an allergic state (Lesslie, 1965).

BCG vaccination of cattle has been abandoned world-wide, not only because of the low protective effect of the vaccine but because BCG vaccination results in a sensitivity to tuberculin and therefore interferes to a marked degree with schemes for eradication of the disease in which tuberculin testing forms the basis (WHO, 1956). In addition, many difficulties are inherent in the application of any vaccination scheme, one of the most important being the essential condition that only animals free from the disease are submitted to vaccination (WHO, 1956). Despite these impediments it has been stated that there is a need to develop a vaccine to control bovine tuberculosis in the tropics and hence for trials of the efficacy of the different BCG strains, candidate mycobacterium of environmental origin and, in due course, recombinant vaccines (Dabom and Grange, 1993).

CHAPTER THREE

3.1 Materials and Method:

3.1.1 Study Site.

The study was conducted at the Accra and Amasaman slaughterhouses in the Tema and Ga Districts of the Greater Accra Region of Ghana respectively. The animals for the study were randomly selected from the Ashaman cattle Market at Ashaman, in the Tema District, individual Farms and the Aveyime cattle Ranch in the Greater Accra Region.

There are three main slaughterhouses in the Greater Accra Region. Accra slaughterhouse, also known as Accra abattoir, GIHOC slaughterhouse, known as Tema slaughterhouse, in the Tema District and the Amasaman slaughterhouse in the Ga district.

It is estimated that over 60% of locally produced meat consumed in the Greater Accra Region come from these three slaughterhouses, about 10% come from other slaughterhouses/slabs in and out of the region with the remaining 30% coming from illegal slaughtering slabs in the region (Monthly Reports, Veterinary Services, 1997).

Accra abattoir and Amasaman slaughterhouses were selected for the study because, GIHOC slaughterhouse and Amasaman slaughterhouse share the same conditions which are different from Accra abattoir (sanitation, method of slaughtering, type of equipment and meat inspection personnel). Also, GIHOC slaughterhouse and Accra abattoir are both situated in the same district (Tema District).

3.1.2 The Accra Abattoir

Accra abattoir was constructed in 1997, with the Social Security and National Insurance trust (SSNIT) as the biggest shareholder. The abattoir was constructed to provide a hygienic place for slaughter of animals for the ever-increasing population of the Accra and Tema Municipalities.

The abattoir therefore is the most modern slaughterhouse in Ghana. It is well equipped for operating as a modern slaughterhouse. The abattoir is also equipped with a good laraige, making ante-mortem examination easy and convenient, but most often the animals are brought to the abattoir very late in the evening and are slaughtered that same night making ante-mortem examination and resting of the animals almost impossible. The abattoir has a very good drainage system. Sewage from the plant is first pumped to a treatment plant before discharge into the sea.

Three permanent Veterinary Personnel and two Personnel from the Environmental Health Unit of the Ministry of Local Government perform meat inspection at the abattoir. All the three Veterinary Personnel have had some training with duration ranging between three weeks to six months. Their working experience range between two to twenty years. The Personnel from the Environmental Health Unit, on the other hand, have not had any training in meat inspection.

3.1.3 Amasaman Slaughterhouse

A private businessman constructed the Amasaman slaughterhouse. It is located at Amasaman, the capital of the Ga District, on the Accra Nsawam road.

It was originally constructed to slaughter about ten cattle a day. It is now slaughtering about forty cattle a day. The facilities available at the slaughterhouse are not up to internationally acceptable level. There is no running water. The drainage system however, is good. No equipment to raise

carcasses for dressing, and there is no meat van to transport carcasses from the slaughterhouse to the market.

Two Veterinary Personnel and two Personnel from the Environmental Health Unit of the Ministry of Local Government conduct meat inspection. Unlike the personnel at Accra abattoir, none of the four Personnel have had any special training in meat inspection.

3.1.4 Ashaman Cattle Market.

The Ashaman cattle market is located about one kilometre away from Tema, on the Tema Akosombo road. The Tema Municipal Assembly constructed it. Cattle, sheep and goats from all over the country and other countries like, Bukina Faso and Niger are brought to the market for sale. Some of the animals are brought for slaughter whilst others are for breeding. The capacity of the market is over two thousand animals. Both healthy and sick animals are brought to the market for sale. It is not uncommon to find dead and almost decomposed animals in and around the market. Due to poor records on the animals, it is often very difficult to trace back to the origin of an animal if found to be sick of a disease, including tuberculosis. Due to lack of compensation to owners of animals that die at the market, animals are often quickly slaughtered when the owners detect that they are about to die. They do this so that they can sell the carcasses to retrieve at least part of the cost of the animal. There are Veterinary Personnel at the market who issue either movement or slaughter permit to people who purchase animals at the market depending on the purpose for which the animal was purchased.

3.1.5 Individual Farms/Ranches

In Ghana, cattle are kept in small groups of an average size of one hundred. There are few cattle ranches in Ghana. The only ranch where animals were selected for the study was the Aveyime cattle ranch at Aveyime, on the Sege Bator road. All cattle in the region are kept on free range. Animals walk long distances every day in search of food. There are often few water sources for the animals especially during the dry seasons. Most of the rivers and dams, which serve as water sources for the animals dry up during the dry season, with the remaining few serving a large number of animals from different localities. The water sources therefore become a potential source of infection. Herdsmen and villagers use water from the same dams serving as water source for the animals for drinking, bathing and washing.

In the same way, during the dry season, the pasture is dry, forcing herdsmen to walk very long distances with their animals in search of food. In so doing, they always come in contact with other animals from other localities. This practice leads to cross infections of bacterial diseases including BTB. During this period, movement of animals and herdsmen produces dust, which facilitate aerosol transmission of infectious agents, especially air borne diseases such as BTB.

During the dry season, the grass is often burnt; leaving the grazing ground bare, except few areas along riverbanks, dams and ponds. These areas therefore become over populated.

The pastures become contaminated with faecal and urine material which may contain both bacteria and parasites. The poor nutrition coupled with lose of energy through walking long distances could also lower the resistance of animals to infectious diseases.

3.2. Study Period

The study was conducted from September 1999 to Jan. 2001.

3.3 Study Population:

One hundred and fifty cattle were selected from nine hundred cattle (after the nine hundred cattle were tuberculin tested) for the study. Nine hundred cattle were tuberculin tested instead of one thousand one hundred arrived at by the formula $N = \{(Z^2 P q)/d^2\}$ using Epi Info. Where:

N is the sample size

Z- is the Confidence limit=95% (1.96)

P- is the assumed national prevalence of BTB in cattle =3% (0.03) based on previous works (monthly reports Veterinary services, 1997)

$q = 1 - p = 0.97$

d - is the acceptable deviation from the true value=1% (0.001)

The power of the test was set at 80%.

The population of cattle (yearly number of cattle passing through the Ashaman cattle market+ cattle from all the individual farms in the area studied) involved in the study was estimated at 34,000 based on 1997 Veterinary monthly reports.

From the formula above, the sample size for the present study was estimated to be 66 cattle {33 tuberculin positive and 33 tuberculin negative cattle (3% of the study population)}. All the 33 cattle that were expected to test positive to the tuberculin test were to be selected as cases for the study.

The nine hundred cattle, which were tuberculin tested, were randomly selected from individual farms, Aveyime cattle ranch and Ashaman cattle market. Individual farm owners and the ranch

were selected after they have agreed to remove and slaughter any animal that will react positive to the test.

There was no problem to remove animals tested positive at the cattle market because, the cattle that were brought there were all for sale, and could therefore be easily purchased for the study.

3.4 Tuberculin Test:

Nine hundred cattle of different sexes aged two years and above, selected randomly from the Ashaman cattle market, individual farms and the Aveyime cattle ranch were tested for bovine tuberculosis, using the ICTT. This test was selected because; there is no information about the presence or absence of Johne's disease in the region. Also, the test was selected to differentiate avian tuberculosis from BTB (Blood and Radostits, 1989).

Avian and mammalian tuberculin were injected simultaneously into two separate sites on the same side of the neck, 12cm apart, and one above the other, using the McIntock tuberculin testing equipment. A dose of 10,000 tuberculin units (0.1ml tuberculin containing 2mg of bovine or avian PPD) were used. Before the injection, the two injection sites were shaved with a razor blade. After shaving, a measurement of the thickness of the skin was taken with calipers, and the results recorded. Each animal was given an identification number using permanent paint. The test results were recorded 72 hours after the injection. Care was taken in placing the injection as sensitivity varies from place to place in the skin. The thickness of the skin was measured again after the 72 hours and compared with the previous readings. The greater of the two reactions indicates the organism responsible for the sensitization. Cattle that reacted positive to bovine tuberculin were selected as cases for the study. Cattle that reacted negative to all the two tuberculin were selected as controls for the study. All cattle selected (cases and controls) were

sent to Accra abattoir or Amasaman slaughterhouse for slaughter and for meat inspection. The study was double blinded to prevent biases.

3.5 Meat Inspection

Both the positive cases and controls were given coded numbers before slaughter. Each batch of cattle for the study was presented at the slaughterhouses for slaughter without making the Meat Inspectors aware that the animals presented belonged to the experimental lot. This was done to prevent biases. Before the animals were presented for slaughter, an Assistant took nasal swabs from both the cases and controls to be stained and examined for tubercle bacilli. Faecal samples were also taken for examination for oesophagostomum eggs and larvae. All formalities at the slaughterhouses were followed.

Samples of lesions were taken into sterile bottles and correctly labeled. The number of suspected BTB cases were recorded each day. Findings of the Meat Inspectors were recorded daily. Any organ that was condemned or trimmed was also recorded and preserved. The actions of the Meat Inspectors were also recorded each day.

The Assistant also took note of the findings of Meat Inspectors and recorded them daily. Any organ that was condemned or trimmed was also recorded and preserved for further investigation. An enhanced meat inspection was conducted after the regular Meat Inspectors had completed their inspection. Enhanced meat inspection comprised the following 1. Cutting lymph nodes and lungs into slice of about 2cm. and 10cm respectively. 2. Carefully inspecting the offals for any swelling or granulomas. 3. Debonning of the carcasses. 4. Cutting the bones into pieces to look for any possible lesions. The carcasses and offals were re-inspected to find any possible lesions

that might have been missed by the Meat Inspectors. All the intestines and the mesenteric lymph nodes were re-examined. The intestines were washed and inspected for the presence of adult Oesophagostomum worms. Any suspicious lesions seen were taken and added to those that were identified by the Meat Inspectors and sent to the Central Veterinary Laboratory in Accra and the Noguchi Memorial Institute for Medical Research for microbiological and parasitological investigations. The coded numbers on carcasses were compared to the original numbers on each animal prior to slaughter after all the inspections. In this way, each animal was identified. Final result for the inspection was then recorded.

3.6.1 Laboratory Investigations.

Forty five (45) samples of pimply gut-like lesions, five (5) congested lymph nodes, twenty (20) nasal swabs, and forty (40) Faecal samples were sent to the laboratory for bacteriological and parasitological examination.

3.6.2 Bacteriological examination

Smears were made from all the pimply gut-like lesions and lymph nodes. The following steps were followed.

1. An incision was made with a sterile knife to expose the underlying tissues.
2. Using a sterile wire loop, a small amount of tissue material was scooped from the inside of the pimply gut-like lesion between the edges of dead tissue and the living tissue and spread thinly on a microscopic slide to make a smear.
3. The smear was then left to dry for a period of about thirty minutes.

4. The smear was then gently passed over a Bunsen burner flame to fix it.
5. After fixing, the slide was stained by Ziehl Neelsen staining method.
6. After staining, the smear was made to dry and examined for acid-fast organisms.

The lymph nodes were treated the same way as the pimply gut-like lesions.

The swabs were gently robbed on a microscopic slide after moisturizing it in distilled water, to make a smear. The smears made from swabs from nasal discharges were also stained and examined for acid-fast organisms using the above method.

3.6.3 Parasitological Examination

Direct wet smears were made from all the 40 faecal samples collected from both the cases and the controls and examined for *oesophagostomum* species eggs and larvae. Small amounts of the faecal material was mixed with two drops of distilled water on a microscopic slide (the smear was made light enough to see through). The wet smear made was covered with a cover slip and examined under the microscope using the low power lenses for *Oesophagostomum* species eggs or larvae.

The pimply gut-like lesions found in the intestines were also carefully examined for the presence of *oesophagostomum larvae*.

3.7. Knowledge, Attitude, Beliefs and Practices of Meat Inspectors in Relation to BTB.

The strength of staff involved in meat inspection at all the slaughterhouses in the region was accessed. Through interviews and administration of questionnaire, the knowledge attitude and practices of Meat Inspectors in the region in relation to BTB was accessed.

The educational background and qualifications of Meat Inspectors were accessed as well as their job experience. Problems facing Meat Inspectors that could affect the effectiveness of meat

inspection were also accessed. All the assessment was done through interviews and administration of questionnaire. The number of suspected and confirmed tuberculosis cases from each of the slaughterhouses in the region during the period of the study were accessed.

3.8 Limitations

The tubercle bacilli identified in the pimply gut-like lesions were not isolated and typed due to lack of the facilities and reagents.

3.9 Ethical Considerations.

Cattle Owners agreed to remove and slaughter any tuberculin positive animal before their animals were selected for the study. After testing the cattle for tuberculosis, Herdsmen and Cattle Owners around were interviewed to ascertain what they know about bovine tuberculosis. Based on what they know, Herdsmen and Cattle Owners received information about BTB and the risk of infection posed by cattle. They were also informed on how to prevent being infected and getting other people also infected. Cattle Owners whose animals were slaughtered were compensated for the losses and information was kept confidential.

CHAPTER FOUR

4.0 RESULTS:

Nine hundred cattle were tested for tuberculosis using the single intradermal comparative tuberculin test. Four hundred out of the nine hundred were selected from the Ashaman cattle market, 300 from individual farms and 200 from the Aveyime cattle ranch. Out of the 900 cattle tested, 75 tested positive for bovine tuberculosis. Out of the 75 cattle that tested tuberculin positive 45 were from the Ashaman cattle market, 22 from individual farms, whilst 8 were from the cattle ranch (table 1.) The results of 18 cattle were doubtful (the animals reacted equally to both bovine and avian tuberculin antigen), whilst 807 were negative. The ages of the tuberculin positive cattle ranged between 2 and 9 years. Only two out of the 75 cattle that tested positive showed signs of emaciation. The higher percentage of tuberculin positive cattle was found at the Ashaman cattle market (11.25%), followed by the individual farms (7.33%) with the Aveyime cattle ranch of the least.

4.1. Results of Meat Inspection.

Meat Inspectors who have been assigned to inspect meat at the two slaughterhouses inspected all the 150 cattle routinely after slaughter in batches (Table 6). The 150 cattle were also subjected to an enhanced meat inspection. The meat inspectors passed the carcasses and offal of 147 out of the 150 cattle for human consumption at the first inspection without any comments. The offal of 1 out of the remaining 3 cattle was condemned as a result of multiple lesions suspected to be tuberculous lesions. The skin of 1 animal was also condemned because it was suspected to be infected with dermatophilosis (streptothricosis). About *Zi* a kilogram of muscle was trimmed

from the gluteal muscles of the third animal as a result of an abscess believed to have been caused by an injection.

During the enhanced meat inspection, small swollen circumscribed round lesions with a diameter ranging 0.01- 0.5cm containing yellowish cheesy material upon incision, which are often termed “pimply gut”, were found in the small and large intestines of the offal of 74 cattle. The “pimply gut” like lesions was also found in the offal of the animal that was condemned by meat inspectors. All the 75 animals, which showed the presence of the described lesions, were also positive for the tuberculosis test prior to slaughter. None of the controls tuberculin test negative cattle showed any of the described lesions (Table 2).

4.2. Microscopic Examination of Smears for Acid Fast Bacteria and Heiminthes.

Microscopic examination of smears of “pimply gut” lesions stained with Ziehl Neelson showed the presence of acid-fast organisms in 35 (77.7%) out of the 45 samples submitted to laboratories for examination (table 3 fig 1). Acid Fast Organisms were identified in 3 (60%) out of the 5 lymph nodes submitted for Microscopic examination. Microscopic examination of nasal swabs did not show acid-fast organisms.

Macroscopic examination of the pimply gut-like lesions did not show any oesophagostomum larvae.

Microscopic examination of faecal samples did not show the presence of oesophagostomum eggs or larvae

4.3 Cases of BTB at Slaughterhouses.

During the period of the study, a total of 37,802 cattle were slaughtered at both Accra and Amasaman slaughterhouses. 19,733 (52.2%) cattle were slaughtered at Accra abattoir, whilst 18,069 (47.8%) cattle were slaughtered at Amasaman slaughterhouse. Out of the 37802 cattle slaughtered at both slaughterhouses during the period of the study, 34,028 (90%) came through the Ashaman cattle market. During the period of the study (Sept 1999 to Jan. 2001), 150 suspected cases of tuberculosis were reported at the Accra abattoir. There was no suspected tuberculosis case reported at Amasaman slaughterhouse during the period (Table 4 &5).

4.4. Strength Of Staff At Slaughterhouses In The Greater Accra Region

There were 25 Personnel involved in meat inspection in the Greater Accra Region. Out of the 25 Meat Inspectors operating in the region, 13 were assigned to the two slaughterhouses where the study was conducted (Table 7). There were 9 Veterinary Personnel and the remaining 16 were from the Environmental health unit of the Ministry of Interior. Out of the 25 personnel, only 5 have had specialized training in meat inspection, after their basic training at either Pong Tamale Veterinary College or the School of Hygiene. All the 5 personnel with specialized training in meat inspection were from the Veterinary Services Department. Two of the 5 trained meat inspectors were assigned to the Accra abattoir.

4.5. Knowledge, Attitude, Beliefs and Practices (KABP) Of Meat Inspectors In Relation to BTB.

Table 8 shows the results of knowledge, attitude, beliefs and practices (KABP) of Meat Inspectors in relation to BTB. Even though all the 13 Meat Inspectors assigned to the two slaughterhouses listed BTB among the first 5 most important diseases to look for at meat

inspection, very little attention and time was spent to examine carcasses and offals for signs of the disease. Out of the 13 Meat Inspectors interviewed, only 2 responded that they used more than 10 minutes to examine each carcass. Only 7 said they pay special attention in looking for TB lesions. All the 13 Inspectors interviewed said they did not conduct ante-mortem examinations prior to slaughter of animals. Seven out of the 13 Meat Inspectors interviewed said they have diagnosed BTB at slaughterhouses and could recognize the lesions when they saw one. Out of the 13 Meat Inspectors interviewed, 11 said they used only one knife during meat inspection. When asked if that will not lead to cross contamination of carcasses, especially after making an incision through a TB lesion, all the 11 said they wash the knives after cutting through a suspected TB lesion. It was established that even when the meat inspectors came across suspected BTB lesions, they only trimmed off the affected part and passed the carcass for human consumption without bothering to send the lesions to the laboratory for confirmation.

Among the several reasons why suspected samples were not sent to the laboratory were:

- 1- Lack of facilities for the transportation of the samples.
2. Delays in making results available to the slaughterhouses.
3. Lack of cooperation from the Butchers or cattle owners.

All the 13 Meat Inspectors said that they often came across the “pimply gut” lesions in cattle during meat inspection. However, they said they do not attach any importance to them because they did not know the lesions could be due to mycobacterium. They also said that offal with numerous “pimply gut” lesions would be condemned or disposed of not because they think humans will be infected but for aesthetic reasons.

Table 1.
Results Of Tuberculin Test for Cattle from Farms and the Cattle Market

LOCATION	NUMBER OF CATTLE TESTED	NUMBER POSITIVE	% POSITIVE	P<
Ashaman Cattle Market	400	45	11.25	0.05
Individual Farms	300	22	7.33	0.05
Aveyime Cattle Ranch	200	8	4.00%	0.05
TOTAL	900	75	8.33%	

Table 2
A 4x4 Table Showing the Relationship Between Tuberculin Test Result on Cattle and the Presence of Pimply Gut like Lesions at Post Mortem

POST MORTEM FINDINGS	TUBERCULIN TEST POSITIVE	TUBERCULIN TEST NEGATIVE	TOTAL
Pimply gut present	75	0	75
Pimply gut not present	0	75	75
TOTAL	75	75	150

Table 3.
Microscopic Examination of Lesions for Mycobacterium/Oesophagostomum eggs/ larvae

N/N OF SAMPLES	TYPE OF SAMPLES	TEST CONDUCTED	NO POSITIVE	NO NEGATIVE	% POSITIVE
45	Pimply gut-like L.	Microscopic	35	10	77.7
20	Lymph nodes	⁷⁵	3	17	15.0
40	Feecal material	⁷²	0	40	0

Fig1

Microscopic examination of smears from pimply gut-like lesions for Mycobacterium

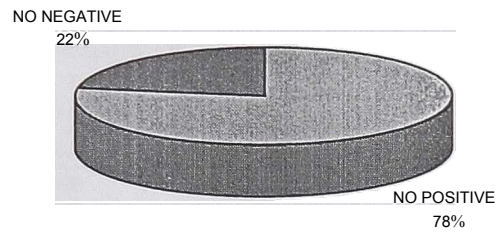


Table 4.

Monthly Incidence of Suspected Bovine Tuberculosis cases at Accra Abattoir (Jan. -Dec. 2000)

MONTH	NUMBER OF ANIMALS SLAUGHTERED	SUSPECTED TB CASES	%
January	1909	15	0.78
February	1852	19	1.03
March	1842	20	1.09
April	1674	13	0.78
May	1596	11	0.69
June	1340	18	1.34
July	1595	20	1.25
August	1464	13	0.89
September	1475	12	0.81
October	1579	5	0.32
November	1764	4	0.22
December	1643	4	0.24
Total	19733	150	0.76

Table 5.

Monthly Incidence of Suspected Bovine Tuberculosis cases at Amasaman slaughterhouse (Jan. -Dec. 2000).

MONTH	NUMBER OF ANIMALS SLAUGHTERED	SUSPECTED TB CASES	%
January	1500	0	0
February	1560	0	0
March	1346	0	0
April	1440	0	0
May	1451	0	0
June	1401	0	0
July	1465	0	0
August	1486	0	0
September	1445	0	0
October	1452	0	0
November	1766	0	0
December	1757	0	0
Total	18069	0	0

Table 6.
Batch Submission of Cattle with Known Tuberculin Status For Meat Inspection

BATCH NO	NUMBER OF ANIMALS	SLAUGHTER HOUSE	REMARKS
1	10	Accra abattoir	5 with pimple gut
2	8	³	4 „ „
3	10	³³	5 „ „ and 1 with skin lesions
4	6	⁵⁷	3
5	8	³³	4 „ „
6	6	Amasaman	3 „ „
7	10	³³	
8	8	Accra abattoir	4 ⁵¹
9	10	³³	
10	8	³⁵	4 „ „
11	6	Amasaman	3 „ „
12	10	³⁷	
13	8	Accra abattoir	4 55
14	10	³³	
15	8	³³	4 5, 53
16	8	³³	4 „ „
17	10	³³	
18	6	³³	3 „ „ and abscess
TOTAL	150	150	75

Table 7.
Strength of Staff at Accra and Amasaman Slaughterhouses

SLAUGHTERHOUSE	NO OF STAFF	NO TRAINED
Accra abattoir	8	2
Amasaman slaughterhouse	5	0
Total	13	2

Table 8.**KABP of Meat Inspectors In Relation to Bovine Tuberculosis**

STATEMENT	NO INTERVIEWED	POSITIVE RESPOND	NEGATIVE RESPOND
TB is among the first five most important diseases at slaughter house	13	13	0
Listed TB as number 1 disease looked for	13	11	1
Use more than one knife for inspection	13	2	11
Spent more than 10 minutes on carcass	13	0	11
Pay special attention to look for TB	13	7	5
Ante mortem on animals before slaughter	13	0	13
Personally diagnosed TB at Inspection	13	7	6
Stamp carcasses after inspection as an identification	13	0	13
Know pimply gut like lesion could be TB	13	0	13

Table 9.**Conditions at Accra Abattoir and Amasaman Slaughter House**

ITEM	ACCRA ABATTOIR	AMASAMAN SLAUGHTER HOUSE
Location	Tema District,	Ga District
Distance from Accra		
Method of slaughtering	raising	on the ground
Availability of water	enough	inadequate
Availability of electricity	available	available
Drainage system	very good	good
Sanitation	good	average
Availability of meat van	available	not available
No of qualified Inspectors	two	nil
Record keeping	good	poor

CHAPTER FIVE

5.1 DISCUSSION.

Based on the intradermal comparative tuberculin test, the present study has established that, bovine tuberculosis is prevalent in Ghanaian cattle. The prevalence of BTB was 8.33% in the 900 cattle from small individual farms (300), the Ashaman cattle market (400) and a large ranch (200), all in southern Ghana. The prevalence established by the study may not be the true reflection of the prevalence of BTB in Ghana, as the animals submitted at the market were often old with some showing signs of ill health and likely to be infected with BTB which is a chronic disease. However, the 7.33% prevalence established by the study at individual farms coupled with a prevalence of 13.8% established by an earlier study in some parts of the country (Bonsu *et al*, 2000), strongly suggest that the prevalence of BTB in Ghana could be high. The low prevalence of 4.0% established at the Aveyime cattle ranch may be due to an earlier intervention of the test and slaughter programme embarked on by the ranch before this study. The affected animals in the present study comprised animals of both sexes, aged two years and above. Out of the 75 animals that reacted positive to tuberculin test, only two (2.6%) showed signs of emaciation, the rest of the animals were of good bodily condition and were therefore presented for slaughter. This clearly underlines the limitation of clinical diagnosis of BTB

Bovine tuberculosis is transmissible to humans (Crofton, 1992, Archa and Szyfres, 1987, Blood and radostits, 1989), and so this high prevalence of the infection in cattle presented for slaughter represents a potential risk to human infection. Studies elsewhere have indicated a high prevalence of tuberculosis due to *M. bovis* in humans in areas where the prevalence of bovine tuberculosis is high (Alfredson and Skjierve, 1993, Archa and Szyfres , 1987, Carmicael, 1939).

Unpasteurised milk is the main source of human infection of *M. bovis*, but meat, which harbours *M. bovis*, the causative agent of BTB, if not well-cooked or roasted before consumption, could lead to human infection (Blood and Radostits, 1989, Crofton, 1992). It is also possible for individuals handling the meat to be infected through aerosol and contact where there is broken skin.

In an earlier study conducted in the Dangme West District in the Greater Accra Region of Ghana, where a prevalence rate of 13.8% was established, it was found that milk is frequently drunk without boiling or pasteurization (Bonsu *et al.*, 2000). Other studies conducted in some parts of the country indicate that despite a high prevalence of BTB in the country, there is substantial lack of knowledge about the disease among cattle owners and herdsmen (Otuipri *et al.*, 2000). This means that there is considerable risk when infected cattle are not detected and remain in the herd. The risk is higher when infected animals are producing milk for human consumption. There is also a risk when such animals are slaughtered, and the lesions caused by the disease could not be detected and the carcass passed for human consumption. It is therefore essential to reliably identify infected animals prior to or after slaughter and ensure that their carcasses are properly destroyed. Thus a reliable diagnostic tool is crucial to the prevention of human infection.

The present study has shown that, whereas the comparative tuberculin test is effective in identifying TB infected cattle, routine meat inspection could identify only one out of the 75 tuberculin test positive cattle (1.3%). This means that nearly all cattle infected with *M. bovis* are likely to remain undetected by routine meat inspection and the meat could pose a considerable risk to human health. The finding of the study is in conformity with the findings of Kazwala and

others. In a study conducted in Tanzania to find out the number of tuberculin positive cattle with TB lesions, detection of tuberculous lesions using routine meat inspection procedures identified only 26.5% of the 913 positive cattle (Kazwala *et al.*, 1997). Ritacco and de Kantor (1991) reported a similar result in Latin America. Carmichael (1941) in his extensive survey examined 1500 tuberculin positive cattle after slaughter and found out that only 2.5% had tuberculous lesions. In Cape Coast, (Ghana), Turkson and Boadu (1999) could not detect any TB lesions in 4 tuberculin positive cattle after slaughter, however, pimply gut-like lesions were detected in all the 4 cattle.

All the studies reviewed were conducted in developing countries where animals are often kept in free range, a practice, which does not favour infections by the aerosol route. In these countries, water and feed for cattle can easily be contaminated by *M. bovis*. For example, during the dry season in Ghana, one small pond may serve as the only source of drinking water for several hundreds of animals from different areas. In such a situation, if one infected animal drinks from the pond and contaminates it, many non-infected animals could easily be infected. Also, animals with TB lesions in the intestine shed tubercle bacilli in their feces (Maddock, 1936, Bakulov *et al.*, 1987). This may contaminate the grazing grounds serving as source of infection for non-infected animals. Non-infected animals may therefore get infected with *M. bovis*, either through the contaminated grass they pick as they go grazing or through the contaminated water they drink. The oral route of infection in these areas is therefore more likely than the aerosol or respiratory route. It is documented that there is direct connection between the route by which an animal is infected with tuberculous bacilli and the location of lesions. If an animal gets infected through aerosol (respiratory route), the primary location of lesions will be in the lungs and/or

associated organs. On the other hand, if the infection was through the mouth (oral route), then the lesions will be in the intestines and/or associated organs (Crofton, 1992). It is therefore most likely that the location of BTB lesions in cattle if infected, will be in the intestines and /or associated organs.

The literature indicates that 90-95% of BTB lesions are found in the respiratory system (Herenda *et al*, 2000). In countries where intensive management is practiced, and animals are often kept in an enclosed environment in large numbers, and where water and feed are not likely to get contaminated with *M bovis*, this could be true and Cattle are most likely to be infected with *M. bovis* through the respiratory system. Lesions are therefore likely to be located in the lungs and associated organs.

So whilst the respiratory route may be the major route of BTB infection in developed countries, the oral route may be the most important in developing countries. In the same way, whilst the lungs and associated organs may be the common sites for BTB lesions in the developed countries, the intestine and it's associated organs may be the most common sites of BTB lesions in developing countries.

In the present study, over 80% of BTB lesions were found in the intestines contrary to the reports in literature and meat inspectors missed these lesions and the carcasses passed for human consumption. Infected animals were as young as 2 years and as old as 9 years. The presence of many young infected cattle suggests that the disease is endemic and animals get infected early in life. Meat inspection records of animals slaughtered at the two main slaughterhouses revealed

that BTB is commonly diagnosed at slaughter, even-though the prevalence was very low due to inability to identify BTB lesions in some carcasses of cattle. This data also affirms the endemicity of BTB in cattle in the Greater Accra region of Ghana.

The major objective of the study was to test the hypothesis that *“pimply gut-like lesions frequently found in the intestines of cattle at meat inspection are largely due to tuberculosis and not the gut worm, Oesophagostomum and are missed at meat inspection posing a significant risk to human infection*

To test this hypothesis, groups of BTB positive (75) and negative (75) cattle based on the comparative tuberculin test were presented for slaughter at the two main slaughterhouses in the Greater Accra region of Ghana. Of the 150 cattle of known tuberculin status slaughtered in the present study, only 1 (1.3% was suspected of having BTB at meat inspection, 74 were passed as BTB negative and so fit for human use. This means that 98.7% of cattle infected with BTB based on the comparative tuberculin test undertaken before slaughter were missed at meat inspection. This supports the suggestion that post -mortem inspection of animals at slaughter largely fails to detect cattle infected with BTB (Kazwala *et al.*, 1997, Turkson and Boadu, 1999, Stumpff *et al.*, 1985).

The comparative tuberculin test is a deferential test. It is enough confirmation that an animal has been infected with either *M. bovis*, or *M. tuberculosis*, if during the comparative tuberculin test; an animal reacts to the *M. bovis* antigen but not to the *M. avium* antigen. That is why the test is used in the test and slaughter programme for the elimination of BTB. On the other hand, the

animal is infected with *M. avium* or Johne's disease if the animal reacts to the *M. avium* antigen but not to the *M. bovis*. The test is therefore normally used on suspected tuberculin positive animals after an initial screening test has been performed, using other tuberculin test methods, or in an area where Johne's disease is suspected (Blood and Radostits, 1989).

This means that the 75 animals that tested positive to the comparative tuberculin test in the present study were likely to be either infected with *M. bovis* or *M. tuberculosis*. Even though *M. tuberculosis* infection is possible in cattle, it is extremely rare and the disease is not progressive. Also there are no recorded cases of cow to cow or cow to man infection. Again lesions of *M. tuberculosis* in cattle are self-limiting (Collins and Grange, 1987). The animals that reacted positive to the *M. bovis* antigen in the present study cannot therefore be attributed to *M. tuberculosis*, because the percentage of the animals that reacted positive was high (8.33%) and the lesions found in tuberculin positive animals were numerous and of different sizes, which means that the lesions were not self-limiting. It can therefore be concluded that, most of the tuberculin positive animals in the present study, could have been infected with *M. bovis*.

Enhanced meat inspection on all the 150 cattle (75 positive and 75 negative for BTB) revealed small, round, raised lesions of diameter 0.01 to 0.5cm, exclusively in the intestines of 75 out of the 150 cattle slaughtered. These lesions were exclusively restricted to cattle, which reacted positive to bovine tuberculin test prior to slaughter, but were absent in those which were negative in the tuberculin test. There was therefore a strong association of the presence of these intestinal lesions with comparative tuberculin positivity prior to slaughter ($P < 0.001$). Meat Inspectors, who often attributed them to *Oesophagostomum radiatum* infestation, classified these lesions as

“pimply gut” lesions. The lesions are frequently seen in cattle at slaughter by Meat Inspectors and the carcasses of cattle with such lesions are passed for human consumption.

Further examination of these lesions in the laboratory however, revealed the presences of acid-fast bacteria in 77.7% of cases. The causative agent of BTB *Mycobacterium bovis* is an acid-fast bacterium. Currently, routine confirmation of the diagnosis of tuberculosis in all health facilities in Ghana is based on the detection of acid-fast bacilli in sputum. Thus the use of this method for the suspected BTB lesions in the present study is justified. In addition the CTT is a highly specific test and recommended Although the *Mycobacterium* present in the lesions were not specifically isolated and identified as *M. bovis*, taken together with the comparative tuberculin positive reaction of the cattle prior to slaughter to the specific antigen of the causative agent of BTB, *Mycobacterium bovis*, and the very strong association between the presence of the lesions and positive reaction to the comparative tuberculin test, it can be concluded that: (1). cattle with pimply gut-like lesions react to tuberculin test.

(2). the high percentage of pimply gut-like lesions (77.7%) showing acid fast organisms indicate that pimply gut-like lesions found in the intestines of cattle at meat inspection are largely due to *Mycobacterium bovis* and not the gut worm, *Oesophagostomum* species.

(3). the high percentage of tuberculin positive cattle (99.7%) missed by Meat Inspectors indicates that these pimply gut-like lesions are confused with lesions that are caused by *Oesophagostomum radiatum*,. (4). Since meat can be a source of infection of *M bovis* to humans, this inability of Meat Inspectors to detect BTB lesions poses a significant risk to human infection of the disease.

The results of this study may offer the major explanation for the absence of tuberculous lesions in tuberculin test positive cattle at slaughter and inspection as reported by many researchers (Stumpff 1985, Kazwala *et al.*, 1997, Turkson and Boadu, 1999,). The explanation for the absence of lesions are may be that, inspectors look for lesions from only the lungs, lymph nodes and the liver, the supposed common sites for TB lesions (Herenda *et al.*, 2000), without paying much attention to the offal or intestines. Others may simply mistake the lesions in the intestines where they are present for pimply gut and therefore will not attach any importance to them.

The diagnosis of BTB at slaughterhouses needs skill and a trained eye. Meat Inspectors who are assigned to slaughterhouses must therefore be given formal specialized training in meat inspection for effective diagnosis of BTB and other diseases. (Herenda *et al.*, 2000). The detailed meat inspection identified BTB infected cattle were confirmed by the results of the present study. In the year 2000, 19,733 cattle were slaughtered at Accra abattoir, 150 cases of tuberculosis were reported during the period (Table 4). During the same period, 18,069 cattle were slaughtered at the Amasaman slaughterhouse, but there was no reported case of tuberculosis at the Amasaman slaughterhouse (Table 5). This difference occurred even though all the animals slaughtered at both slaughterhouses came from the same source (Ashaman cattle market). It was established, that out of the eight Meat Inspectors assigned to carry out meat inspection at the Accra abattoir, two had specialized formal training in meat inspection, whilst none of the 5 Meat Inspectors assigned to the Amasaman slaughterhouse, had any specialized training in meat inspection. The skills and the training of the 2 Meat Inspectors at the Accra abattoir may have contributed to the better recognition of BTB lesions, hence the high incidence of BTB cases recorded during the period under consideration at the Accra abattoir. Presently, out of the 25 Meat Inspectors in the

Greater Accra region, only 5 have specialized training in meat inspection. This may be the major reason for the big difference between the low prevalence of BTB in the Greater Accra Region reported at slaughterhouses (0.001 to 0.5 %) and the high prevalence reported from the field by the results of tuberculin test in the same region (2 to 13.8%). Due to lack of training, the diagnosis of BTB at our slaughterhouses is not effective as the reports from the two main slaughterhouses in the Greater Accra Region indicate.

The other reason for the low prevalence of BTB reported at slaughterhouses is that, the main organs routinely examined by meat inspectors for tuberculous lesions at meat inspection are the lungs, liver, and lymph nodes. As the results of the present study indicated, Meat Inspectors in the slaughterhouses do not seriously consider tuberculosis when examining the intestines. The situation is similar in most African countries. The situation is the same as , exemplified by a study in Tanzania, where the Meat Hygiene Ordinance (1962) recommends the examination of lymph nodes of the head, thoracic and abdominal cavities for tuberculous lesions (Kazwala *et al.*, 1997). Since the practice in Ghana is similar; it is obvious that BTB lesions will be easily missed at meat inspection.

In addition to the sites of examination, the technique and concentrated examination will also assist in identifying suspected lesions of BTB in cattle (Kazwala *et al.*, 1997). The results of the present study showed that, even though Meat Inspectors were aware of the public health implications of passing meat infected with *M. bovis*, very little time was spent for inspecting carcasses for signs of the disease. As indicated by Kazwala (1997), there is a need for thorough inspection to increase the chance of BTB lesion detection at meat inspection. So the very shorter

time spent on inspection may have contributed to the very poor detection rate of signs of the disease at the slaughterhouses in the present study.

Since modern meat inspection recommends minimum handling of meat at meat inspection to prevent microbial contamination by Meat Inspectors (*Hathaway et al., 1988*), how then can effective diagnosis of BTB at our slaughterhouses be achieved? The only way to achieve effective diagnosis of BTB is to keep proper records on animals so that animals coming from more areas of the country with high endemicity of BTB can be handled separately as recommended by FAO. The introduction of the Hazard Analysis Critical Control Point (HACCP) concept in meat inspection as recommended by the FAO can also help in an effective diagnosis of BTB and other zoonotic diseases at the slaughterhouses (*Herenda et al., 2000*). There is therefore the need for a nation-wide survey to establish the prevalence of BTB so that animals coming from low prevalence areas could be dealt with separately at slaughter, with minimum handle as advised by modern inspection

The Government of Ghana, through the peri-urban dairy cattle development project and the poverty reduction programme, is encouraging the use milk and milk products from local cows in Ghana. This calls for the control of bovine tuberculosis in cattle in Ghana to reduce the public health risks in the use of milk and milk products. The test and slaughter programme has been used in all countries where bovine tuberculosis has been controlled. The single intradermal comparative tuberculin test, the test used in the present study, is the final test in this programme. All cattle that react positive to the test are slaughtered. The test is repeated every year until there are no more reactors. The reason for employing this test in the programme is that, it is capable of

differentiating *M bovis* and *M tuberculosis* from non-pathogenic mycobacterium, so that animals infected with non -pathogenic mycobacterium are not slaughtered (Blood and Radostits 1989). The present study has confirmed that the single intradermal comparative tuberculin test is an effective test in identifying BTB infected cattle and should be used in Ghana.

The test and slaughter programme is recommended to countries where the prevalence of BTB is below 5% (Blood and Radostitis, 1989). The results of the present study suggest that the prevalence of BTB in Ghana is higher than the 5% limit. The results of the study should therefore form the bases of any future BTB control programme in Ghana

Also, meat inspection plays a major role in detecting disease outbreaks before they attain epidemic proportions. An example is the recent outbreak of foot and mouth disease in Europe, which was first detected at a slaughterhouse during meat inspection. To play this important role, meat inspection must be carried out effectively. The present study has revealed that Meat Inspectors in Ghana are not adequately trained and lack vital equipments for proper conduct of their responsibilities. There is therefore the need to train and equip Meat Inspectors to make meat inspection effective in controlling diseases of animals and in particular those of zoonotic importance.

5.2 CONCLUSIONS/ RECOMMENDATIONS:

In conclusion, it can be stated that routine meat inspection at slaughterhouses is not effective in the diagnosis of bovine tuberculosis. Over 90% of cattle in the present study with tuberculosis pass through meat inspection undetected, posing a significant risk to human infection. The study results have also shown that the prevalence of BTB is much higher than is reported by Meat Inspectors and this requires efforts to control or eliminate the disease.

The study results have also shown that pimply gut-like lesions, commonly found in the intestines of cattle at slaughter are largely due to Mycobacterium and not the nodular worm, Oesophagostomum, and are missed by Meat Inspectors posing a risk to human infection.

There is therefore the need for:

- Further work to establish the type of mycobacterium identified in the pimply gut-like lesion by the present study, to make an informed decision on how best human infection of BTB could be prevented.
- Technical Officers who are being posted to the slaughterhouses need to be trained before they are sent to the slaughterhouses.
- There is a need to provide the needed logistics for effective meat inspection in the country.

REFERENCES:

1. Acha P N and szyfres B eds(1987). Zoonoses and communicable diseases common to man and animals(2nd ed) Washington DC, Pan Amer Health Org (Scientific publication),503-963.
2. Alfredson S and Skjerve E(1993). An abattoir based case control study of risk factors for mycobacteriosis in Norwegian swine. Preventive medicine 15, 253-59.
3. Anon (1989). TB infection in Dutch alpacas. Animal Pharm; 175, 12
4. Archer J F(1981). Bovine lymph node survey. Advances in veterinary public health 1
38-40
5. Bakulov I A, Butkin E I, Bedemikov B A, Urkov G G(1987). Epidemiology and microbiology. Kalos 3, 142
6. Bates D K(1986). Developments in veterinary public health as they affect meat quality. Kajian Vet. Malasyia 18, 229-349
7. Berends B R, Snijders J M A, Van Logtestin J G(1993). Efficacy of current EC meat inspection procedures and some proposed revisions with respect to microbiological safety: a critical review. Veterinary Record, 133,411-5

8. Blanco D C and Radostits O M(1989) Veterinary Medicine (7th ed) London: Balliere Tindall Ltd. 710-740
9. Blood and Radostits (1989). Veterinary medicine. E L B S, (5th ed). Macmillan New York. 19, 524- 530.
10. Bonsu O-A, Laing E, Akanmori B. D .(2000). Prevalence of tuberculosis in cattle in the Dangme West district of Ghana, Public health Implications. Acta tropica 76, 9-14
11. Bouvet E, Casalino E, Mendoza-Sassi G (1987) A nosocomial outbreak of multi drug resistant *Mycobacterium bovis* among HIV infected patients. A case control study. AIDS 7, 1453-1460.
12. Carmichael J(1941). Bovine tuberculosis in the tropics with special reference to Uganda Part II Experimental tuberculosis in zebu cattle. Vet. J 97, 329-339
13. Carmichael J(1939). Bovine tuberculosis in the tropics with special reference to Uganda Part I J. Comp Pathol 52, 322-335.
14. Cayla J A, Jansa J M, Plasencia J, Pavellada N(1991). Impact of tuberculosis on new AIDS definition in Bcelona. Bull Int. Union Tuberc. Lung Dis. 66, 43 -45

15. Codex Alimentarius commission (1991). Report of the 6th session of the committee on meat hygiene. 62-65.
16. Collins D M, de Lisle G W, Collins J D, Costello E(1994). DNA restriction fragment typing of *Mycobacterium bovis* isolates from cattle and badgers in Ireland. Vet. Rec. 721-726
17. Collins C H and Grange J M,(1983). A review: The bovine tubercle bacillus. Journal of Applied Bacteriology, 55, 12-29.
18. Collins C H and Grange J M(1987). Zoonotic implications of *Mycobacterium bovis* infection. Ir. Vet. J. **41**, 363-366.
19. Cooper G L, Grange J M, McGregor J A, McFadden J J(1989). The potential use of DNA probes to identify and type strains within the *Mycobacterium tuberculosis* complex Applied microbiol 8,127-130.
20. Cosivi O., Meslin F. X., Daborn C.J.& Grange J.M (1998) The Epidemiology of *Mycobacterium bovis* in animals and humans with particular reference to Africa, sci. tech. **14** 3.
21. Crofton J(1992) Clinical tuberculosis, Macmillan U. K. 10,131-134.

22. Curtis P E (1990) Environmental medicine concept for farm animal clinicians. Vet record 645-646.
23. Dabom C J. Grange J M(1993). HIV/AIDS and its implications for the control of animal tuberculosis. Br. Vet J. 149, 405 -417
24. Dankner W M, Waeker N J, Essey M A, Moser K,. Thompson M, Davis C H(1993) *Mycobacterium bovis* infections in San Diego; A Clinicoepidemiological study of 73 patients and a forgotten pathogen Medicine (Baltimore) 72,11-37.
25. Doyle T M and Stuart P (1958) Vaccination of cattle with BCG Br Vet J 114, 3-10
26. Duffield B J and Young D A(1985). Survival of *Mycobacterium bovis* in different environmental conditions. Vet Microbiol **10**, 193-197.
27. Elbers A R W, Tielen M J m Snijders J M A, Cromwijk W A J , Hunneman W A(1992). Epidemiological studies on lesions in finished pigs in the Netherlands. Preventive Veterinary Medicine 14,217-31
28. Elwood D C and Waddingon F G(1972). A second experiment to challenge the resistance of tuberculosis in BCG vaccinated cattle in Malawi Br Vet J **128**, 619-626.

29. Festenstein F and Grange J M(1991). Tuberculosis and the acquired immune deficiency Syndrome-Review J Applied Bacteriol 71, 19-30.
30. Francis J(1972). Route of infection in tuberculosis Aust. Vet J 48,578
31. Francis J(1949). Tuberculosis. The control of an infectious disease. Lancet 257-553.
32. Francis J(1947). Bovine tuberculosis, a contrast with human tuberculosis. London Staples Press Ltd 220
33. Gallangher, J, Muirhead R. H, Burn, k. J. (1980) Tuberculosis in wild baggers(melesmeles) in Glloucestershire. Vet Record 105,54-551.
34. Ghobashy H M (1997). Animal tuberculosis in Egypt Egypt, in animal tuberculosis in Africa and middle East 53-54.
35. Glosser , J.W. (1988). Back to the future: The animal health monitoring system -A political necessity being addressed in the United States Acta. Veterinair Scandinavia 29, 42-8
36. Gracey, J.F and Collins, D.S. (1992). Meat hygiene. BailliereTindall. Londol. 390-425.

37. Grange J.F and Collins (1987). Bovine tubercle bacilli and disease in animals and man. *Epidemiological information* ,**92** 221-34.
38. Grau, F. H. (1986). Microbial ecology of meat and poultry in: *Advances in meat research* v.2 (macmillan).1-39.
39. Grossklaus, D. (1987). The future role of the veterinarian in the control of zoonoses. *The Veterinary Quaterly* **9**, 321-31
40. Guha A N and Sarkar P B(1970). Study of tuberculosis amongst cattle in Calcutta. *Ind Vet J.* **47**, 196-200
41. Hardie R M and Watson J M(1992). *Mycobacterium bovis* in England and Wales: past present and future. *Epidemiol Infection* **109** 23-33.
42. Hathaway, S.C and Mckenzie, A.I(1991). Postmortem meat inspection programs: separating science and cost-effective meat inspection. *Veterinary record* Jan. 24,78.
43. Hathaway, S.C., Pullen, M.M., Mckenzie, A.I(1988). A model for risk assessment of organoleptic postmortem inspection procedures for meat and poultry, *J. of the American vet. med. Assoc.* 960-6.

44. Herenda D, Chambers P G, Ettriqui A, Seneviratna P, da Silva T J P (2000) Manual on meat inspection for developing countries, FAO Health Paper 119 1-15
45. Himes E M, Luchsinger D W , Jarganin J L, Thoen C O, Hood H B, Ferrin D A(1980). Tuberculosis in fennec foxes Journal of American Vet. Med. Assoc. **177**, 825-826.
46. Holm J and Holm M (1945) National examination for tuberculosis. Acta tubercle Scand 19., 17-107
47. Huitema H(1969), The eradication of bovine tuberculosis in the Netherlands and the significance of man as a source of infection in cattle. The Royal Netherlands Tuberculosis Association, selected papers, **12**, 62-67
48. ICMSF(1988). HACCP in microbiological safety and Quality. International Commission on Microbiological specifications for foods.(Blackwell,Oxford).
49. Kazwala R. R., Kambarage D. M., Dabom C.J., Nyange J. & Sharp J.M.(1997) The prevalence of bovine tuberculosis in indigenous cattle of the southern highlands of Tanzania. Country report . 27-38 in bovine tuberculosis in Africa and the Middle East Rabat Morocco.
50. Kleeberg H H(1960). The tuberculin test in cattle. J. S. Afr. Vet. Assoc. **31** ;213-225.

51. Kyrval J, Zachrau R, Poulsen, A(1995). The technical design and setup of visual meat inspection facilities on the slaughter chain. J. S. Afr. Asso. **65** 320-330
52. Latini M. S, Latini O.A. Lopez M.L, Cecconi J.O(1990). Tuberculosis bovina en seres humanos Rev. Argent torax 51 13-16
53. Lesslie I W Magnus K Stewart CJ(1972). The prevalence of bovine tuberculosis infection in man in the English rural population. Tubercle. **53** 198-204.
54. Lesslie I W(1965). Vaccination of cattle with BCG . Vet Rec. 77, 1565-1566
55. Maddock E.C.G(1936). Experiments on the infectivity for healthy calves of bovine tubercle bacilli discharged in dung upon pasture. Part 1 from tubercular calves fed with emulsion of tubercle bacilli 1934-5. Part 2 from tubercular cows passing tubercle bacilli in their dung 1935-6 Journal hygienicamg) 594-601.
56. Madie, P(1992). Do we still need meat inspection. Veterinary continuing Education Massey University **145**, 77-85
57. Madlar E M (1940) Pulmonary tuberculosis in cattle. Am. Rev. Tubec. 41. 283-306
58. Mason P R, Gwanzura L, Lowe O, Kolk A H (1993) The use of monoclonal antibodies to identify *Mycobacteria* grown in culture in Zimbabwe. Tubercle lung Dis. 74., 195-199

59. Mckay W M(1943). Congenital tuberculosis in bovine Vet J. 98;47-53.
60. Modie P A(1977). Tuberculin reactions in BCG vaccinated cattle Br. Vet J. 133, 624-625
61. Monagham M L M and Hannan J (1983). Abattoir survey of bovine kidney disease. Vet record 113, 55-7
62. Monthly reports Veterinary Services, (1997) Accra,
63. Morris R S, Pfeiffer D U, Jackson R(1994). The Epidemiology of *Mycobacterium bovis* infections. Vet Microbiol 40:153-177.
64. Morse D R Brothwell D R Ucko PJ(1964). Tuberculosis in ancient Egypt Am rev respiratory Disease 90 524- 41.
65. Murray, G(990). Ante-mortem and post mortem meat inspection: an Australian inspection services perspective. Australian veterinary Journal, 63, 211-5
66. Narin JP Raviglione MC Kochi A(1992). HIV associated tuberculosis in developing countries: Epidemiology and strategies for prevention. Tubercle lung disease 73 311-321.

67. Ostetag (1999) The use of fresh milk of tuberculous animals. The journal of comparative pathology and therapeutics 12, 240-5099.
68. O'Reilly L M, Castello E (1988) Bovine tuberculosis with special reference to the epidemiological significance of pulmonary lesions. Ir Vet News 10 11-21
69. Otopri, Laing E, Akanmori B D (2000) Detection and management of zoonotic diseases at Kumasi slaughterhouse in Ghana. Acta Tropica 76., 15-19
70. Plum, (1939). Epidemiological trends of tuberculosis. Amer Rev. 42; 382-400
71. Radostits O M, Blood D C, Gay C C (1994). Veterinary Medicine (8th ed) a text book of the diseases of cattle, sheep, pigs, goats, and horses. London; Ballere Tindall, 1094; 830-850.
72. Rakower J(1953). Tuberculosis among Jews; Mortality and morbidity among different Jewish ethnic groups. Tuberculosis among Yemenite Jews. Etiologic factors. Am Rev Tubber 67;85-93.
73. Ram T. Shama R M(1955). Tuberculosis infection in Harayana Hissar cattle Indian J Vet Sci Anim Husb 25; 99104.

74. Ritacco V and de Kantor I N(1991). Zoonotic tuberculosis in Latin America. J Clin Microbiol **30**: 3299-3300
75. Roth R B(1940). The environmental factor in relation to high Negro tuberculosis rates. Am Rev. Ubere 38; 197-204.
76. Sauret j. Jolis R. Ausina V. Castro E. Comudella R(1992). Human tuberculosis due to *Mycobacterium bovis* : a report of ten cases. Tubercle Lung disease 73; 388-391..
77. Schliesser T(1992). *Mycobacterium bovis* infection in man in Moussa AA M lotfio Mahir in: Proceedings of the international conference on animal tuberculosis in Africa and the middle East Cairo 193-194.
78. Shama A K Vanamya P R, Parihav N S(1985). Tuberculosis in cattle; A retrospective study based on necropsy. Ind. J Vet Pathol **9**; 14-18.
79. Shimao T(1995). Disaster awaits Asia as Asia are neglected . TB& HIV 2.
80. Sigurdson J(1945). Studies on the risk of infection with bovine tuberculosis to the rural population. With special reference to pulmonary tuberculosis. Acta Tuberc. Scand Suppl XV: 1- 250

81. Stamp J T, Wilson A(1946). Some aspects of pathogenesis of bovine tuberculosis based on abattoir returns. Vet Rec. 58; 11-15.
82. Stead W W, Senner J W Reddick W T, Lofgren J P(1990). Racial differences in susceptibility to infection by *Mycobacterium tuberculosis*. N Engl J Med. 322; 422-427.
83. Steele J H and Ranney A F (1958) animal tuberculosis. Am Review Respiratory Disease 77, 908-921
84. Stenhouse W, Hoy W A(1930). The viability of *Mycobacterium bovis* on pasture land, in stored faeces and in liquid manure. I. The viability of *Mycobacterium bovis* on pasture land. J Hyg (Camb) 30; 413-419.
85. Stumpff C D Essey M A Pemberton R. Roswell NM Thompson RE(1985). Tuberculosis in new Mexico: Preliminary Report Proceedings Us Animal Health Assoc 89 480- 484.
86. Turkson and Boadu (1999) Unpublished Reports, Accra
87. Van Logtestijn, J.G(1993). Integrated quality. Meat safety : a new approach. Meat international, 2. 123-8.
88. Warren E L (1994) Medical microbiology and immunology, 3th edition, Appleton and Lang Connecticut, 21, 114-119

89. WHO (1993) Report of the WHO on zoonotic tuberculosis with participation of FAO,
Geneva, 15th Nov. 1993.
90. WHO (1956. WHO/FAO Expert committee on Zoonoses second report
Geneva,.(technical report series 169)
91. Wilesmith J W. Williams D R (1986). Tuberculosis lesions in reactor cattle. Vet rec.
11951.
92. Wilson R. Crouch, E.A.C. (1987). Risk assessment and comparisons an introduction .
Science, 205.45-50.
93. Zukeman L(1980). Badgers, cattle and tuberculosis. Report to Rt. Hon. Peter Walker MP
London HMSO 107

Appendix 1

Checklist questions for an interview.

- I. 1. Mention five most common diseases which you come across during meat inspection which you think are of public health importance.
2. What is bovine tuberculosis?
3. Is bovine tuberculosis a problem in your area?
4. How often do you diagnose tuberculosis in cattle?
5. What are the organs you examine for bovine tuberculosis?
6. How many suspected cases of BTB have you come across this year (2000)?
7. How many of the cases were sent for confirmation, and how many of them were confirmed?
8. If some were confirmed, Who confirmed it?
9. How many animals were slaughtered this year(2000) at the slaughter house where you work?
10. How often do you come across pimple gut-like lesions in the intestines of cattle?
- II. 11. What do you think are the cause of these lesions?
12. What action do you take when you come across these lesions?
13. What other cases which are found at meat inspection do you think can be confused with tuberculosis?
14. Do you think the correct diagnosis of BTB. is important? If yes, why?
15. Do you spend more or less than 10 minutes on a carcasses during meat inspection?
16. What do you think need to be done but have not been done to encourage meat inspectors to sent suspected cases of tuberculosis for confirmation?

17. Have you had any training on meat inspection after Pong Tamale Veterinary College or the School of Hygiene?
18. If yes, what was the duration of the course?
19. What training do you think you need to improve on your meat inspection skills?
20. How will you rate the effectiveness of meat inspection in Ghana? 1. Effective, 3. Not effective 4. Can not tell.
21. How will you rate the conditions at the legal slaughter houses/slabs? 1. Excellent 2. Good. 3. Poor 4. Very poor.